Part: Plant Physiology

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Plant Cell

I. Functions of the cell wall: *The cell wall serves a variety of purposes including:*

1- maintaining/determining cell shape (analogous to an external skeleton for every cell). Since protoplasts are invariably round, this is good evidence that the wall determines the shape of plant cells.

2- Supports and mechanical strength (allows plants to get tall, hold out thin leaves to obtain light).

3- Prevents the cell membrane from bursting in a hypotonic medium (*i.e.*, resists water pressure).

4- Controls the rate and direction of cell growth and regulates cell volume.

5- Ultimately responsible for the plant architectural design and controlling plant morphogenesis since the wall dictates that plants develop by cell addition.

6- Has a metabolic role (*i.e.*, some of the proteins in the wall are enzymes for transport, and secretion).

7- Carbohydrate storage - the components of the wall can be reused in other metabolic processes (especially in seeds). Thus, in one sense the wall serves as storage for carbohydrates.

8- Signaling - fragments of wall, called oligosaccharins, act as hormones. Oligosaccharins, which can result from normal development or pathogen attack, serve a variety of functions including: (a) stimulate ethylene synthesis; (b) induce phytoalexin synthesis; (c) induce chitinase and other enzymes; (d) increase cytoplasmic calcium

levels and (d) cause an "oxidative burst". This burst produces hydrogen peroxide, superoxide and other active oxygen species that attack the pathogen directly or cause increased cross-links in the wall making the wall harder to penetrate.

II. Wall Components - Chemistry

The main ingredient in cell walls is polysaccharides (or complex carbohydrates or complex sugars) which are built from monosaccharides (or simple sugars). Eleven sugars are common in these polysaccharides including glucose and galactose. Carbohydrates are good building blocks because they can produce a nearly infinite variety of structures. There are a variety of other components in the wall including protein, and lignin.

A. Cellulose

Cellobiose (glucose-glucose disaccharide) is the basic building block. Cellulose readily forms hydrogen bonds with itself (intra-molecular H-bonds) and with other cellulose chains (inter-molecular H-bonds). A cellulose chain will form hydrogen bonds with about 36 other chains to yield a microfibril. This is somewhat analogous to the formation of a thick rope from thin fibers. Microfibrils are 5-12 nm wide and give the wall strength - they have a tensile strength equivalent to steel. Some regions of the microfibrils are highly crystalline while others are more "amorphous".

B. Cross-linking glycans (=Hemicellulose)

The diverse group of carbohydrates used to be called hemicellulose. Characterized by being soluble in strong alkali. They are linear (straight), flat, with a β-1,4 backbone and relatively short side chains. Two common types include xyloglucans and glucuronarabinoxylans. Other less common ones include glucomannans, galactoglucomannans, and galactomannans. The main feature of this group is that they don't aggregate with themselves - in other words, they don't form microfibrils. However, they form hydrogen bonds with cellulose and hence the reason they are called "*cross-linking glycans*". There may be fructose sugar at the end of the side chains which may help keep the molecules planar by interacting with other regions of the chain.

C. Pectic polysaccharides

These are extracted from the wall with hot water or dilute acid or calcium chelators (like EDTA). They are the easiest constituents to remove from the wall. They form gels (*i.e.*, used in the jelly making). Another diverse group of polysaccharides that are particularly rich in galacturonic acid (galacturonans = pectic acids). Polymers of primarily β 1,4 galacturonans (= polygalacturonans) are called homogalacturons (HGA) and are particularly common. These are helical in shape. Divalent cations, like calcium, also form cross-linkages to join adjacent polymers creating a gel. Pectic polysaccharides can also be cross-linked by dihydrocinnamic or diferulic acids. The HGA's (galacturonans) are initially secreted from the Golgi as methylated polymers; the methyl groups are removed by pectin methylesterase to initiate calcium binding.

Although most pectic polysaccharides are acidic, others are composed of neutral sugars including arabinans and galactans. The pectic polysaccharides serve a variety of functions including determining wall porosity, providing a charged wall surface for cell-cell adhesion (middle lamella), cell-cell recognition, pathogen recognition and others.

D. Protein

Wall proteins are typically glycoproteins (polypeptide backbone with carbohydrate side chains). The proteins are particularly rich in the amino acids hydroxyproline (hydroxyproline-rich glycoprotein, HPRG), proline (proline-rich protein, PRP), and glycine (glycine-rich protein, GRP). These proteins form rods (HRGP, PRP) or beta-pleated sheets (GRP). The wall proteins also have a structural role since: (1) the amino acids are characteristic of other structural proteins such as collagen and gelatin; and (2) to extract the protein from the wall requires destructive conditions. Protein appears to be cross-linked to pectic substances and may have sites for lignification. The proteins may serve as the scaffolding used to construct the other wall components.

Another group of wall proteins is heavily glycosylated with arabinose and galactose. These arabinogalactan proteins, or AGP's, seem to be tissue-specific and may function in

cell signaling. They may be important in embryogenesis and growth and guidance of the pollen tube.

E. Lignin

The polymer of phenolics, especially phenylpropanoids. Lignin is primarily a strengthening agent in the wall. It also resists fungal/pathogen attack.

F. Suberin, wax, and cutin

A variety of lipids are associated with the wall for strength and waterproofing.

G. Water

The wall is largely hydrated and comprised of between 75-80% water. This is responsible for some of the wall properties. For example, hydrated walls have greater flexibility and extensibility than non-hydrated walls.

III. Morphology of the Cell Wall - *there are three major regions of the wall:*

1- **Middle lamella**

The outermost layer, the glue that binds adjacent cells, is composed primarily of pectic polysaccharides.

2- **Primary wall**

Wall deposited by cells before and during active growth. The primary wall of cultured sycamore cells is comprised of pectic polysaccharides (30%), cross-linking glycans (hemicellulose 25%), cellulose (15-30%) and protein (20%). The actual content of the wall components varies with species and age. All plant cells have a middle lamella and primary wall.

3- **Secondary Wall**

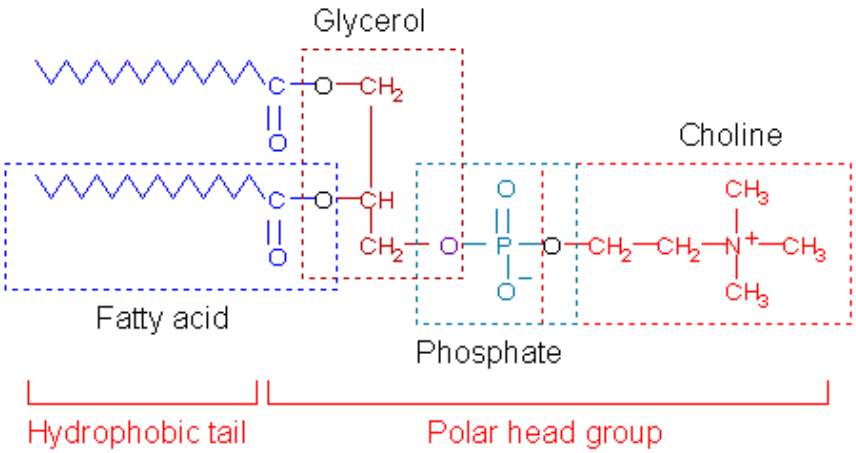
Some cells deposit additional layers inside the primary wall. This occurs after growth stops or when the cells begin to differentiate (specialize). The secondary wall is mainly for support and is comprised primarily of cellulose and lignin.

III. Organelles in Both Plant and Animal Cells

First, we will study the components of the protoplast, but will especially concentrate on those structures unique to plants.

A. Plasma or Cell membrane

Cell boundary; selectively permeable; bilayer of phospholipids with inserted protein. Phospholipids are unique molecules - they are amphipathic, meaning that they have both hydrophilic and hydrophobic regions. They have a glycerol backbone; one of the hydroxyls is bonded to phosphate and another charged group, the other two hydroxyls are esterified to fatty acids. The fatty acids range in length from C14-C24. One fatty acid is usually unsaturated and the other is saturated. The unsaturated fatty acid is kinked which helps to keep plant cell membranes fluid at cool temperatures. As a result plant phospholipids usually have a higher degree of unsaturation than animals. Hydrophobic interactions between the tail regions of the phospholipids hold the membrane together. Some proteins are found: (1) just on the outside or inside surfaces of the membrane (peripheral proteins - non-covalent interactions and anchored proteins - covalently bound to lipids, etc); or (2) embedded in the membrane (integral protein), many of which span the membrane (trans-membrane proteins). Hydrophilic regions of the integral proteins are oriented to the outside of the membrane whereas hydrophobic regions are embedded within the phospholipid bilayer. Lipid soluble materials can readily pass through but charged or ionized substances (hydrophilic) pass through very slowly, if at all. The function of the membrane is to: (1) regulate traffic; (2) separate the internal from the external environment; (3) serve as a platform on which some reactions can occur; (4) participate in some reactions (*i.e.*, the membrane components are important intermediates or enzymes); and (5) provide some structural integrity for the cell.



Structure of a phospholipid

B. Nucleus

The cell "brain". Surrounded by a double membrane (two phospholipid bilayers). The nuclear membrane has pores. The structure of the pores is complex and comprised of more than 100 proteins. The pore opening is surrounded by a series of proteins and these are attached to a series of radial spokes. Nucleoplasm - matrix within the nucleus. DNA, which is found in the nucleus, may be condensed into chromosomes or not (chromatin). There may be one or more nucleolus (site of ribosome production). The nucleus is 5-20 μm in diameter. There is a layer of intermediate filaments (see below) just inside the nuclear envelope; called the nuclear lamina.

C. Cytoplasm/cytosol

The cytosol is the gel-like matrix within the cell in which the other structures are embedded. The cytoplasm refers to the cell materials inside the membrane.

D. Mitochondria

These organelles, like the nucleus and plastids, are double-membrane bound. They vary in shape from tubular (like sausages) to spherical. They reproduce by fission, have their own ribosomes and DNA (a circular loop like prokaryotic cells). The inner

membrane has a larger surface area so it must be folded into finger-like projections (called cristae) to fit inside the outer membrane. Mitochondria are found in all eukaryotic cells. They are the sites of cellular respiration - the process by which energy is released from fuels such as sugar. The mitochondria are the power plant of the cell. They are small (1-5 μm) and generally numerous (500-2000 per cell). A popular misconception is that "plants have chloroplasts, animals have mitochondria." Plant cells, at least green plant cells (*i.e.*, leaf cells) have both. Root cells only have mitochondria. Mitochondrial DNA which comprises about 200 kbases, codes for some of the genes required for cellular respiration including the 70S ribosomes and components of the electron transport system. The inner membrane differs from the plasma membrane in that it has a higher protein content (70%) and unique phospholipids (*i.e.*, cardiolipin).

E. Ribosome

Sites of protein synthesis (translation). Two subunits; one large and the other small. Made in the nucleus from rRNA and protein. Ribosomes are tiny (0.25 μm) and numerous (5-50 X 10^{10} per cell). Since ribosomes are not surrounded by a membrane, they are not considered to be "true" organelles. Some ribosomes are 'free' (produce proteins that remain in the cell) while others are attached to the ER (produce proteins for export). To export a protein, the mRNA and subunits of the ribosome bind together. A signal recognition particle (SRP) binds to specific amino acids in the newly forming protein. The SRP, which is bound to the protein/mRNA/ribosome, then binds to a receptor in the ER membrane. As the protein is made it is released into the lumen of the ER and the SRP sequence of the protein is snipped off.

F. Endoplasmic reticulum

A series of membranous tubes and sacs (cisternae) run throughout the cell. Rough ER has ribosomes associated with it and is laminar while smooth ER lacks ribosomes and is tubular. The ER has several functions including (1) synthesis of lipids and membranes (smooth ER); (2) serving as a site for the synthesis of proteins by the ribosomes (rough ER); (3) transport (a type of cell 'highway' system); and (4) support.

G. Peroxisomes

Membrane sac containing enzymes for metabolizing waste products from photosynthesis, fats and amino acids. Hydrogen peroxide is a product of metabolism in peroxisomes. Catalase which breaks down the peroxide is also present and serves as a marker enzyme for these organelles.

H. Glyoxisomes

Membrane sac containing enzymes for fat metabolism. Especially common in seeds. Also contain catalase.

I. Golgi apparatus

Pancake- or pita bread-like stack of membranes. Particularly important in cells that produce materials for export (secretion). They have a polarity (cis-imports vesicles from ER; trans - exports vesicles). The Golgi is the site of processing and packaging cellular components. Vesicles containing proteins, lipids and other materials, fuse with the Golgi (cis side), and release contents, which then get processed, sorted, packaged and re-released from the other side (trans face). The Golgi also is active in synthesizing many cell components, especially carbohydrates and is involved in tagging proteins with carbohydrates and other side chains for sorting them to their final destination. There are two models for the movement of materials thru the Golgi: (1) Vesicle Migration Model - in this case, a vesicle fuses with the cis side, then ultimately a new vesicle pinch off this stack and fuses with the next one, and so on, until the vesicle reaches the trans side; and (2) Escalator Model - a vesicle fuses with the cis side and never leaves this stack. Rather, the stack on the trans side releases vesicles and then disintegrates while a new stack forms on the cis side. The original vesicle is now in the "second" stack, and so on until it reaches the trans side. Vesicles are tagged with various proteins to direct them to the appropriate locations.

J. Microtubules

Hollow tubes are made of a mix of alpha and beta tubulin, which are globular proteins. There are essentially 13 columns of proteins. The tubes are about 25 μm in diameter.

Microtubules are involved in the cell cytoskeleton (for support), cell movements (cilia, flagella) and cell division (spindle). Assembly of microtubules is prevented by colchicine, an inhibitor derived from *Crocus* bulbs. Low calcium concentration favors the formation of microtubules.

K. Microfilaments

Protein strands. Solid. Made from G-actin. Involved with the cell cytoskeleton. Main function is support. They are about 7 nm in diameter.

L. Intermediate filaments

These are similar to microfilaments. They are also made of protein in the keratin family; about 10 nm in diameter.

M. Cilia/flagella

For cellular movements. Cilia = many, short; flagella = few, long. Have a 9+2 arrangement of microtubules. Prongs on the tubules are ATPases (dynein) to hydrolyze ATP to provide energy for movement. These are not particularly common in plants.

N. The Cytomembrane system

The membranous organelles (ER, vesicles, Golgi, cell membrane) comprise a group of organelles that cooperate and function together. For example, imagine the synthesis of cellulose in the cell wall of a plant. Cellulose synthesis requires the enzyme cellulose synthase. Ribosomes (rough ER) → makes enzyme → passes through RER to smooth ER → packaged into a vesicle → pinches off→ to Golgi (cis face) → processed → repackaged into a vesicle → pinches off (trans face) → cell membrane→ fuses→ releases contents → cellulose synthase makes cellulose.

O. Others

- Microbodies - a general term for any single membrane-bound organelle typically derived from the ER that contains catalase and/or hydrogen peroxide-producing enzymes. This includes the peroxisomes and glyoxisomes;

- Microsomes - a "biochemical" term for the fraction that is obtained from high speed centrifugation of cell homogenates. It includes membrane fragments and ribosomes.
- Oleosome (spherosomes) - these are lipid bodies. The coolest thing about them is that they are encased by one-half of a cell membrane; in other words, just a single phospholipid layer.

IV. Organelles Unique to Plants - Plastids

Plastids are double membrane-bound organelles in plants. They contain their own DNA (in the nucleoid region) and ribosomes. They are semi-autonomous and reproduce by fission similar to the division process in prokaryotes. If plastids only arise from other plastids and can't be built "from scratch", then where do they come from? The egg. Plastids are inherited cytoplasmically, primarily through the female; however, there are examples of paternal inheritance of plastids. The plastid DNA carries several genes including the large subunit of rubisco and those for resistance to some herbicides. The chemistry of the membranes differs from the plasma membrane. Plastid membranes are comprised of glycosylglycerides rather than phospholipids (the phosphate in the polar head group in glycosylglycerides is replaced with galactose or a related sugar).

There are several types of plastids including:

1- Proplastids - small, precursors to the other plastid types, found in young cells, actively growing tissues;

2- Chloroplasts - sites of photosynthesis (energy capture). They contain photosynthetic pigments including chlorophyll, carotenes and xanthophylls. The chloroplast is packed with membranes, called thylakoids. The thylakoids may be stacked into a pancake- like piles called grana (granum, singular). The "liquidy" material in the chloroplast is the stroma. A chloroplast is from 5-20 nm in diameter and there are usually 50-200 per cell. The chloroplast genome has about 145 Kbase pairs, it is smaller than that of the mitochondria (200 kbases). About 1/3 of the total cell DNA is extranuclear (in the chloroplasts and mitochondria);

1- Chromoplasts - non-photosynthetic, colored plastids; give some fruits (tomatoes, carrots) and flowers their color;

2- Amyloplasts - colorless, starch-storing plastids;

3- Leucoplast - another term for amyloplast;

4- Etioplast - plastid whose development into a chloroplast has been arrested (stopped). These contain a dark crystalline body, a prolamellar body, which is essentially a cluster of thylakoids in a somewhat tubular form.

Plastids can dedifferentiate and convert from one form into another. For example, think about the ripening processing in tomato. Initially, green tomatoes have oodles of chloroplasts which then begin to accumulate lycopene (red) and become chromoplasts. Usually, you find only chromoplasts or chloroplasts in a cell, but not both.

V. Organelles Unique to Plants - Vacuoles

This is the large, central cavity containing fluid, called cell sap, found in plant cells. The vacuole is surrounded by a membrane (tonoplast). Back to the water balloon in the box model - imagine the vacuole to be analogous to another water balloon inside our protoplast balloon. This water balloon is a separate entity that can be physically removed from the cell. The vacuole is penetrated by strands of cytoplasm - transvascular strands.

The tonoplast and plasma membrane have different properties such as thickness (tonoplast thicker).

Virtually every plant cell has a large, well-developed vacuole that makes up to 90% or more of the cell volume. Wow! Meristematic and embryonic cells are exceptions. Young tissues have many small vacuoles. As the cell grows the vacuoles expand and eventually coalesce. These small vacuoles appear to be derived from the Golgi.

The central vacuole contains water, ions, organic acids, sugars, enzymes, and a variety of secondary metabolites. Among the hydrolytic enzymes are proteases (digest protein),

ribonucleases (digest RNA) and glycosidases (break links between monosaccharides). These enzymes are typically not used for recycling cellular components but rather leak out on cell senescence. There are smaller lytic vacuoles, which contain digestive enzymes, that are used for this purpose. Another type of vacuole, protein bodies, are vacuoles that store proteins.

Enzymes

The **enzyme is** from the Greek ένζυμο, *énsymo,* which means ***én*** ("in") and ***simo*** ("yeast").

- ✧ Enzyme proteins regulate metabolic reaction rates.
- ✧ i.e., they control metabolism molecules that accelerate or catalyze chemical reactions (A--->B) in cells by breaking old covalent bonds and forming new covalent bonds.
- ✧ A biological catalyst… but, different from a chemical catalyst.
- ✧ Enzymes act only up a specific substrate and do not change the direction of reactions.
- ✧ Enzymes convert substrates to products with out changing themselves

catalysis***** = acceleration of the rate of a chemical reaction by a catalyst

Some important dates in early Enzyme History

1833 Payen and Peroz - alcohol precipitate of barley holds heat-labile components (**proteins**) that convert starch to sugars

1878 Kuhn - coins term 'enzyme' : Greek "in yeast"

1897 Hans and Eduard Buchner - yeast 'juice' + sugars (jelly) = bubbled gas and ETOH

1898 Ducleaux - uses the suffix "ASE" for enzyme naming

1900 E. Fischer - stereospecificity of enzymes is discovered

1st enzyme crystallized UREASE, 1926 James Sumner

$$2\ NH_2\text{-}CO\text{-}NH_2 + 2\ H_2O \text{ -----> } 4\ NH^+_4 + 2\ CO_2$$

Sumner's bioassay = injects rabbits with urease from Jack beans and the ammonia produced killed the bunnies.

Enzyme Parts List

The activity of an enzyme depends, at the minimum, on a specific protein chain. In many cases, the enzyme consists of the protein and a combination of one or more parts called cofactors. This enzyme complex is usually simply referred to simply as the enzyme.

Apoenzyme: The polypeptide or protein part of the enzyme is called the **apoenzyme** and may be inactive in its original synthesized structure. The inactive form of the apoenzyme is known as a **proenzyme or zymogen**. The proenzyme may contain several extra amino acids in the protein which are removed and allows the final specific tertiary structure to be formed before it is activated as an apoenzyme.

Cofactors: Some enzymes do not need any additional components to show full activity. However, others require non-protein molecules called cofactors to be bound for activity. Cofactors can be either inorganic (*e.g.*, metal ions and iron-sulfur clusters) or organic compounds (e.g. flavin and heme). Organic cofactors can be either prosthetic groups, which are tightly bound to an enzyme, or coenzymes, which are released from the enzyme's active site during the reaction. Coenzymes include NADH, NADPH and ATP. These molecules act to transfer chemical groups between enzymes. An example of an enzyme that contains a cofactor is carbonic anhydrase.

Enzymes that require a cofactor but do not have one bound are called *apoenzymes* or *apoproteins*. An apoenzyme together with its cofactor(s) is called a *holoenzyme* (this is the active form). Most cofactors are not covalently attached to an enzyme but are very tightly bound. However, organic prosthetic groups can be covalently bound (*e.g.*, thiamine pyrophosphate in the enzyme pyruvate dehydrogenase). The term "holoenzyme" can also be applied to enzymes that contain multiple protein subunits, such as DNA polymerases, here the holoenzyme is the complete complex containing all the subunits needed for the activity.

Coenzyme: Coenzymes are small organic molecules that transport chemical groups from one enzyme to another. Some of these chemicals such as riboflavin, thiamine and folic acid are vitamins, this is when these compounds cannot be made in the body and must be acquired from the diet. The chemical groups carried include the hydride ion (H^-) carried by NAD or $NADP^+$, the acetyl group carried by coenzyme A, formyl, methenyl or methyl groups carried by folic acid and the methyl group carried by S-adenosylmethionine.

Since coenzymes are chemically changed as a consequence of enzyme action, it is useful to consider coenzymes to be a special class of substrates, or second substrates, which are common to many different enzymes. For example, about 700 enzymes are known to use the coenzyme NADH.

Coenzymes are usually regenerated and their concentrations are maintained at a steady level inside the cell: for example, NADPH is regenerated through the pentose phosphate pathway and *S*-adenosylmethionine by methionine adenosyltransferase.

Another type of cofactor is an inorganic metal ion called a **metal ion activator**. The inorganic metal ions may be bonded through coordinate covalent bonds. The major reason for the nutritional requirement for minerals is to supply such metal ions as Zn^{+2}, Mg^{+2}, Mn^{+2}, Fe^{+2}, Cu^{+2}, K^{+1}, and Na^{+1} for use in enzymes as cofactors.

Final Enzyme (Apoenzyme + Cofactor = Holoenzyme): The type of association between the cofactor and the apoenzymes varies. In some cases, the bonds are rather loose and both come together only during a reaction. In other cases, they are firmly bound together by covalent bonds. The activating role of a cofactor is to either: activate the protein by changing its geometric shape, or by actually participating in the overall reaction.

The overall enzyme contains a specific geometric shape called the **active site** where the reaction takes place. The molecule acted upon is called the **substrate**.

Enzyme Nomenclature and Classification

Enzymes are commonly named by adding the suffix "-ase" to the root name of the substrate molecule it is acting upon. For example, **Lipase** catalyzes the hydrolysis of a lipid triglyceride. **Sucrase** catalyzes the hydrolysis of sucrose into glucose and fructose.

A few enzymes discovered before this naming system was devised are known by common names. Examples are pepsin, trypsin, and chymotrypsin which catalyzes the hydrolysis of proteins.

The latest systematic nomenclature system known as the International Enzyme Commission (IEC) system is based on the type of reaction catalyzed. There are six broad groups of enzymes in this system as shown in the following Table.

For example, when using this system, "urease" becomes "urea amidohydrolase." Do not be overly concerned about enzyme names, but be able to recognize a substance as an enzyme by its "-ase" ending. Some types of reactions that are being catalyzed will be self-evident

EC MAJOR CLASSES of Enzymes
1. Oxidoreductases... [dehydrogenases] catalyzes oxidation-reduction reactions, often using coenzyme as NAD^+/FAD
Alcohol dehydrogenase [EC 1.1.1.1] ethanol + NAD^+ ---> acetaldehyde + NADH
2. Transferases... catalyze the transfer of the functional group
Hexokinase [EC 2.7.1.2] D-glu + ATP ---> D-glu-6-P + ADP
3. Hydrolyases… catalyze hydrolytic reactions adds water across C-C bonds
Carboxypeptidase A [EC 3.4.17.1] [aa-aa] $_n$ + H_2O ---> [aa-aa] $_{n-1}$ + aa
4. Lyases... cleave C-C, C-O, C-N & other bonds often generating a C=C bond or ring
Pyruvate decarboxylase [EC 4.1.1.1] pyruvate ---> acetaldehyde + CO_2
5. Isomerases… [mutases] catalyze isomerizations
Maleate isomerase [EC 5.2.1.1] maleate ---> fumarate [HFCS]
6. Ligases… condensation of 2 substrates with the splitting of ATP
Pyruvate Carboxylase [EC 6.4.1.1] PYR + CO_2 + ATP ---> OAA + ADP + P

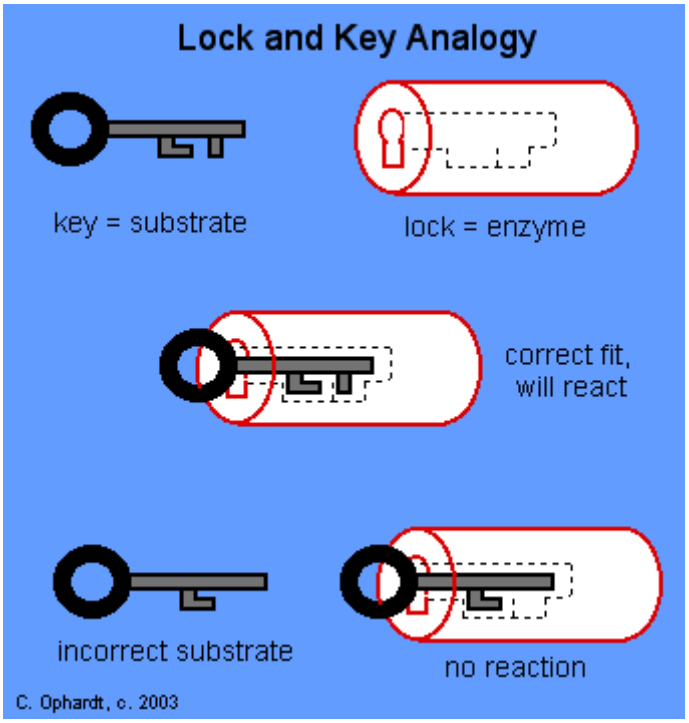
Specificity

Enzymes are usually very specific as to which reactions they catalyze and the substrates that are involved in these reactions. Complementary shape, charge and hydrophilic/hydrophobic characteristics of enzymes and substrates are responsible for this specificity. Enzymes can also show impressive levels of stereospecificity, regioselectivity and chemoselectivity.[19]

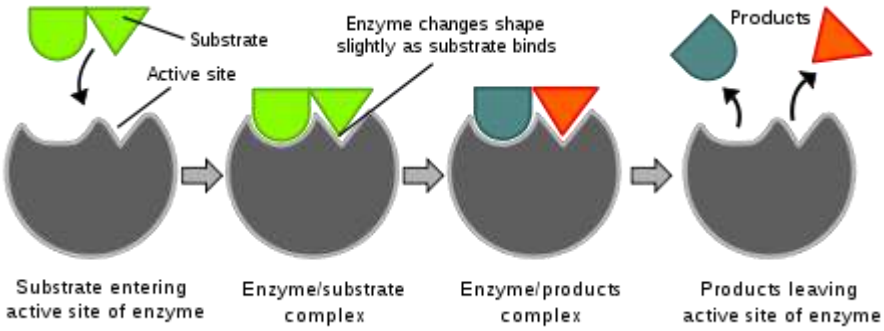
Some enzymes that produce secondary metabolites are described as promiscuous, as they can act on a relatively broad range of different substrates. It has been suggested that this broad substrate specificity is important for the evolution of new biosynthetic pathways.[25]

Lock and key" model

Enzymes are very specific, and it was suggested by Emil Fischer in 1894 that this was because both the enzyme and the substrate possess specific complementary geometric shapes that fit exactly into one another.[26] This is often referred to as "the lock and key" model. However, while this model explains enzyme specificity, it fails to explain the stabilization of the transition state that enzymes achieve. The "lock and key" model has proven inaccurate, and the induced fit model is the most currently accepted enzyme-substrate-coenzyme figure.



Induced fit" model



Diagrams to show the induced fit hypothesis of enzyme action.

In 1958, Daniel Koshland suggested a modification to the lock and key model: since enzymes are rather flexible structures, the active site is continually reshaped by interactions with the substrate as the substrate interacts with the enzyme. As a result, the substrate does not simply bind to a rigid active site; the amino acid side chains which make up the active site are molded into the precise positions that enable the enzyme to perform its catalytic function. In some cases, such as glycosidases, the substrate molecule also changes shape slightly as it enters the active site. The active site continues to change until the substrate is completely bound, at which point the final shape and charge is determined.

Enzyme Kinetics: Basic Enzyme Reactions

Enzymes are catalysts that increase the speed of a chemical reaction without themselves undergoing any permanent chemical change. They are neither used up in the reaction nor do they appear as reaction products.

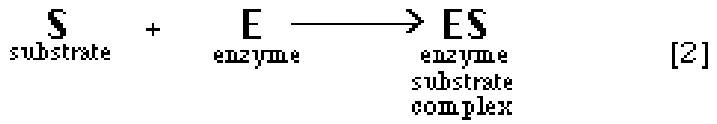
The basic enzymatic reaction can be represented as follows

$$S + E \longrightarrow P + E \qquad [1]$$

where E represents the enzyme catalyzing the reaction, S the substrate, the substance is changed, and P is the product of the reaction.

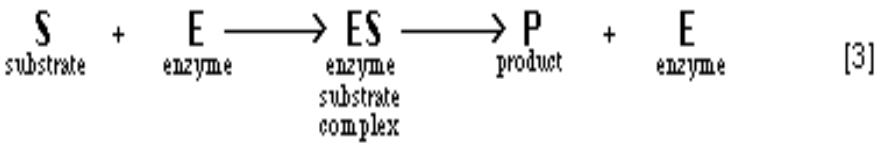
Enzyme Kinetics: The Enzyme Substrate Complex

A theory to explain the catalytic action of enzymes was proposed by the Swedish chemist Savante Arrhenius in 1888. He proposed that the substrate and enzyme formed some intermediate substance which is known as the enzyme substrate complex. The reaction can be represented as:



[2]

If this reaction is combined with the original reaction equation [1], the following results:



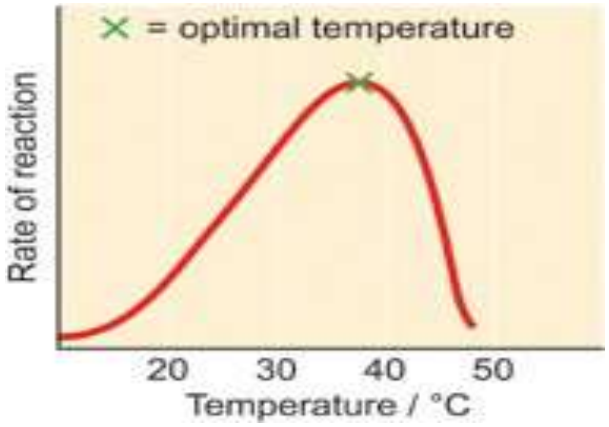
[3]

The existence of an intermediate enzyme-substrate complex has been demonstrated in the laboratory, for example, using catalase and a hydrogen peroxide derivative.

Factors Affecting Enzyme Activity

Knowledge of basic enzyme kinetic theory is important in enzyme analysis in order both to understand the basic enzymatic mechanism and to select a method for enzyme analysis. The conditions selected to measure the activity of an enzyme would not be the same as those selected to measure the concentration of its substrate. Several factors affect the rate at which enzymatic reactions proceed - temperature, pH, enzyme concentration, substrate concentration, and the presence of any inhibitors or activators.

Temperature

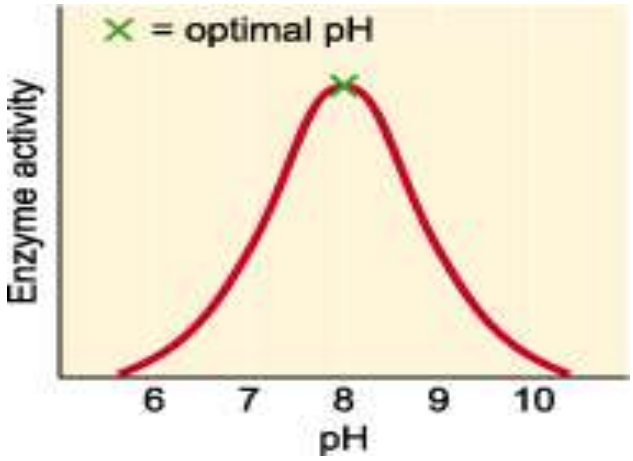


As the temperature rises, reacting molecules have more and more kinetic energy. This increases the chances of a successful collision and so the rate increases. There is a certain temperature at which an enzyme's catalytic activity is at its greatest (see graph). This optimal temperature is usually around human body temperature (37.5 ^{o}C) for the enzymes in human cells.

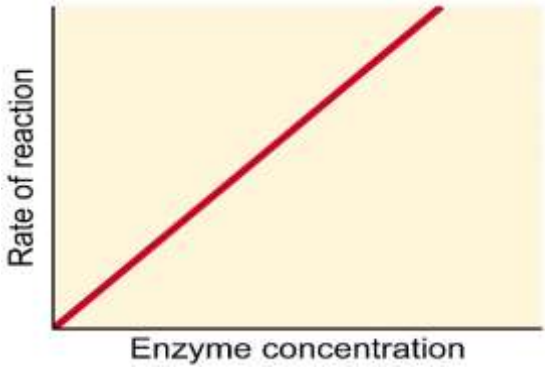
Above this temperature, the enzyme structure begins to break down (**denature**) since at higher temperatures intra- and intermolecular bonds are broken as the enzyme molecules gain even more kinetic energy.

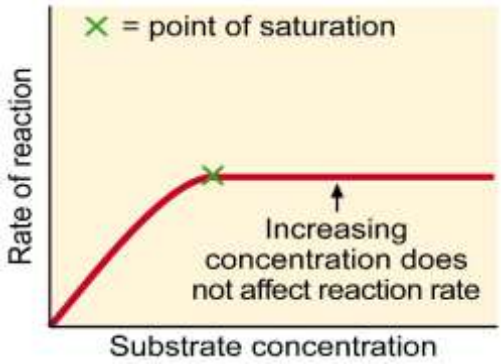
pH

Each enzyme works within quite a small pH range. There is a pH at which its activity is greatest (the optimal pH). This is because changes in pH can make and break intra- and intermolecular bonds, changing the shape of the enzyme and, therefore, its effectiveness.



Concentration of enzymes and substrate





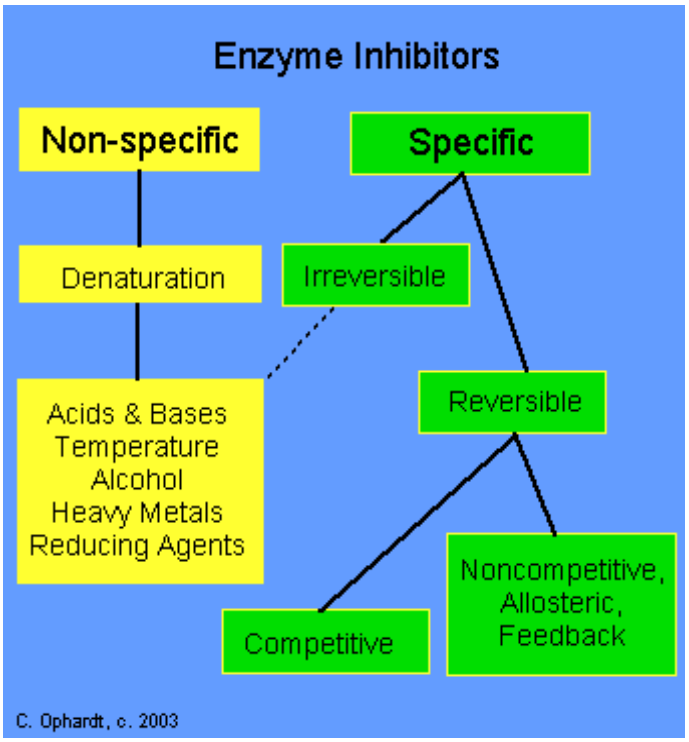
The rate of an enzyme-catalyzed reaction depends on the concentrations of the enzyme and substrate. As the concentration of either is increased the rate of reaction increases (see graphs).

For a given enzyme concentration, the rate of reaction increases with increasing substrate concentration up to a point, above which any further increase in substrate concentration produces no significant change in reaction rate. This is because the active sites of the enzyme molecules at any given moment are virtually saturated with substrate. The enzyme/substrate complex has to dissociate before the active sites are free to accommodate more substrate. (See graph).

Provided that the substrate concentration is high and that temperature and pH are kept constant, the rate of reaction is proportional to the enzyme concentration. (See graph).

Enzyme Inhibitors

Enzyme inhibitors are molecules that interact in some way with the enzyme to prevent it from working in the normal manner. There are a variety of types of inhibitors including nonspecific, irreversible, reversible - competitive and noncompetitive. Poisons and drugs are examples of enzyme inhibitors.



Nonspecific Inhibitors

A nonspecific inhibition affects all enzymes in the same way. Non-specific methods of inhibition include any physical or chemical changes which ultimately **denature** the protein portion of the enzyme and are therefore irreversible.

Temperature: Usually, the reaction rate increases with temperature, but with enzyme reactions, a point is reached when the reaction rate decreases with increasing temperature. At high temperatures the protein part of the enzyme begins to denature, thus inhibiting the reaction.

Acids and Bases: Enzyme activity is also controlled by pH. As the pH is decreased or increased, the nature of the various acid and amine groups on side chains is altered resulting in changes in the overall shape structure of the enzyme.

Specific Inhibitors:

Specific Inhibitors exert their effects upon a single enzyme. Most poisons work by specific inhibition of enzymes. Many drugs also work by inhibiting enzymes in bacteria, viruses, or cancerous cells and will be discussed later.

Competitive Inhibitors:

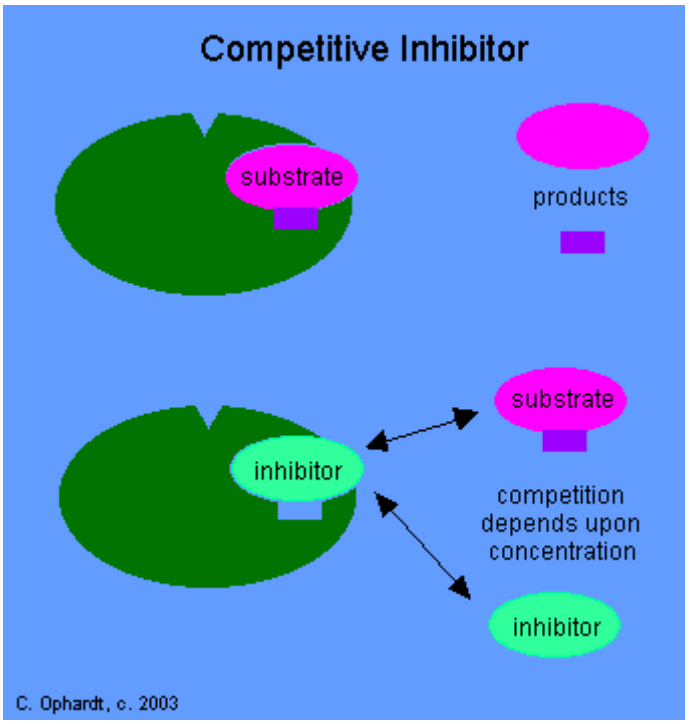
A competitive inhibitor is any compound that closely resembles the chemical structure and molecular geometry of the substrate. The inhibitor competes for the same active site as the substrate molecule. The inhibitor may interact with the enzyme at the active site, but no reaction takes place. The inhibitor is "stuck" on the enzyme and prevents any substrate molecules from reacting with the enzyme. However, a competitive inhibition is usually reversible if sufficient substrate molecules are available to ultimately displace the inhibitor. Therefore, the amount of enzyme inhibition depends upon the inhibitor concentration, substrate concentration, and the relative affinities of the inhibitor and substrate for the active site.

Example: Ethanol is metabolized in the body by oxidation to acetaldehyde, which is in turn further oxidized to acetic acid by aldehyde oxidase enzymes. Normally, the second reaction is rapid so that acetaldehyde does not accumulate in the body.

A drug, **disulfiram (Antabuse) inhibits** the aldehyde oxidase which causes the accumulation of acetaldehyde with subsequent unpleasant side-effects of nausea and vomiting. This drug is sometimes used to help people overcome their drinking habit.

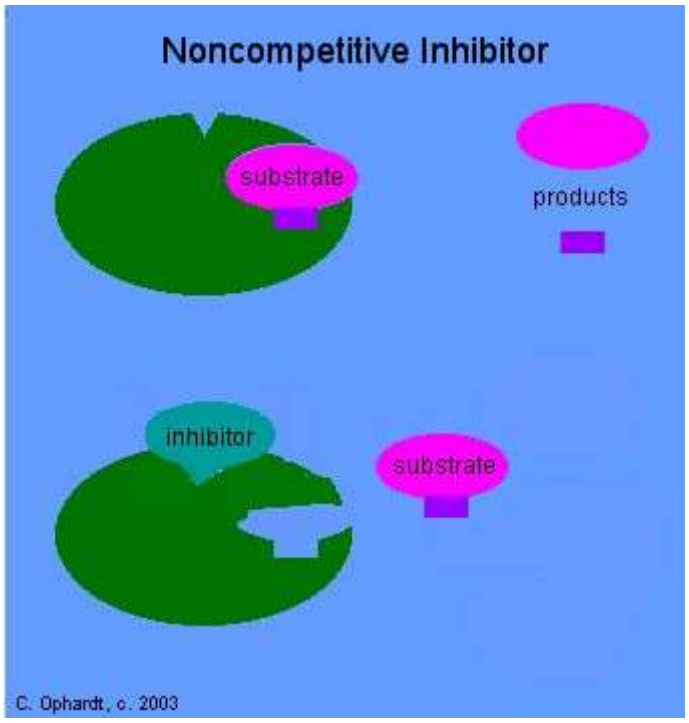
Methanol poisoning occurs because methanol is oxidized to formaldehyde and formic acid which attack the optic nerve causing blindness. Ethanol is given as an antidote for methanol poisoning because ethanol competitively inhibits the oxidation of methanol. Ethanol is oxidized in preference to methanol and

consequently, the oxidation of methanol is slowed down so that the toxic by-products do not have a chance to accumulate.



Noncompetitive Inhibitors:

A noncompetitive inhibitor is a substance that interacts with the enzyme, but usually not at the active site. The noncompetitive inhibitor reacts either remotely from or very close to the active site. The net effect of a noncompetitive inhibitor is to change the shape of the enzyme and thus the active site, so that the substrate can no longer interact with the enzyme to give a reaction. Noncompetitive inhibitors are usually reversible but are not influenced by concentrations of the substrate as is the case for a reversible competitive inhibitor. See the graphic.



Irreversible Inhibitors form strong covalent bonds with an enzyme. These inhibitors may act at, near, or remote from the active site. Consequently, they may not be displaced by the addition of excess substrate. In any case, the basic structure of the enzyme is modified to the degree that it ceases to work.

Since many enzymes contain sulfhydryl (-SH), alcohol, or acid groups as part of their active sites, any chemical which can react with them acts as an irreversible inhibitor. Heavy metals such as Ag^{+}, Hg^{2+}, and Pb^{2+} have strong affinities for -SH groups.

Nerve gases such as diisopropylfluorophosphate (DFP) inhibit the active site of acetylcholine esterase by reacting with the hydroxyl group of serine to make an ester.

Oxalic and citric acids inhibit blood clotting by forming complexes with calcium ions necessary for the enzyme metal ion activator.

Photosynthesis: Light-Dependent Reactions

(or, Life is a photochemical phenomenon)

I. Overview of photosynthesis

Photosynthesis can be defined as the light-driven synthesis of carbohydrates. The equation for this reaction, which you've seen many times is:

$$\mathbf{CO_2 + H_2O + light + chloroplast \rightarrow (CH_2O)_n + O_2}$$

From this simple equation we can make some elegant conclusions:

A. Photosynthesis is a redox reaction.

Some definitions

(a) Reduction – a gain of electrons;

(b) Oxidation – a loss of electrons;

(c) helpful mnemonics to remember: "oil rig" - oxidation is loss, the reduction is gain, or "Leo says grrrr" - loss equals oxidation, gain reduction;

(d) Redox reaction - reaction in which one component is oxidized and the other is reduced. Electrons must come from somewhere and go somewhere.

1- The reduction sequence of carbon: carbon dioxide (most oxidized form of carbon) → carboxyl (organic acid) → carbonyl (aldehydes, ketones) → hydroxyl (alcohols) → methyl → methane (most reduced form of carbon). Note: each step requires the addition (or removal) of two electrons and two protons for reduction (oxidation). Two steps also require the addition/removal of water.

2- How can you tell if a molecule has been oxidized or reduced? (1) look for a change in valence (*i.e.*, $Fe^{2+} \rightarrow Fe^{3+}$ represents oxidation because an electron was lost, increasing the total positive charge); (2) In many biological redox reactions, oxidation is usually accompanied by a loss of protons (hydrogen ions) and reduction is accompanied by a gain of protons; and (3) look for a decrease in the number of oxygen atoms.

3- Biological redox reactions may require electron donors and/or acceptors. These include (1) NAD^+; (2) $NADP^+$; and (3) FAD; which are coenzymes (organic compounds, other than the substrate, required by an enzyme for activity):

$$NAD(P)^+ (ox) + 2e^- + 2H^+ \rightarrow NAD(P)H\ (red) + H^+$$

$$FAD(ox) + 2e^- + 2H^+ \rightarrow FADH_2\ (red)$$

4- Reducing Potential - potential for components to participate in a redox reaction; to predict the direction and tendency of electrons to flow between two electron carriers. The take-home-lessons are: (1) the more negative the reducing potential the better the electron donor; (2) the more positive the reducing potential the better the electron acceptor; (3) spontaneous electron passage occurs from a carrier with more negative reducing potential to one with a more positive reducing potential.

B. CO_2 is reduced to a carbohydrate.

C. Water is oxidized (to oxygen).

D. Water supplies the electrons for the reduction; water is cleaved in the process yielding oxygen as a byproduct.

E. Light provides the energy for the reduction.

F. Photosynthesis is an energy conversion process that ultimately converts light energy to chemical energy (carbohydrates). In a broad sense, it is an example of the 1st Law of Thermodynamics - energy cannot be created nor destroyed, but it can be changed from one form to another.

G. BLACK BOX summary model for photosynthesis. Diagram in class that shows two boxes (light-dependent and light-independent reactions). This model further shows that during the light-dependent and light-independent reactions that there are three major types of energy conversions during photosynthesis:

Conversion 1: Radiant energy (sunlight) → electrical energy (passage of electrons via a series of carriers). This reaction series is part of the light-dependent reactions (z-scheme, non-cyclic electron flow)

Conversion 2: Electrical energy → "Labile" chemical energy (ATP, NADPH; unstable, not readily stored). During this step, ATP and NADPH are produced as the end result of non-cyclic electron flow.

Conversion 3: "Labile" chemical energy → Stable chemical energy (carbohydrate). This last step is the light-independent reactions or Calvin-Benson cycle. This process requires ATP and NADPH.

II. Chloroplasts - *specialized organelles that carry out the process of photosynthesis*

A. Structure.

Remember the cell unit? Terms that you should know are thylakoid (or lamellae), lumen (intermembrane space), envelope, double membrane, stroma, granum, granal thylakoids (or lamellae), stromal thylakoids (lamellae), and starch grains. Chloroplasts may contain fat globules (plastoglobuli). Stacked (or appressed) regions - portion of granum in which thylakoids are adjacent to one another. Non-stacked (non-appressed) regions - regions of the chloroplast where the thylakoids are not adjacent to one another.

B. Chemistry - *Chloroplasts contain:*

1- DNA - circular loop; 120-160 kilobases that code for about 120 proteins;

2- RNA;

3- ribosomes;

4- proteins - some are coded by the nuclear genome, others by the chloroplastic genome. For example, rubisco, an important enzyme, has 2 different subunits, one from each source. The nuclear genes are essential for chloroplast function;

5- pigments - make up about 7% of the chloroplast. These are molecules with a color that absorb light. Two major groups of pigments in higher plants, chlorophylls and carotenoids/xanthophylls. These occur in the thylakoids because they are highly hydrophobic (fat soluble)

D. Pigments

1. Chlorophylls

These molecules look like a tennis racket. The head of the racket is a **porphyrin** ring system, made of four **pyrrole** units linked together (**tetrapyrrole**). It has a long hydrocarbon tail, called **phytol** (C-20), that is derived from the **terpene** pathway (diterpene), built from the **isoprene** skeleton. **Magnesium** is chelated in the ring. The tail is important for orienting the molecule in the membrane. The interaction of the chlorophyll with the membrane is non-covalent and is important because it ultimately determines the physical properties of the chlorophyll.

- ✓ chlorophyll a - methyl group
- ✓ chlorophyll b - formyl group
- ✓ phaeophytin - chlorophyll without the magnesium
- ✓ chlorophyllide - chlorophyll without the tail

2. Carotene/xanthophylls

Both are terpenoid pigments, **tetraterpenoids** (C-40). Carotenes are hydrocarbons, xanthophylls are oxygenated. These pigments are orange and yellow.

3. Chlorophyll biosynthesis

a- ALA (Δ-aminolevulinic acid) is the first well-established precursor

b- ALA is derived from α-ketoglutarate (or glutamate) (a Kreb's cycle intermediate, from the mitochondrion)

c- 2 ALA condense to form a unit of payroll

d- 4 payrolls condense to form porphyrin (tetrapyrrole)

e- Magnesium is inserted

f- A photoreduction step occurs (converts protochlorophyllide → chlorophyllide)

e- the tail is added

4. Light and the Greening Process

Recall that etiolated plants (grown in the dark) are yellowish but turn green rapidly when placed in the light. Light is required, among reasons:

a- convert etioplasts → chloroplasts;

b- photo-reduce protochlorophyllide to chlorophyllide; and

c- activate enzymes for ALA synthesis.

III. Conversion 1: Photons to electrons

A. Nature of light

Light is part of the electromagnetic spectrum - radiation emitted by the sun. Acts as discrete particles (called photons) traveling as waves. Wavelength - the distance between any two crests (or troughs). Symbolized by lambda (λ); frequency - number of waves passing a point in one second (υ). Frequency is inversely related to wavelength $\upsilon = c/\lambda$ where c = speed of light (3×10^{10} cm sec^{-1}). The energy of a photon is quantum.

B. Which photons are important in photosynthesis?

Radiations between 400-700 nm are photosynthetically active (termed PAR). Specifically, red (600's) and blue (400's) light are important.

C. Photons must be absorbed to be used in a photochemical reaction.

In other words, only those molecules that absorb quanta participate in photosynthesis. So, which molecules absorb red and blue light? Chlorophyll a & b absorb light in the red and blue regions of the visible spectrum. Note that the absorption spectra match the action spectrum of photosynthesis and hence, implicates (though doesn't prove) that they are involved in the process. (Subsequent work has shown chlorophylls to be the major photosynthetic pigments).

D. Quantity and Quality

1- Light quality - refers to the wavelengths of light that are important. Photosynthetically active radiations (PAR) range from 400 - 700 nm with peaks in red and blue.

2- Light quantity - refers to the amount of light (PAR) received; units of mol m^{-2} s^{-1}, called the photon fluence rate; or units of energy, J m^{-2} s^{-1}.

E. What happens when chlorophyll absorbs light?

The chlorophyll molecule becomes excited (this takes only 10^{-15} sec = femptosec) and an electron moves to an outer energy level. This is diagrammed:

CHL (ground state) → CHL* (excited state)

Blue light excites an electron to a higher energy level than red light. Imagine the "bell ringer" at a carnival. The electrons change spin at the first (S1) and second (S2) excited singlet states. Electrons don't stay excited long (10^{-9} sec), because they either:

1- return to the ground state and release their absorbed energy as heat (***thermal deactivation***);

2- return to ground state and release their extra energy as light (***fluorescence***);

3- transfer their energy to another molecule; kind of like hitting pool balls (***resonance transfer***); or

4- change spin and revert to a triplet state (same spin as ground state) and be used in a photochemical reaction (***photochemistry***).

F. Why excite electrons?

The ultimate purpose of exciting electrons from chlorophyll is to provide the energy needed to transfer electrons from water to $NADP^+$. Recall that spontaneous electron transfers proceed from a carrier with a more negative redox potential to a more positive one. The redox potential of water/oxygen = +0.82 eV while for NADP/H = -0.32 eV. Thus, photosynthetic electron flow is not a spontaneous process and requires energy.

G. How much energy is required to transfer electrons from water to $NADP^+$?

First, let's calculate the actual redox difference (ΔEm) between water and NADPH:

ΔEm = Em (acceptor) - Em (donor). Or, ΔEm = -0.320 - (0.820) = -1.14 = ca. -1.2 eV.

The actual amount of energy involved is calculated from the equation:

$\Delta G = -n F Em$

where F = Faraday constant = 96,000 J/coulombs, and n = number of electrons involved in the reaction (which equals one for each photon). Substituting in the equation:

$\Delta G = - (1) \times 96000 \times (-1.14) = 109440$ J mol^{-1} (=109.4 kJ mol^{-1})

To summarize, approx. 110 kJ mol^{-1} is required to reduce NADPH from water.

H. Do red and blue photons have enough energy?

Let's calculate the energy in red photons. Assume red photons have a wavelength of 660 nm = 6.6×10^{-5} cm.

The energy of a photon is expressed by the following equation:

$E = h\upsilon$

where h = Planck's constant which relates energy to frequency of oscillation and is 6.6255×10^{-34} J sec $photon^{-1}$; and υ = pulses sec^{-1}.

Since $\upsilon = c/\lambda$ (see A above), we can substitute back in the original equation:

$E = hc/\lambda$

$E = ((6.625 \times 10^{-34}$ j sec $photon^{-1})(3 \times 10^{10}$ cm $sec^{-1}))/6.6 \times 10^{-5}$ cm

$= 3.01 \times 10^{-19}$ j $photon^{-1}$

multiply by Avogadro's number

$= 3.01 \times 10^{-19}$ j $photon^{-1} \times 6.02 \times 10^{23}$ photon mol^{-1}

= 181,000 j mol^{-1}

= 181 kj mol^{-1}

IV. Chloroplast complexes:

There are four major complexes in the chloroplast. These are physically distinct from one another and can be isolated from the chloroplast by electrophoresis and ultracentrifugation.

A. Photosystem II (PSII) Complex

1- large multi-subunit protein complex

2- occurs in the stacked regions of the granal thylakoids

3- integral proteins - coded by the chloroplast genome; including D1 (33 k) and D2 (31 KD)

4- peripheral proteins - coded by nuclear genome; bind Ca^{2+} and Cl^{-}

5- P680 reaction center - a unique chlorophyll a, maximum red light absorption at 680nm; maybe two chlorophyll a molecules; this is ***the*** chlorophyll that "looses" electrons

6- manganese ions (Mn^{2+})

7- phaeophytin, plastoquinone

LHCII - Light harvesting pigment complex associated with PSII. It is comprised of (a) 250 chlorophyll a and b, in approximately equal amounts; (b) several carotenoids; (c) proteins - each pigment is associated with protein (*ca*. 15 pigments/protein); the protein is coded by the nuclear genome

B. Cytochrome b/f Complex

1. occurs in stacked and non-stacked regions
2. cytochrome b (b-type cytochrome, not associated with protein)
3. cytochrome f (c-type cytochrome, associated with protein)
4. non-heme iron-sulfur protein (Fe-SR)

C. Photosystem I (PSI) Complex

1- occurs in non-stacked regions (stromal thylakoids)

2- about 11 polypeptides - including 1a & 1b that are coded by a single operon in the chloroplast genome, bind p700

3- 50-100 chl a

4- electron carriers

5- LHCI - contains about 100 chlorophylls; 4:1 ratio of chl a: chl b.; the protein is encoded by nuclear genome

6- P700 reaction center chlorophyll a

D. ATP synthase/Coupling Factor Complex

1. occurs in non-stacked regions
2. stalk - CFo (4 polypeptides)
3. head - CF1 (5 polypeptides)
4. nine polypeptides, some nuclear, some chloroplastic

V. The Z-Scheme (Or, the Light-Dependent Reactions; Or, Non-cyclic photophosphorylation).

A. Overview

During the light-dependent reactions of photosynthesis, electrons are transferred from water to $NADP^+$. This reaction is depicted as follows:

$$\mathbf{H_2O \rightarrow NADP^+}$$

As the electrons move from water to NADP+, they pass through three of the four complexes described above - Photosystem II (PSII), a cytochrome b/f complex (cyt b/f), and Photosystem I (PSI). After electrons

are removed from the water, they are sequentially shuttled from PSII to the cyto b-f complex to PSI and then finally to NADP+. Thus:

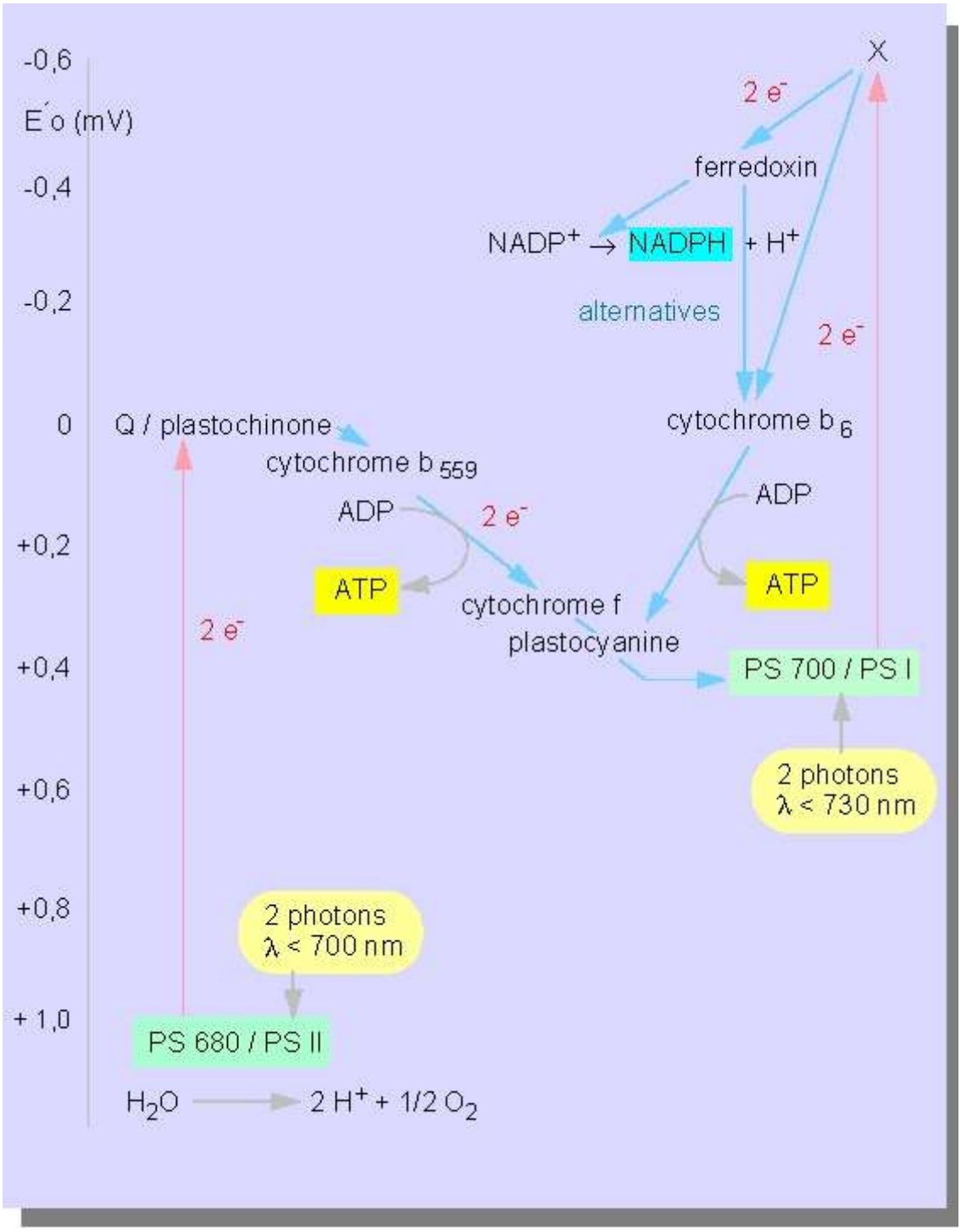
$$\mathbf{H_2O \rightarrow PSII \rightarrow Cytb/f \rightarrow PSI \rightarrow NADP^+}$$

Since PSII, cyt b/f, and PSI are physically separated from one another, there must be a means to transfer electrons between the complexes. A mobile form of plastoquinone (PQ) transfers electrons from PSII to cyt b-f. A copper-containing protein, plastocyanin (PC), transfers electrons from the cytochrome b-f complex to PSI. Thus, the reaction sequence is modified as follows:

$$\mathbf{H_2O \rightarrow PSII \rightarrow PQ \rightarrow Cytb/f \rightarrow PC \rightarrow PSI \rightarrow NADP^+}$$

The transfer of electrons from PSI to NADP+ is mediated by a soluble complex found in the stroma, ferredoxin (Fd). Thus our revised equation:

$$\mathbf{H_2O \rightarrow PSII \rightarrow PQ \rightarrow Cytb/f \rightarrow PC \rightarrow PSI \rightarrow Fd \rightarrow NADP^+}$$



Z - Scheme of Photosynthesis

The transfer of electrons from water to PSII involves an "oxygen-evolving complex" (OEC), part of PSII, that is rich in chloride and manganese ions. Thus,

H_2O → OEC → PSII → PQ → Cytb/f → PC → PSI → Fd → $NADP^+$

B. Origin of the name

Derived from the zig-zag arrangement of components with regard to redox potential. But, why don't we call it the N-scheme?

C. Oxygen evolving complex

The energy of a single photon is not sufficient to split water. Experiments suggest that 4 photons are required to split two water molecules. Since only one electron can be excited at a time (Einstein's Law of Photochemical equivalents), this presents a minor problem.

The solution – a water-oxidizing "clock". Single electrons are transferred through a series of intermediate stages sequentially increasing the electron deficit to a total of four. At this point, the original oxidation state is restored by extracting four electrons from the water.

Diagram of the water-oxidizing clock - in class

A series of five intermediate states, S0 - S4 are postulated;

1- Initially the clock is in the So state, and may be associated with Mn II

2- S1, which may be associated with Mn III, is the most stable form;

3- S2 may be associated with Mn IV;

4- S3 may be associated with a histidine (one of the amino acids in the D1 protein);

5- The nature of S4 isn't clear;

6- Conversion from one state to the next requires one photon and results in the loss of one electron to P680; and

7- the loss of 4 total electrons generates a strong enough potential to split water.

Evidence:

1- after a dark equilibration period, oxygen is released after the third light flash and then after every fourth flash;

2- explains the occurrence of Mn in photosystem II.

D. PQ Shuttle (Q cycle)

1- PQH_2 is reduced on the stromal side of the thylakoid in PSII

2- PQH_2 shuttles over to the lumen side of thylakoid and gets oxidized when it transfers its electrons to the cyt b/f complex

3- One electron is given to Fe-S protein, which in turn, passes it to cyt f and then to PC. The other electron is given to cyt b which then partially reduces another PQ.

4- The "leftover" protons are dumped into the lumen

A second PQH_2 from PSII shuttles to the cyto b/f complex and essentially repeats step 3 - one electron is passed to Fe-S then to cyt f and to PC. The other electron from PQH_2 is given to cyt b and then to PQ to fully reduce it to PQH_2 which must also grab two protons from the stroma.

E. Herbicides and electron transport

1- Urea derivatives - DCMU (diuron) blocks electron flow at a point after Q. They bind to the Qb binding site, preventing PQ from doing so. Interestingly, there are resistant varieties, that have a single amino acid substitution in the D1 binding protein.

2- Viologen dyes - paraquat/diquat - accept electrons from the reducing side of PSI - thus, they interrupt electron flow and also convert oxygen to superoxide - which causes damage to membranes, etc.

VI. How many photosystems? Two

Emerson observed that the rate of photosynthesis was greater than the sum of the rates when red light (660 nm) and far red light (710 nm) were given separately. This synergistic effect, called the Emerson

enhancement Effect, suggested two cooperating systems which has been the conventional wisdom for a long time.

VII. Photophosphorylation

The production of ATP using the energy of sunlight is called **photophosphorylation**. Only two sources of energy are available to living organisms: sunlight and oxidation-reduction (redox) reactions. All organisms produce ATP, which is the universal energy currency of life.

In photophosphorylation, light energy is used to create a high-energy electron donor and a lower-energy electron acceptor. Electrons then move spontaneously from donor to acceptor through an electron transport chain.

ATP is made by an enzyme called ATP synthase. The structure of this enzyme and its underlying gene is remarkably similar in all known forms of life.

ATP synthase is powered by a transmembrane electrochemical potential gradient, usually in the form of a proton gradient. The function of the electron transport chain is to produce this gradient. In all living organisms, a series of redox reactions is used to produce a transmembrane electrochemical potential gradient, or a so-called proton motive force (pmf).

Redox reactions are chemical reactions in which electrons are transferred from a donor molecule to an acceptor molecule. The underlying force driving these reactions is the Gibbs free energy of the reactants and products. Gibbs free energy is the energy available ("free") to do work. Any reaction that decreases the overall Gibbs free energy of a system will proceed spontaneously.

The transfer of electrons from a high-energy molecule (the donor) to a lower-energy molecule (the acceptor) can be *spatially* separated into a series of intermediate redox reactions. This is an electron transport chain.

The fact that a reaction is thermodynamically possible does not mean that it will occur. A mixture of hydrogen gas and oxygen gas does not spontaneously ignite. It is necessary either to supply activation energy or to lower the intrinsic activation energy of the system, in order to make most biochemical reactions proceed

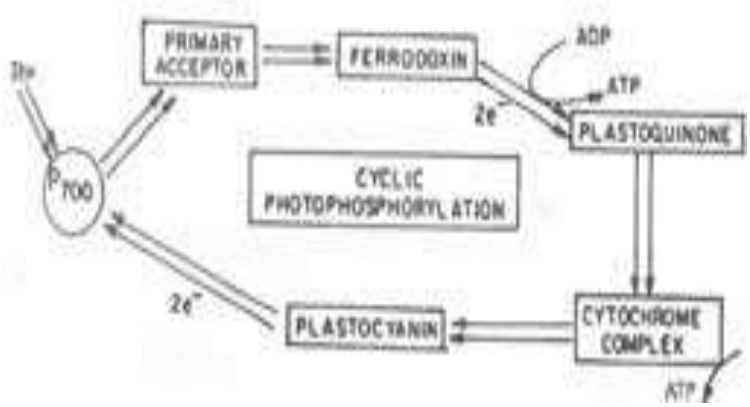
at a useful rate. Living systems use complex macromolecular structures to lower the activation energies of biochemical reactions.

It is possible to couple a thermodynamically favorable reaction (a transition from a high-energy state to a lower-energy state) with a thermodynamically unfavorable reaction (such as a separation of charges, or the creation of an osmotic gradient), in such a way that the overall free energy of the system decreases (making it thermodynamically possible), while useful work is done at the same time. Biological macromolecules that catalyze a thermodynamically favorable reaction *if and only if a thermodynamically unfavorable reaction occurs simultaneously* underlie all known forms of life.

Electron transport chains (most known as ETC) produce energy in the form of a transmembrane electrochemical potential gradient. This energy is used to do useful work. The gradient can be used to transport molecules across membranes. It can be used to do mechanical work, such as rotating bacterial flagella. It can be used to produce ATP and NADPH, high-energy molecules that are necessary for growth.

Cyclic photophosphorylation

In **cyclic electron flow**, the electron begins in a pigment complex called photosystem I, passes from the primary acceptor to plastoquinone, then to cytochrome b6f (a similar complex to that found in mitochondria), and then to plastocyanin before returning to chlorophyll. This transport chain produces a proton-motive force, pumping H^+ ions across the membrane; this produces a concentration gradient that can be used to power ATP synthase during chemiosmosis. This pathway is known as cyclic photophosphorylation, and it does not produce O_2, as well as ATP.

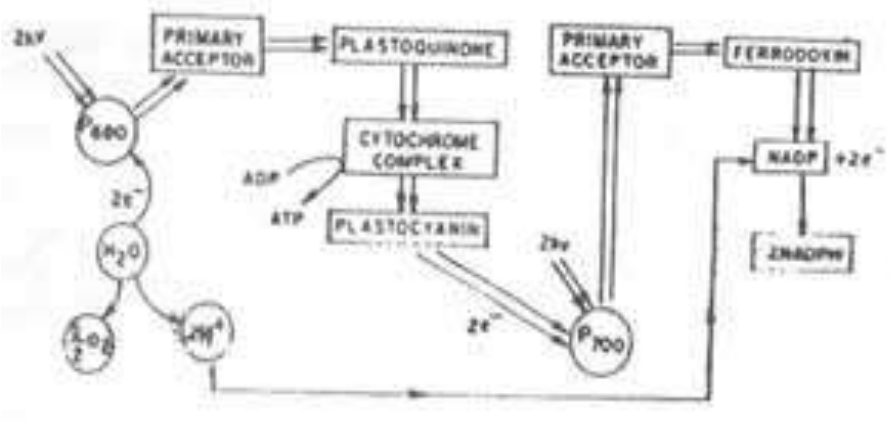


Cyclic Photophosphorylation

Unlike non-cyclic photophosphorylation, NADP+ does not accept the electrons, but they are sent back to photosystem I. NADPH is NOT produced in cyclic photophosphorylation. In bacterial photosynthesis, a single photosystem is used, and therefore is involved in cyclic photophosphorylation

Non-cyclic photophosphorylation

The other pathway, noncyclic photophosphorylation, is a two-stage process involving two different chlorophyll photosystems. Being a light reaction, Noncyclic photophosphorylation occurs on thylakoid membranes inside chloroplasts. First, a water molecule is broken down into $2H^+ + 1/2\ O_2 + 2e^-$ by a process called photolysis (or *light-splitting*). The two electrons from the water molecule are kept in photosystem II, while the $2H^+$ and $1/2O_2$ are left out for further use. Then a photon is absorbed by chlorophyll pigments on surrounding the reaction core center of the photosystem. The light excites the electrons of each pigment, causing a chain reaction that eventually transfers energy to the core of photosystem II, exciting the two electrons which are transferred to the primary electron acceptor. The deficit of electrons is replenished by taking electrons from another molecule of water. The electrons transfer from the primary acceptor to plastoquinone, then to plastocyanin, providing the energy for hydrogen ions (H^+) to be pumped into the thylakoid space. This creates a gradient, making H^+ ions flow back into the stroma of the chloroplast, providing the energy for the regeneration of ATP.



Non-Cyclic Photophosphorylation

The photosystem II complex replaced its lost electrons from an external source, however, the two other electrons are not returned to photosystem II as they would in the analogous cyclic pathway. Instead, the still-excited electrons are transferred to a photosystem I complex, which boosts their energy level to a higher level using a second solar photon. The highly excited electrons are transferred to the acceptor molecule, but this time are passed on to an enzyme called Ferredoxin- NADP reductase|$NADP^+$ reductase, for short FNR, which uses them to catalyst the reaction (as shown):

$$NADP^+ + 2H^+ + 2e^- \rightarrow NADPH + H^+$$

This consumes the H^+ ions produced by the splitting of water, leading to a net production of $1/2O_2$, ATP, and $NADPH+H^+$ with the consumption of solar photons and water.

The concentration of NADPH in the chloroplast may help regulate which pathway electrons take through the light reactions. When the chloroplast runs low on ATP for the Calvin cycle, NADPH will accumulate and the plant may shift from noncyclic to cyclic electron flow.

Carbon Reactions (Calvin cycle, C4 and CAM)

I. The final frontier - *Photosynthetic Carbon Reduction (step 3)*

- called "dark reactions" because reactions don't require light - however, note that these reactions can (and normally do) occur in the light. In one sense they can be considered "light-dependent" since they require the ATP and NADPH generated during the Z scheme.
- called the Calvin cycle - after the fellow and his colleagues who worked out most of the reactions. If you had done it, you too would own a Nobel Prize.
- occurs in the stroma
- there are three major steps: fixation → reduction → rearrangement/recharging/release

A. Carbon dioxide fixation

Carbon dioxide is fixed (trapped, bound) to form an organic compound (phosphoglyceric acid, PGA)

- carbon dioxide condenses with RuBP (ribulose bisphosphate; C5) to form 2 molecules of PGA (C3)
- first product of carbon fixation is PGA (Calvin's experiments)
- catalyzed by the enzyme ribulose bisphosphate carboxylase (rubisco).
- rubisco is the most abundant protein on earth; it makes up 50% of leaf protein
- the reaction mechanism is diagrammed in the text (on overhead)

B. Reduction

Step in which the temporary chemical (ATP) and reducing (NADPH) potentials that were generated in the light-dependent reactions are used to reduce the PGA (an acid) to a carbonyl (glyceraldehyde 3-phosphate; abbreviated G3P or GAP)

- PGA is reduced to G3P
- this is a two-step reaction sequence
- first, PGA is phosphorylated with ATP to 1,3-bisphosphoglycerate which is subsequently reduced to G3P (note a phosphate is lost during this reaction). NADPH provides the electrons for the reduction

- energy requirements - at this point in the cycle, for each carbon dioxide fixed, two ATP and two NADPH are required (one for each of the two PGA's)

C. Rearrangement/Recharging/Release

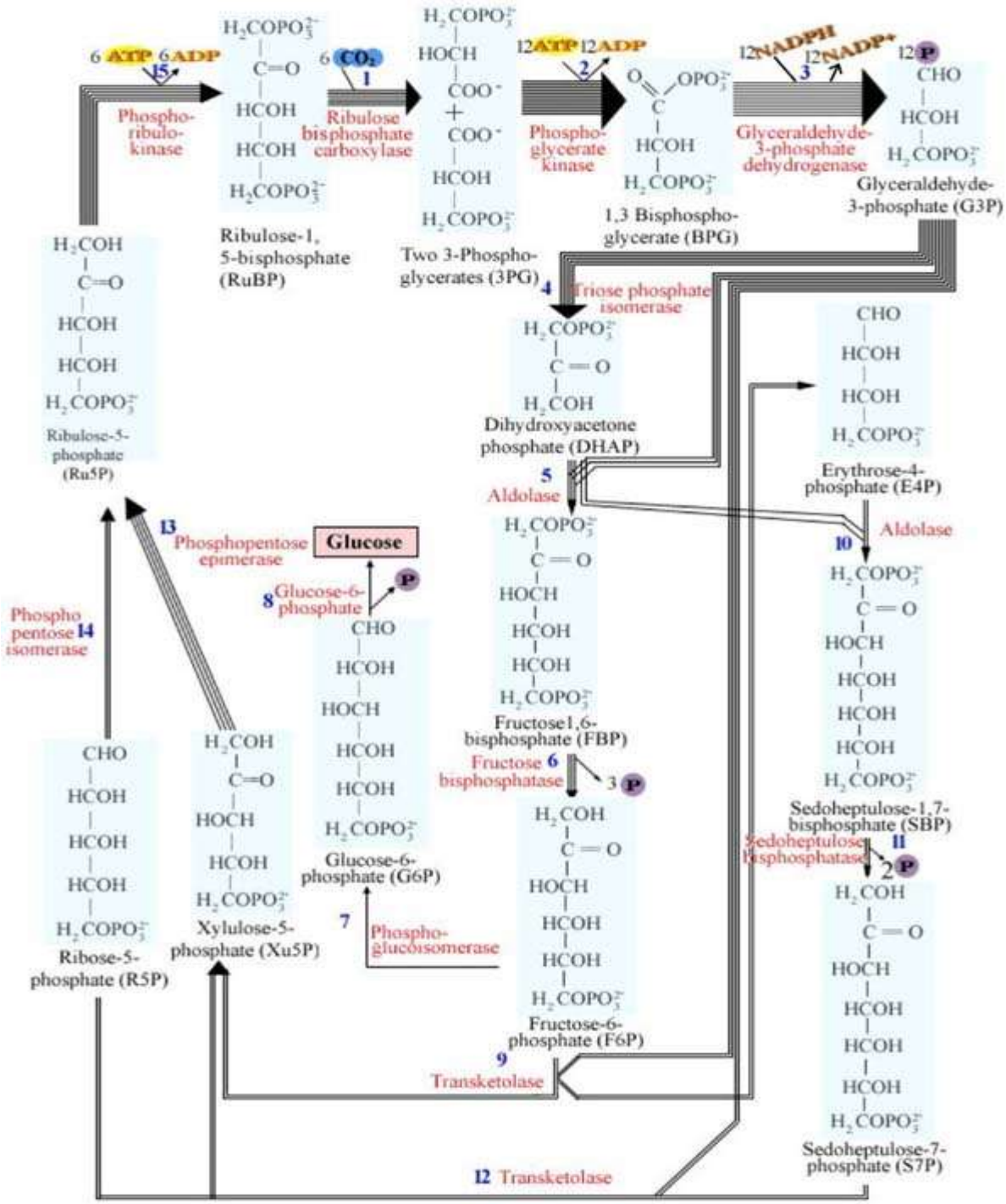
Complex series of reactions (rearrangement) that result in the net removal of a C3 carbohydrate from the cycle (release) and the production of the precursor to the starting material (recharging):

- ✧ the cycle must turn 3 times for the production of one net triose
- ✧ the end product of the cycle is ribulose-5-P (RuP)
- ✧ ATP converts ribulose-5-P to RuBP
- ✧ ATP comes from the Z scheme

E. Summary

The fixation of 1 carbon dioxide requires 3 ATP and 2 NADPH.

see the next diagram for details



Calvin cycle

II. Regulation of the Calvin cycle

We will not cover this in class except to say that regulation of the cycle is important. There are several regulatory controls:

1. rubisco - light activated;
2. allosteric regulation - rubisco has a binding site for CO2;
3. rubisco activase - protein that "activates" rubisco; and
4. Fd/thioredoxin - several enzymes require a reduction to become activated.

III. C3 Plants

Plants that exhibit the type of photosynthetic carbon reduction that we described above are termed C3 plants. In other words, the first product of carbon dioxide fixation is a 3-carbon compound (PGA). Thus, when radioactively labeled carbon dioxide is fed to a plant, the first place that it shows up is PGA.

IV. Photorespiration

Light stimulated the production of carbon dioxide in the presence of oxygen

- not associated with mitochondrial respiration
- requires light
- not accompanied by ATP synthesis
- wastes energy (i.e., ATP, NADPH)

A. Observations on photorespiration

1. Not all plants photorespire.
2. Plants that photorespire typically show light saturation.
3. Plants that photorespire have a higher CO_2 compensation point. In other words, it takes a greater amount of carbon dioxide to break even.
4. Oxygen inhibits photosynthesis in plants that photorespire (called the Warburg effect) –

B. The problem - rubisco

Unlike most enzymes, rubisco is not substrate specific - it also has an oxygenase function. In addition to its normal substrate (carbon dioxide), rubisco also binds oxygen to RuBP. Although rubisco has a higher affinity for binding carbon dioxide (Km = 9 μM), if enough oxygen is present, it acts as a competitive inhibitor (the Km for oxygen is 535 μM).

C. The reaction catalyzed by ribulose bisphosphate carboxylase/ oxygenase

When rubisco binds oxygen to RuBP, the RuBP is essentially split in half into a 3-carbon piece and a 2-carbon fragment according to the following reaction:

RuBP + oxygen + rubisco → PGA (C3)+ phosphoglycolate (C2)

Compare this to the normal reaction:

RuBP + oxygen + rubisco → 2 PGA (C3)

Thus, rubisco has oxygenase activity as well as carboxylase.

D. What determines which process will occur? Oxygenase activity occurs when:

1. carbon dioxide levels are low - during periods of active photosynthesis; and
2. oxygen levels are high - due to the activity of PSII; high light intensity.

The ratio of [carbon dioxide]/[oxygen] ultimately determines the product of the rubisco reaction.

if [carbon dioxide/oxygen] = high; then it favors normal Calvin cycle
if [carbon dioxide/oxygen] = low; then it favors oxygenase activity

V. Photosynthetic carbon oxidation (PCO), or, Glycolate cycle

The purpose of this pathway is to metabolize and reclaim the carbon in phosphoglycolate

A. Overview of the major steps:

1. The products of rubisco oxygenase activity are phosphoglycolate and PGA;
2. PGA enters the Calvin cycle as normal;

3. Phosphoglycolate is dephosphorylated to glycolate and is then shuttled out of the chloroplast into the peroxisome;
4. Recall that peroxisomes are single membrane-bound organelles that contain catalase. They also have the marker enzyme glycolate oxidase;
5. In the peroxisome, the glycolate is oxidized to glyoxylate by glycolate oxidase. This is a redox reaction. Oxygen gets reduced to hydrogen peroxide;
6. Catalase converts the potentially destructive hydrogen peroxide to oxygen and water;
7. Glyoxylate is converted to glycine (an amino acid) by a transamination reaction. Glycine is transported out of the peroxisome into the mitochondrion. Two glycine molecules condense to form serine releasing carbon dioxide. This process requires NADH;
8. Serine is further metabolized in the peroxisome to glycerate;
9. Glycerate enters the chloroplast, is phosphorylated and enters the Calvin cycle;

B. The Highlights - The glycolate cycle:

- is oxidative;
- occurs in three organelles;
- reclaims some (75%), but not all, of the carbon from glycolate;
- carbon dioxide is released in the mitochondria and is hence the reason this is a type of "respiration".

C. Why do plant photorespire?

From a Darwinian perspective, we'd expect that this process would have been selected against. However, the fact that so many plants do it, suggests that it may have an unappreciated function. Possibilities include: (a) salvage the carbon lost during rubisco oxygenase action; (b) mechanism to help prevent destruction by excess light.

VI. C4 Photosynthesis, or, How maize avoids photorespiration

Plants that avoid photorespiration have a unique modification of photosynthesis. They are called C4 plants because the first product of carbon dioxide fixation is a 4-carbon compound, not PGA as it is in C3 plants.

Examples: Many plants have this specialized modification. Found in many different and unrelated groups of plants which indicates that it evolved independently several times. Even within a genus, some members can be C4 others C3.

C4 photosynthesis is common in grasses like maize, sorghum, crabgrass and members of the Centrospermae (a closely related group of plants that includes Chenopodiaceae, Amaranthaceae, Aizoaceae, Nyctaginaceae, Portulacaceae, Zygophyllaceae). Not all grasses are C4; for example, Kentucky blue grass (*Poa pratensis*; common lawn grass) is C3.

A. How do C4 plants avoid photorespiration?

The answer is simple - C4 plants separate the site of oxygen production (PSII) from rubisco (Calvin cycle). But how? PSII and rubisco placed differently:

1. Cells. In typical C3 plants, the chloroplasts are dispersed throughout the mesophyll. Usually, there is a well-defined palisade and spongy layer. In contrast, C4's have a more or less uniform mesophyll layer with a well-developed bundle sheath around each vein. This is called Kranz anatomy because the bundle sheaths appear like a wreath surrounding the vein. In C4 plants, the Calvin cycle activity occurs primarily in the bundle sheath cells, whereas PSII activity occurs in the mesophyll cells.
2. Chloroplasts - The chloroplasts of C4 are dimorphic. Bundle sheath cell (BSC) chloroplasts are agranal. Recall that PSII occurs in the appressed regions of the chloroplasts. Thus, agranal chloroplasts have little PSII activity; but, they do have hi PSI activity. The mesophyll cell (MC) chloroplasts have typical granal stacking, but low rubisco activity. B. Since C4 plants have separated the Calvin cycle PSII, there must be a mechanism to get carbon dioxide into the BSC since:

1. there is relatively slow diffusion to deep, interior regions of the leaf, especially considering;
2. the ambient level of carbon dioxide is low.

In order to solve this problem, plants required a mechanism to:

1. fix carbon dioxide in regions of the leaf where it occurs in high concentration (i.e., MC). The enzyme that catalyzes this reaction is phosphoenolpyruvate carboxylase (PEPcase). This enzyme binds carbon dioxide (actually bicarbonate) to PEP to form oxaloacetate (reaction diagram). This reaction occurs in

the cytoplasm. Note that OAA is a C4 compound. Hence these plants are called C4 - because the first product of carbon fixation is a four-carbon compound.

2. transport the fixed carbon dioxide (which is in the form of a C4 compound like malate or aspartate) from the MC to the BSC. OAA is converted to another C4 compound that, in turn, migrates to the BSC where it is decarboxylated and used in the Calvin cycle. The "leftover" C3 shuttles back to the MC to pick up another carbon dioxide and repeat the process.

C. General scheme - on overhead, covered in class

D. Details

Note that there are at least three different types of C4 plants. They differ in the specific form in which carbon dioxide is transported.

E. Advantages of C4 metabolism

Plants that exhibit this type of photosynthesis are characteristic of hot, tropical environments that have a high light fluence. The advantage of C4 in these circumstances is that C4 metabolism:

1. avoids the photorespiratory loss of carbon
2. improves the water use efficiency of the plants
3. results in higher rates of photosynthesis at high temperatures
4. improves the efficiency of nitrogen utilization (because C3 requires lots of rubisco)

VII. Crassulacean Acid Metabolism - CAM plants

A. Origin of the name

Crassulacean refers to the Stonecrop family (Crassulaceae) and related succulents in which this process is common. To date, plants in more than 18 different families including Cactaceae (Cactus family) and Bromeliaceae (Pineapple family) have been shown to carry out CAM metabolism. *Acid* is derived from the observation that these plants accumulate large amounts of organic acids in the dark.

Plants with CAM metabolism evolved in dry, hot, high-light environments. This is largely a mechanism to conserve water. Plants in dry environments can’t afford to compromise - they lose too much water opening

their stomates during the day. CAM plants solved this problem by opening up the stomates at night to obtain carbon dioxide. This strategy is just the reverse of "normal" plants. But, this presents another problem - ATP and NAPDH, which are products of the light-dependent reactions, are not available when the carbon dioxide is fixed. The solution to this problem was to store the carbon dioxide during the night until ATP and NADPH were available the following day. Thus, there is a temporal separation of initial carbon fixation via PEPcase and the Calvin cycle (C4 plants have a spatial separation).

B. PEPcase

This is the initial enzyme that fixes carbon dioxide. The product is ultimately malate which accumulates in the vacuole during the night (hence the "acid" term).

C. Sequence of events.

Night → stomates open → nocturnal transpiration (lower than diurnal) and carbon fixation by PEPcase → OAA produced → reduced with NADPH to malate → shuttled into vacuole → acid content of vacuole increases → starch depleted to provide PEP for carboxylation → day → stomates close → transpiration decreased → acid content decreases → malate decarboxylated to provide carbon dioxide for Calvin cycle → starch content increases

Aerobic respiration

Aerobic respiration requires oxygen to generate energy (ATP). It is the preferred method of pyruvate breakdown from glycolysis and requires that pyruvate enter the mitochondrion to be fully oxidized by the Krebs cycle. The product of this process is energy in the form of ATP (Adenosine Triphosphate), by substrate-level phosphorylation, NADH and $FADH_2$.

Simplified reaction:

$$\mathbf{C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_{2\,(g)} + 6H_2O}$$

$$\mathbf{\Delta G = -2880\ kJ\ per\ mole\ of\ C_6H_{12}O_6}$$

The reducing potential of NADH and $FADH_2$ is converted to more ATP through an electron transport chain with oxygen as the "terminal electron acceptor". Most of the ATP produced by aerobic cellular respiration is made by oxidative phosphorylation. This works by the energy released in the consumption of pyruvate being used to create a chemiosmotic potential by pumping protons across a membrane. This potential is then used to drive ATP synthase and produce ATP from ADP. Biology textbooks often state that 38 ATP molecules can be made per oxidized glucose molecule during cellular respiration (2 from glycolysis, 2 from the Krebs cycle, and about 34 from the electron transport system). However, this maximum yield is never quite reached due to losses (leaky membranes) as well as the cost of moving pyruvate and ADP into the mitochondrial matrix and current estimates range around 29 to 30 ATP per glucose.

Aerobic metabolism is 19 times more efficient than anaerobic metabolism (which yields 2 mol ATP per 1 mol glucose). They share the initial pathway of glycolysis but aerobic metabolism continues with the Krebs cycle and oxidative phosphorylation. The post-glycolytic reactions take place in the mitochondria in eukaryotic cells, and in the cytoplasm in prokaryotic cells.

Glycolysis

Glycolysis is responsible for the production of ATP (adenosine triphosphate) through the degradation of glucose. It is a fundamental reaction performed by all organisms where glucose is turned into pyruvate.
There are ten steps to glycolysis and each step is facilitated by a different enzyme.
All reactions occur in the cytoplasm and can take place with or without oxygen.

The general reaction for glycolysis is as follows:

glucose + 2P*i* + 2ADP + 2NAD → 2 pyruvate + 2ATP + 2NADH + $2H^+$ + $2H_2O$

The net energy gain for this reaction is 2ATP and 2NADH

(glycolysis is not very efficient).

The first five steps of glycolysis require free energy, they are **endergonic**.

Step One

Glucose to G6P.

This step is mediated by the enzyme hexokinase

Step Two

G6P to F6P

This step is mediated by the enzyme phosphoglucomutase

Glucose ring changes its shape to form a fructose ring.

Step Three

F6P to FBP

This step is mediated by the enzyme phosphofructokinase

ATP molecule is used. P is transferred to create FBP

Step Four

FBP to DAP + G3P

This step is mediated by the enzyme aldolase

The fructose ring opens and breaks into two different sugar phosphates.

Step Five

DAP and G3P to two G3P

This step is mediated by the enzyme isomerase

DAP rearranges to form G3P

Why phosphorylate to get glycolysis going?

1) Phosphate can be used later to convert ADP to ATP
2) The addition of phosphate changes the 3D shape of the glucose molecule so that G6P can be recognized by enzymes.
3) Phosphate helps to trap glucose within the cell.

G6P is unrecognizable to the transporter.

Step Six

2 G3P to 2 BPG

This step is facilitated by the enzyme phosphate dehydrogenase

G3P's are oxidized.

Each gains a P group to yield BPG.

$2NAD^+$ is reduced to form $2NADH + 2H^+$

There is a **huge drop in free energy** here

Step Seven

2 BPG to 2 3PG

This step is facilitated by the enzyme phosphoglycerate kinase

BPG gives P to ADP.

Two ATP molecules are made

Step Eight

2 3PG to 2 2PG

This step is mediated by the

enzyme phosphoglyceromutase

The P groups on the two 3PG move.

2 2PG's are formed.

Step Nine

2 2PG >>> 2PEP

This step is mediated by the enzyme enolase

The 2PG loses H_2O and becomes PEP

Step Ten

2PEP to 2 Pyruvates

This step is facilitated by

the enzyme pyruvate kinase

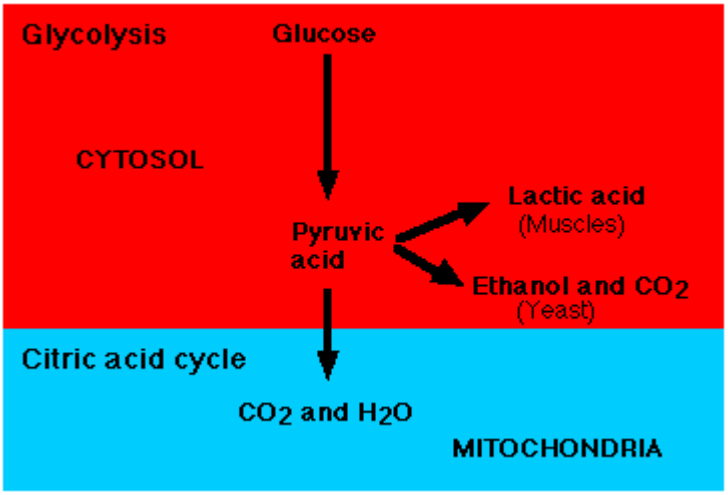
Two PEP transfer their P to ADP.

Two ATP molecules are made.

Without O_2: $NADH + H^+$ is used in fermentation.

With O_2: More NADH (in addition to that formed in glycolysis)

is produced in **pyruvate oxidation** and in the citric acid cycle.



The Fates of Pyruvic Acid

In YEAST

- Pyruvic acid is decarboxylated and reduced by NADH to form a molecule of carbon dioxide and one of ethanol.
- $C_3H_4O_3 + NADH + H^+ \rightarrow CO_2 + C_2H_5OH + NAD^+$
- This accounts for the bubbles and alcohol in, for example, beer and champagne.
- The process is called **alcoholic fermentation**.
- The process is energetically wasteful because so much of the free energy of glucose (some 95%) remains in alcohol (a good fuel!).

In active MUSCLES

- Pyruvic acid is reduced by NADH forming a molecule of **lactic acid**.
- $C_3H_4O_3 + NADH + H^+ \rightarrow C_3H_6O_3 + NAD^+$
- The process is called **lactic acid fermentation**.
- The process is energetically wasteful because so much free energy remains in the lactic acid molecule. (It can also be debilitating because of the drop in pH as the lactic acid produced in overworked muscles is transported out into the blood.)

In MITOCHONDRIA

- Pyruvic acid is oxidized completely to form carbon dioxide and water.
- The process is called **cellular respiration**.
- Approximately 40% of the energy in the original glucose molecule is trapped in molecules of **ATP**.

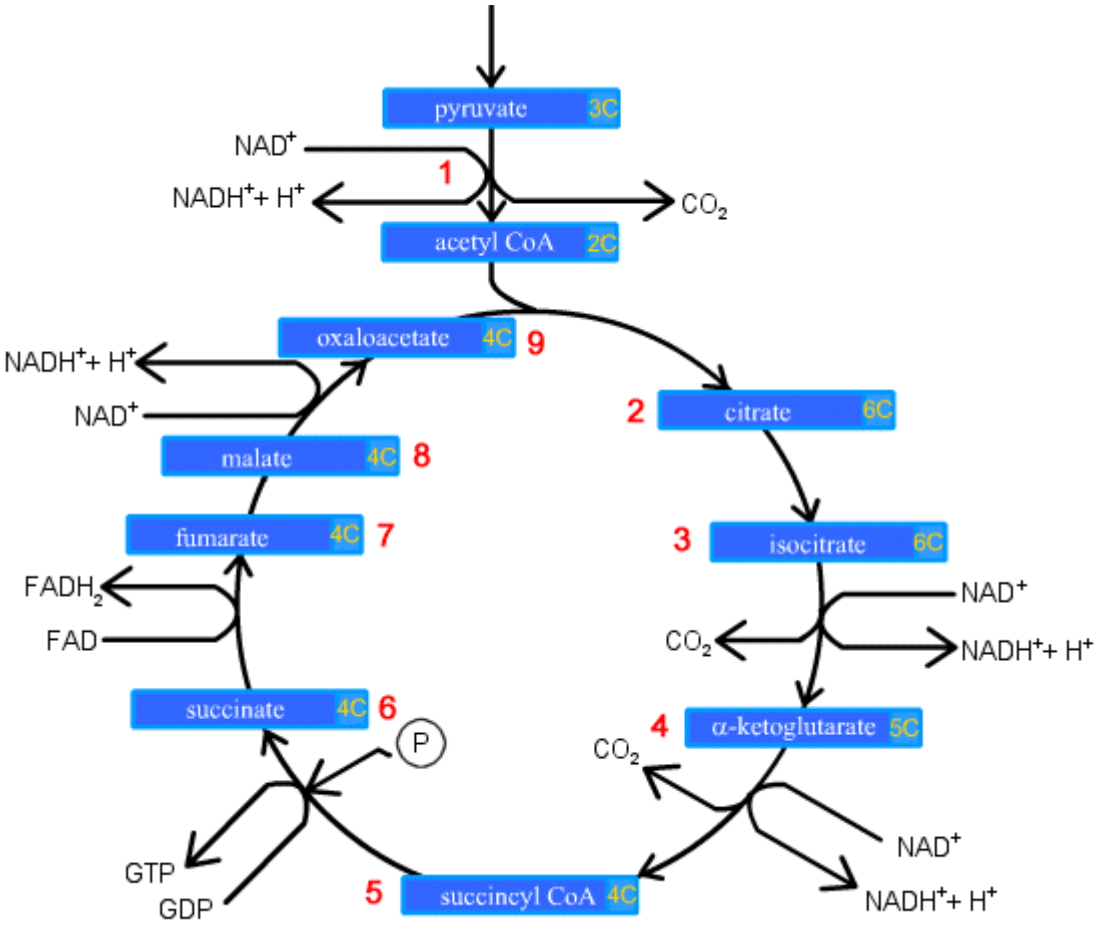
Pyruvate Oxidation

Pyruvate oxidation occurs in the inner membrane of the mitochondria. This process is a source of acetyl-CoA molecules for the citric acid cycle. Pyruvate oxidation occurs in three easy steps.

First, the pyruvate is oxidized (it goes from 3C to 2C acetyl. CO_2 is released as a result). Secondly, NAD^+ is reduced to NADH Finally, the pyruvate dehydrogenase complex attaches CoA to acetyl. The total energy yield for this process is 2NADH.

Krebs Cycle

The pyruvate molecules produced during glycolysis contain a lot of energy in the bonds between their molecules. To use that energy, the cell must convert it into the form of ATP. To do so, pyruvate molecules are processed through the Kreb Cycle, also known as the citric acid cycle.



1. Prior to entering the Krebs Cycle, pyruvate must be converted into acetyl CoA (pronounced: acetyl coenzyme A). This is achieved by removing a CO_2 molecule from pyruvate and then removing an electron to reduce NAD^+ into NADH. An enzyme called coenzyme A is combined with the remaining acetyl to make acetyl CoA which is then fed into the Krebs Cycle. The steps in the Krebs Cycle are summarized below:

2. Citrate is formed when the acetyl group from acetyl CoA combines with oxaloacetate from the previous Krebs cycle.

3. Citrate is converted into its isomer isocitrate.

4. Isocitrate is oxidized to form the 5-carbon α-ketoglutarate. This step releases one molecule of CO_2 and reduces NAD^+ to $NADH_2^+$.

5. The α-ketoglutarate is oxidized to succinyl CoA, yielding CO_2 and $NADH_2^+$.

6. Succinyl CoA releases coenzyme A and phosphorylates ADP into ATP.

7. Succinate is oxidized to fumarate, converting FAD to $FADH_2$.

8. Fumarate is hydrolyzed to form malate.

9. Malate is oxidized to oxaloacetate, reducing NAD^+ to $NADH_2^+$.

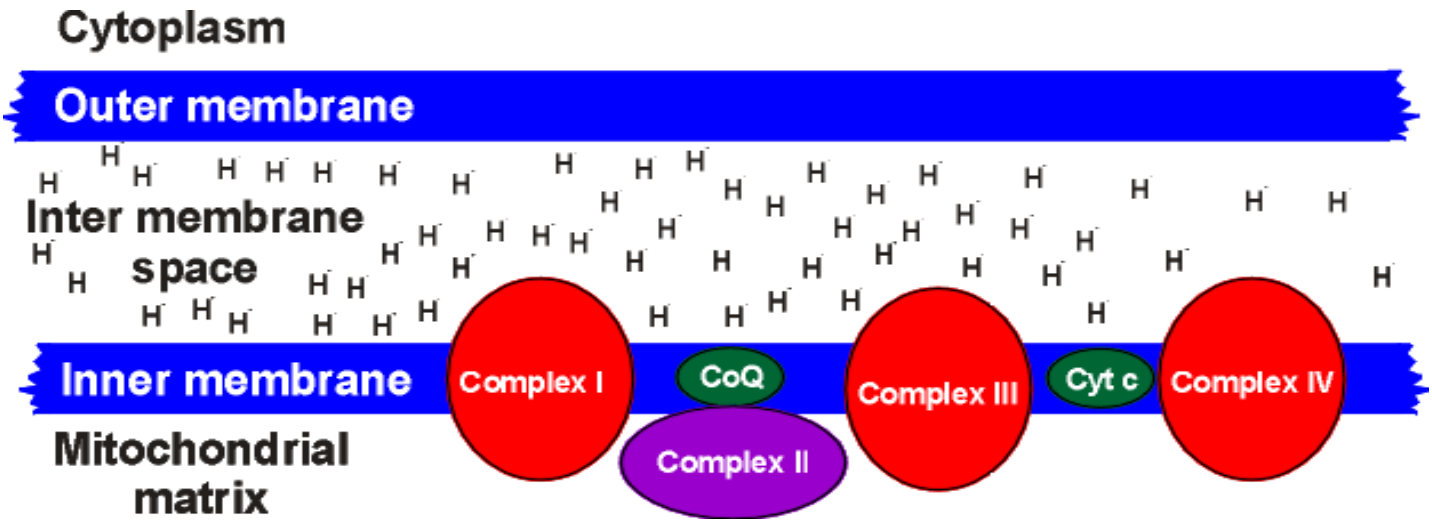
We are now back at the beginning of the Krebs Cycle. Because glycolysis produces two pyruvate molecules from one glucose, each glucose is processed through the Krebs cycle twice. For each molecule of glucose, six $NADH_2^+$, two $FADH_2$, and two ATP.

Electron Transport Chain

What happens to the $NADH_2^+$ and $FADH_2$ produced during the Krebs cycle? The molecules have been reduced, receiving high-energy electrons from the pyruvic acid molecules that were dismantled in the Krebs Cycle. Therefore, they represent energy available to do work. These carrier molecules transport the high-energy electrons and their accompanying hydrogen protons from the Krebs Cycle to the electron transport chain in the inner mitochondrial membrane.

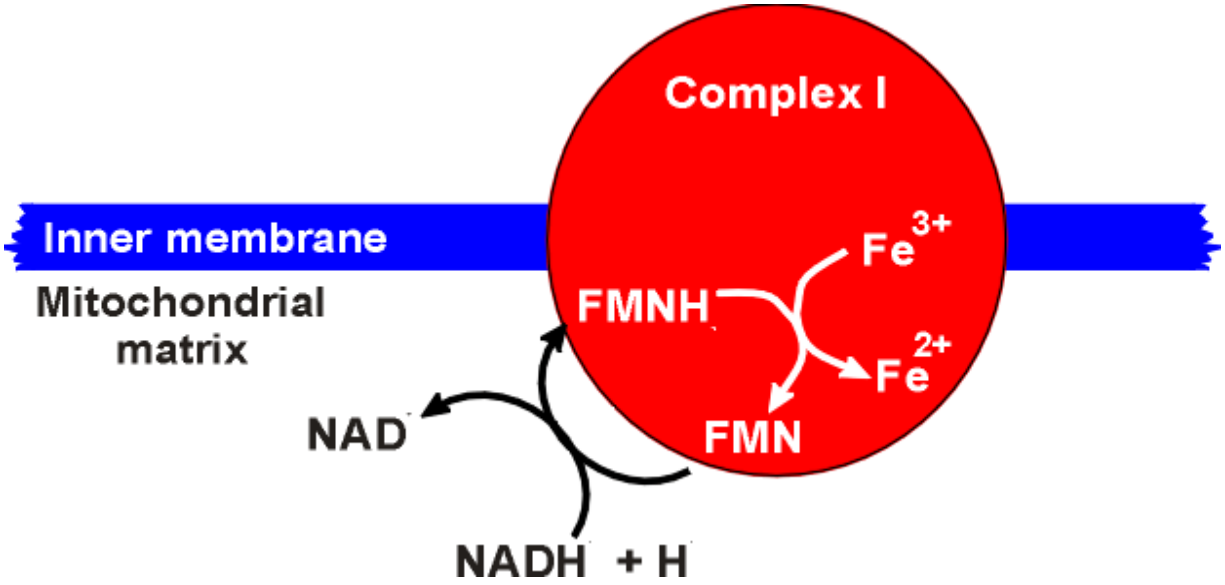
The chain consists of 6 proteins associated with the inner mitochondrial membrane:

1. **NADH dehydrogenase (complex I)**
2. **Succinate coenzyme Q reductase (complex II)**
3. **Coenzyme Q (CoQ) (also called ubiquinone)**
4. **Cytochrome bc1 complex (complex III)**
5. **Cytochrome c (Cyt c)**
6. **Cytochrome oxidase (complex IV**



Step 1

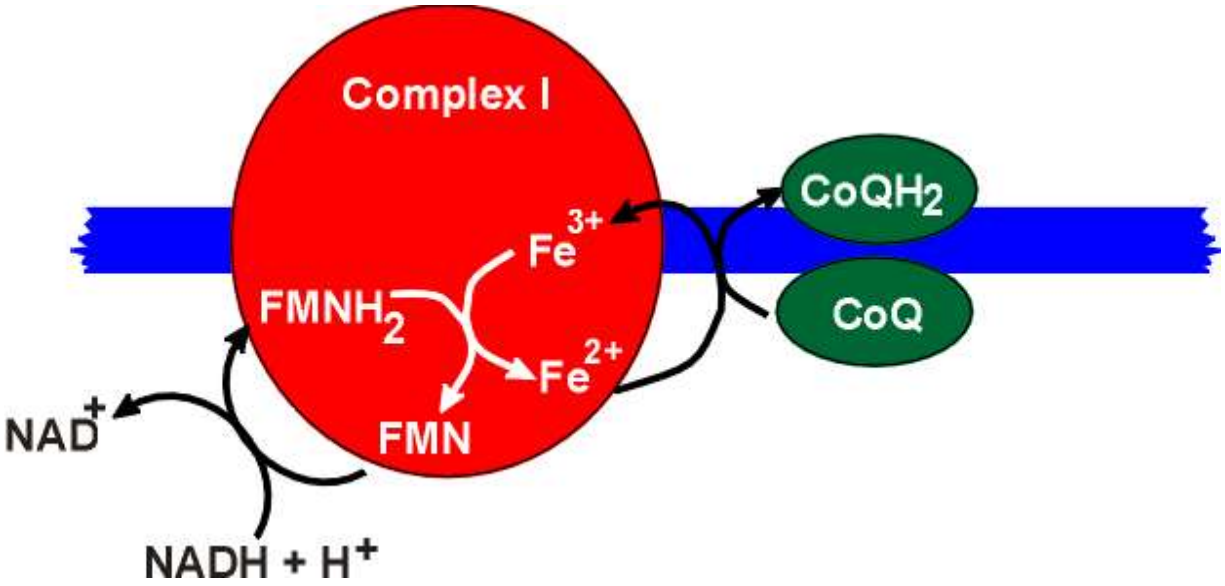
NADH binds to complex I and passes 2 electrons to a flavin mononucleotide (FMN) prosthetic group. The FMN is reduced to $FMNH_2$. Each electron is transferred with a proton.
The electrons are then passed to iron-sulfur proteins (FeS) in complex I (this is non-heme iron). The electron is accepted by Fe^{3+} which is reduced to Fe^{2+}:



(Remember that Fe^{3+} is reduced to Fe^{2+} by electrons)

Step 2

Two electrons from the reduced FeS proteins are then passed to CoQ along with 2 protons. The CoQ is thus reduced to $CoQH_2$ (ubiquinol) while the FeS proteins are oxidized back to Fe^{3+} state.

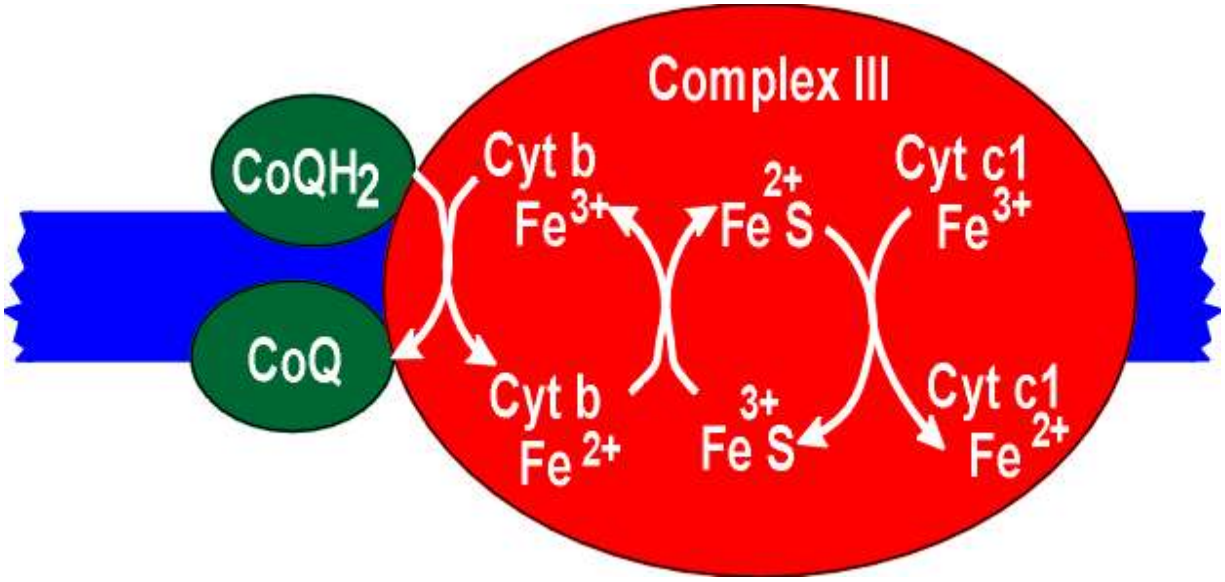


CoQ is small and lipid soluble so it is mobile in the mitochondrial membrane. It diffuses easily and shuttles the electrons to complex III (we will talk about complex II later).

Step 3

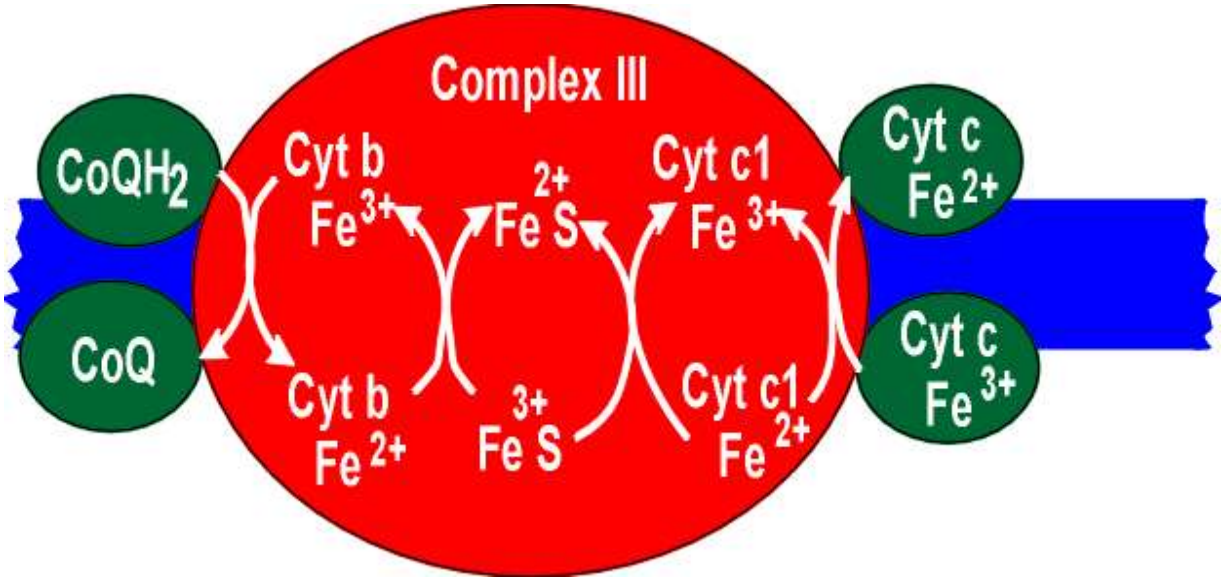
Complex III contains cytochrome b, cytochrome c1 and FeS proteins. Like FeS proteins, cytochromes contain bound Fe atoms (this time the iron is heme). The iron atoms alternate between +3 and +2

oxidation states as they pass on the electrons.
$CoQH_2$ passes 2 electrons to cyt b causing the Fe^{3+} to be reduced to Fe^{2+}. The electrons are passed to the FeS protein and then to cyt c1.



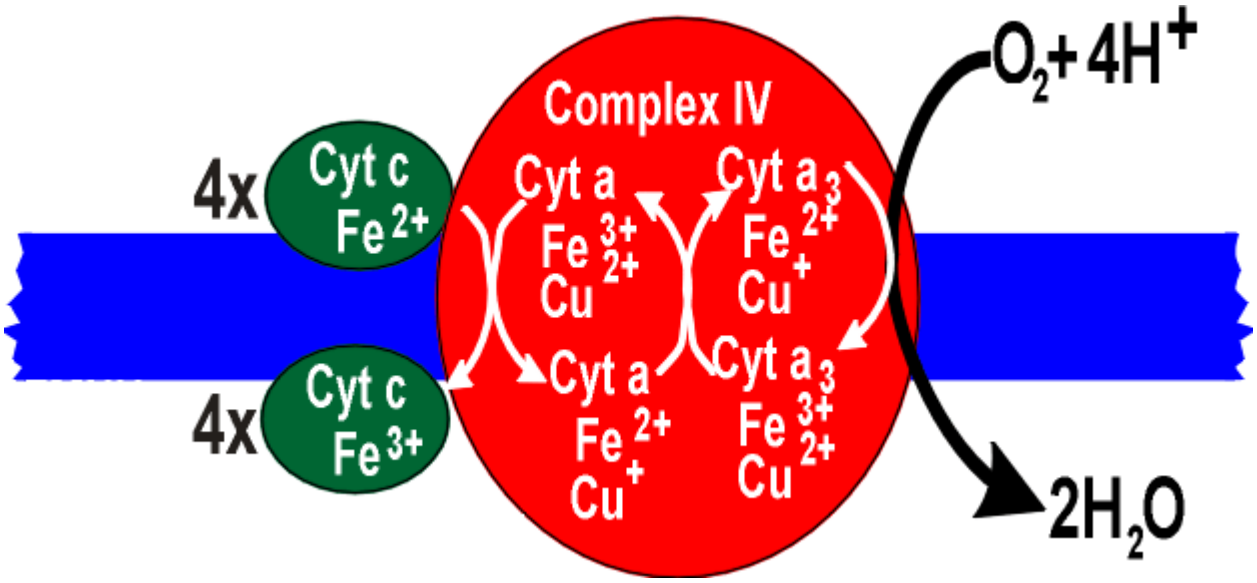
Step 4

Cyt c is another small mobile protein. It accepts electrons from complex III (Fe^{3+} is reduced to Fe^{2+}) and shuttles them to the last electron transport protein in the chain (complex IV).



Step 5

Complex IV contains cytochrome a and cytochrome a_3 (both use Fe and Cu atoms to handle the electrons). Four cytochrome c molecules pass on 4 electrons to complex IV. These are eventually transferred with 4 H^+ to O_2 to form 2 water molecules.



This is a complex reaction mechanism and no attempt has been made in the above diagram to explain how the 4 electrons from 4 Cyt C are conveyed to the O_2 (it doesn't balance with respect to electrons).

Part: Practical Plant Physiology

Prepared by Prof. Dr. Arafat Abdel Hamed Abdel Latef

Experiments

1- Vander Blank's theory

2- Determination of glucose

3- Determination of scurcose

4- Determination of starch

5- Determination of Enzymes activity

6- Factors affecting enzymes activity

7- Determination of pigments

8- Determination of proteins

9- Determination of amino acids

10- Determination of fats

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CARBOHYDRATES METABOLISM

Carbohydrates are a group of important organic compounds present ubiquitously in plants. By being the basic respiratory substrate and forming an integral part of cell walls, carbohydrates are vital to the sustenance of plants both functionally and structurally. Etymologically meaning hydrates of carbon, carbohydrates are principally composed of carbon, hydrogen and oxygen generally in the ratio of 1:2:1. Some of the carbohydrates have nitrogen and sulphur also in addition to C, H and O. Currently however carbohydrates are described as polyhydroxy aldehydes or polyhydroxy ketones and their derivatives.

Carbohydrates constitute the principal respiratory substrate that provides energy for all the metabolic processes in plants. They also constitute the major stored reserve in cells. In addition to these, most of the organic compounds that go to make the skeletal framework of the cell are composed of carbohydrates.

CLASSIFICATION OF CARBOHYDRATES

Carbohydrates may be classified into their principal groups viz. Monosaccharides, oligosaccharides and polysaccharides.

MONOSACCHARIDES: also known as simple sugars, monosaccharides constitute the simplest among carbohydrates. In other words hydrolysis of monosaccharides does not yield simpler carbohydrates, monosaccharides are the basic units for the building up of oligosaccharides and polysaccharides.

Monosaccharides have carbon atoms in each molecule ranging from two to eight. This constitutes the basis for the classification of monosaccharides. All monosaccharides contain either an aldehyde group or a ketone group. Monosaccharides with an aldehyde group are known as aldoses while those with a ketone group are known as ketoses.

- **Carbohydrates metabolism**

Monosaccharides are classified into the following groups based on the number of carbon atoms in each molecule

i. Dioses: Two carbon atoms in each molecule, e. g. Glycollic aldehyde

ii. Trioses: these are the principle 3 carbon compounds present in plant cells. The most important ones are Glyceraldehyde and dihydroxyacetone. Triose sugars exhibit optical activity. This is due to the asymmetrical nature of the carbon atom. The asymmetrical carbon atom imparts isomerism to the trioses. There are two types of isomers (based on optical activity) dextrorotatory and levorotatory. The former turned plane-polarized light to the right while the latter turn it to the left.

iii. Tetroses: Four carbon atoms are present in each molecule,e.g.Erythrose.

iv. Pentoses $C_5H_{10}O_5$: These are 5-carbon sugars that are generally not found in the free state in the cytoplasm. They are however present as constituents of complex carbohydrates. Some of the familiar pentoses are Xylulose, Ribose, Ribulose,Arabinose etc. Xylulose and Arabinose are part of the complex carbohydrates namely Xylans and Arabans.

v. Hexoses $C_6H_{12}O_6$: These are the principal sugars that play a very important role in plant metabolism. Photosynthetic fixation of carbon results in the production of lipids via the glyoxalate cycle. Glucose and Fructose are the most important hexoses found dissolved in the free form. Mannose and galactose are the other two found frequently. Hexoses sugars have several asymmetric carbon atoms resulting in the existence of several isomeric forms. Another important feature of hexoses is their ring structure.

vi. Heptoses: each molecule has 7 carbon atoms e. g. Sedoheptulose.

Oligosaccharides: there are condensation products of two, three, or more monosaccharide units and are generally classified based on the number of

monosaccharide molecule present. Thus there are disaccharides, trisaccharides, tetrasaccharides etc.

i. Disaccharides: Each disaccharide consists of two units of monosaccharides. Some of the common disaccharides are sucrose, lactose, maltose etc. Sucrose is the common sugar that is cane sugar, whereas maltose is produced during seed germination by the action of amylase on starch.

ii. Trisaccharides: Three units of monosaccharides condense to form one unit of a trisaccharide. E.g. Raffianose., Gentianose

iii. Tetrasaccharides: Four units of monosaccharides condense to form one unit of tetrasaccharide. E. g. stachyose.

Metabolism of sucrose

Sucrose is the principal sugar found widely distributed in plant tissues. It is made up of one unit of glucose and one unit of fructose with the elimination of one molecule of water ($C_{12}H_{22}O_{11}$). Sucrose is a non-reducing sugar even though the two units that compose it (Sucrose) are reducing. Sucrose is of great importance in plant metabolism because it is in this form that carbohydrates are transported in higher plants. Radioisotopic studies have clearly shown that the labeled carbon used for photosynthesis soon finds its way into the sucrose molecule.

Sucrose Synthesis in plants: The work of Leloir and Cardini (1955) has shown that UDPG (Uridine diphosphoglucose) plays a key role in the addition of glucose to fructose to form a molecule of sucrose. There are at least two principal pathways of sucrose synthesis. In the first pathway involving the enzyme sucrose synthetase, glucose is transferred from UDPG to fructose

UDPG + Fructose ⟶ Sucrose. + UDP

The second pathway is as follows:

i. Glucose 6 phosphate ⟶ Glucose-1-phosphate

ii. Glucose-1-phosphate combines with uridine triphosphate to produce UDPG and pyrophosphate. The enzyme uridine diphosphoglucose pyrophosphorylase catalyzes this reaction.

iii. UDPG + fructose 6 phosphate → sucrose phosphate + UDP
the enzyme sucrose phosphate synthetase catalyzes this reaction.

iv. Dephosphorylation of sucrose phosphate is brought about by the enzyme phosphatase to yield sucrose.

Sucrose degradation: Sucrose is hydrolyzed by the enzyme invertase to its unit glucose and fructose the enzymatic reaction is believed to proceed irreversibly .

$$\text{Sucrose} + H_2O \xrightarrow{\text{Invertase.}} \text{Glucose} + \text{Fructose}$$

POLYSACCHARIDES: These have a large number of monosaccharide units (polymer). Polysaccharides have a high molecular weight and are generally insoluble in water. Polysaccharides are found in plant cell walls. Starch and cellulose are the principal polysaccharides besides inulin, hemicellulose etc.

STARCH: This is the principal storage reserve for carbohydrates. Starch is usually stored in the form of granules.

Structure of starch: the grains vary in shape, structure and size depending on the source. They have an average size of 1-50μ across and have a central point called the hilum, around which the chains of glucose units in a helical fashion.

Starch molecules are built up by long chains of glucose molecules joined through 1- 4 oxygen bridges. On heating, starch yields two components viz amylase and amylopectose; the former is soluble in water while the latter is insoluble.

Each molecule of amylase consists of an unbranched helical chain of a large number of glucose residues joined to each other by 1-4 oxygen bridges. Each molecule has a molecular weight from 10,000-10,00,000.

Molecules of amylopectin are made up of branched chains of glucose residues with 1- 4 as well as 1- 6 bridges. Branching is caused by the 1-6 oxygen linkages. Molecular weight ranges from 50,000 to 10,00,000.

Starch synthesis: Starch is synthesized either in chloroplasts or in amyloplasts. Light is not necessary for starch synthesis provided the glucose residues and the necessary enzymes are available. The following steps are involved in starch synthesis.

i. **Primer:** Starch synthesis can only begin in the presence of a primer such as maltose, maltotriose etc. The primer then accepts glucose residues and forms a chain.

ii. **(** Glucose 1 phosphate) $_n$ + Primer. → Amylose. + (pi) $_{n.}$ The enzyme starch phosphorylase catalyzes this reaction and helps in the addition of glucose residues one by one to the nonreducing end of the primer. In addition to the above, another enzyme Uridine diphosphate transglycosylase (UDPG glycosylase) also catalyses the formation of 1- 4 oxygen bridges between the glucose units. This enzyme was first isolated from bean, corn and potato. The primer molecules could be maltose or maltotriose or even a starch molecule

UDPG. + primer → UDP + 1,4- glycosyl acceptor

Akazawa, Murata and Minamikawa (1964) found even sucrose can act as a glucose donor in starch synthesis. This reaction takes place as follows.

1) Sucrose. + UDP → UDPG + Fructose
2) UDPG + Primer starch → UDP

According to Murata et al (1963) ADPG (Adenosine diphosphate glucose) may play an important role in starch synthesis than UDPG.

An enzyme called D-enzyme first discovered by Peat, Whelan and Rees (1953) is known to reversibly transfer glucose units to a primer molecule.

All the enzymes mentioned above help in the formation 1- 4 glucosidic bridges and form straight chains. The branches in the chain formed due to 1- 6 bridges are however catalyzed by an enzyme called Q- enzyme which was first isolated from potato extracts (Baun and Gilbert). The Q- enzyme transfers small chains of glucose units onto primers at the 1- 6 position resulting in an amylopectose molecule.

Starch degradation: Amylases are the principal enzymes that bring about the degradation of starch. They hydrolyze the 1- 4 linkages (breaking the oxygen bridge by the addition of a water molecule) between the glucose residues and yield independent glucose molecules. Starch phosphorylase can also reversibly degrade the starch.

CELLULOSE: This is the principal component of the cell wall in most plants. It is a straight-chain polymer made up of D-glucose units bound together with 1-4 bonding. Cellulose may be regarded as the most abundant natural product available from plants.

The details of cellulose synthesis and degradation are not available to the same extent as for starch. Studies conducted on Acetobacter (Cellulose producing bacteria.) indicate that glucose molecules have to be first phosphorylated before they are incorporated into cellulose molecules. UDPG may also play a role in cellulose synthesis.

NITROGEN METABOLISM

Nitrogen is one of the important elements which plays a significant role in a plant's life. Next only to carbohydrates in abundance, N_2 goes into the very structural framework of plants. Proteins, nucleic acids, vitamins, etc have nitrogen in them. Protoplasm also consists of nitrogen. In the form of enzymes, nitrogen plays a pivotal role in all metabolic reactions. Nitrogen is one of the essential elements included in the list of mineral elements, though it is not a mineral in the strict sense. The major part of the atmosphere is N_2 (about 80 %). Despite this, it is one of the oddities of nature that a vast majority of plants (save for a few prokaryotes) prefer to obtain their nitrogen requirements from the soil in the form of nitrates and nitrites.

These inorganic nitrogen compounds get converted into their organic form viz, amino acids. The amino acids combine to form peptide chains which eventually form the proteins.

Sources of nitrogen: There are two major sources of N_2 on this planet- atmosphere and soil. Of these, the former constitutes a vast resource when compared with the latter.

As has been pointed out already, except for a few prokaryotic organisms like bacteria and cyanobacteria the rest of the plants obtain their N_2 requirements from the soil. In the soil, the following are the sources of N_2.

i. Nitrate nitrogen
ii. Ammonia nitrogen
iii. Organic nitrogen
iv. Molecule nitrogen

Nitrate nitrogen

The decomposition of organic matter obtained due to the death of plants and animals results in the formation of nitrate ions (NO_3) in the soil. Nitrate ions are negatively charged and are loosely held over the clay micelles. Before being taken up by the plants the nitrate ions have to be reduced to ammonia. This reduction takes place within the

living cells of the plant and needs the participation of ATP besides a hydrogen donor. The overall reaction is as follows

$$HNO_3 + 8H \rightarrow NH_3 + 3H_2O$$

To reduce nitrate to ammonia 8 hydrogen are required which are provided by respiration and photosynthesis.

According to Evans, Nason and Nicholas (1957), the following are the stages of nitrate conversion to ammonia.

$$HNO_3 \rightarrow HNO_2 \rightarrow (HNO)_2 \rightarrow NH_2 \rightarrow NH_3$$

Various enzymes are involved in this process. These are nitrate reductase, nitrite reductase, nitric oxide reductase, hyponitrite reductase and hydroxylamine reductase.

Ammonia nitrogen: Ammonium ion is a cation and so held tightly to the soil micelles. This is first converted into nitric acid by soil bacteria through a process known as nitrification. Ammonia is made available to the plants either through nitrate reduction or metabolic degradation of proteins and amides. Urea is also a good source of ammonia.

$$NH_2CONH_2 + H_2O \xrightarrow{\text{Urease}} 2NH_3 + CO_2$$

Organic nitrogen: plant and animal debris, excreta and other decaying organic material is the major source of organic nitrogen. Organic nitrogen is not directly absorbed by the roots of plants except for urea. The organic molecules containing nitrogen- which remain adhered to soil particles, break down into amino acids first, followed by oxidation into ammonia and organic acids.

The ammonia thus released, immediately gets oxidized into NO_3, which on absorption by plants again gets converted into ammonia.

When urea is the source of organic nitrogen, it is first hydrolyzed by the enzyme Urease resulting in the production of ammonia and CO_2.

Molecular N_2: N_2 is an inert gas occupying the bulk of the atmosphere. It is not available to the plants in its gaseous form as they (plants) do not have the genetic potential to use it (gaseous N_2). Gaseous nitrogen has to get converted into nitrate or nitrite before it can be negotiated by plants. The process by which gaseous nitrogen gets converted into nitrates or nitrites is called N_2 fixation.

BIOLOGICAL N_2 FIXATION

N_2 fixation in nature to a large extent is mediated by certain prokaryotic organisms like bacteria and blue green algae (cyanobacteria). Among these organisms, some of them live in symbiotic association with the roots of higher plants, while many others live independently. N_2 fixation by biological means, therefore, is of two categories- Non-symbiotic (free-living) and Symbiotic.

Non-symbiotic N_2 fixation: Many bacteria such as **clostridium, Azatobacter,** etc.., live independently in soil. Many blue green algae (Nostoc, Rivularia etc.) possess certain specialized cells called heterocysts. The bacteria mentioned above and the heterocyst possessing cyanobacteria (blue green algae.) are capable of fixation atmospheric N_2 (Non heterocyst forms also can fix N_2. *Nostoc endophytum* and *Calothrix scopulorium*, the two marine algae can also fix N_2 (Steward, 1964). The N_2 fixed by these organisms reaches the soil when these organisms die and is made available to other plants.

The following is a detailed list of various microorganisms that are known to be involved in N_2 fixation.

Anaerobic bacteria: *Bacillus polymyxa*, *Clostridium pasteurianum*, Klebsiella pneumonieae etc.

Aerobic bacteria: *Azatobacter Chroococcum*, *A. agile*, *Azomonas* spp etc.

Photosynthetic bacteria: Species of Rhadospirillum, Chromatium, Chlorobium etc.

Chemosynthetic bacteria: Species of Thiobacillus, Disulphovibrio etc.

Fungi: Pilularia and yeasts

Cyanobacteria (blue grren algae**)**

Unicellular forms: Gloeotheca, Synechococcus etc.

Filamentous non heterocyst forms: Oscillatoria, Lyngbya etc.

Filamentous heterocyst forms:

Nostoc, Anabena, Cylindrospermum, Tolypothrix, Scytonema etc.

Symbiotic N_2 fixation: many bacteria enter into symbiotic associations with the roots of higher plants. The association is called **Bacteriorhiza.** In this association, while the

higher plants are benefited from extra N_2 made available to them by the bacteria, the bacteria in turn get shelter and food. While many species of bacteria enter into the association with the roots of many families of higher plants, the association of *Rhizobiym* with the roots of leguminous plants is the most widely studied. The bacteria which originally inhabit the soil infect the root and come to lie the cells of the nodule which is formed as a result of the bacterial infection.

The strains of the bacterium *Rhizobium* are aerobic, gram-negative, non-spore-forming rods (0.5-0.9 μ wide and 1- 2- 30 μ long). The species within the genus are categorized mainly based on the host they infect (they exhibit host specificity). It is due to this host specificity, sometimes cross-inoculation between hosts is not possible (the strain infection of one host cannot infect another host).

In some instances, however, cross-inoculation groups have been established. According to Alexander (1961) a cross innoculation group " refers to a collection of species of leguminous plants which develop nodules, when exposed to bacteria obtained from the nodules of any member of that particular group "

This means to say that a single bacterial strain can infect all the members of a particular inoculation group. If this particular strain fails to infect any host plant, then that plant is said to belong to a different inoculation group. More than 20 inoculation groups have been established of which about 7 are very prominent (Alexander, 1961)

Based on the rate of growth, the strains of *Rhozobium* may be grouped into two- Fast growers (*R. leguminosarum, R. facjoli etc) and growers (R. iononiacum, R. luninii etc).*

Nodule formation: The bacteria orginally are soil inhabitiants..When they come in contact with the root hairs of a suitable hoat plant,penetrating begins.The root of legume plants produce tryptophan which is converted into **IAA** (Indole acetic acid) by the bacteria. The bacteria produce anotger substance called "curling factor" (this includes IAA also), which brings about twisting and partial deformation of root hairs. The penetration of root hair by a large number of bacteria converts it into an *infection thread* which extends from cortex to pericycle via., the endodermis within the root. The infection threads release the bacteria in the cortical cell.(Chromosome multiplication takes place in these infected cells of the root rendering them polyploid). The bacteria are released into the cells either singly or in groups where they multiply in large numbers. The

infected cells also divide rapidly resulting in the information of a nodule. As has already been pointed out, cell division is accompanied by Chromosome doubling (Wiff and Cooper, 1938). The nodule condists of a central core area where *Rhizobia* are present and cortical region consisting of vascular tissues of the host. IAA seems to be the chief promoter of the cell division in the host cells.

The bacteria are released into the cytoplasm of the host cells by the dissolution of the membrane of the infection thread. Here the bacteria enlarge, become pleomorphic and immobile and are now known as **bacteroids**. Each bacteroid has an envelop of cell membrane surrounded by the cell wall.

The cells of the nodule contain a pink coloured substance called leghaemoglobin, which is akin to true haemoglobin in its capacity to combine with O_2 and CO_2 and get oxidized into a "brown form with a trivalent iron ". The leghaemoglobin is not produced in the bacterial cells but in the host possibly due to the symbiotic interaction between bacteria and root cells. The exact N_2 fixing site in bacteria is not known, but possibly it may lie on the membrane surrounding the bacteroids in the nodule. It is interesting to note that neither the legume plants can develop leghaemoglobin without the presence of bacteria, nor bacteria possess the N_2 fixing capacity outside of symbiotic association. The cells of the root nodules contain a protein called *Nodulin*, the exact function of which is still not known.

According to Collins and Wang (1961). Leghaemoglobin plays an important role in N_2 fixation by transfering O_2 to respiring bacteria thereby enhancing the production of ATP and reducing agents.

Nodule formation in non legumes: While nodule formation is-quite characteristic of leguminous plants, a number of non leguminous plants aldo exhibite bacterial nodule formation. Symbiotic bacteria are found in the root nodules of *Alnus, Casuarina, Myrica, Petria, Ceanothus, Dioscorea, Coroba, Comptonia etc.* Members of actinomycetales are found in the nodules. Root nodules are also found in the gymnospermous members like *Cycas, Podocarpus etc*. Blue green alga *Anabena cycadacearum* is found in the coralloid root of Cycas. Bacterial leaf nodules are found in members like Diiscorea, Pavetta, Psychotria etc. Klebsiella and Enterobacter are seen in the leaf nodules of Psychotria. In the water fern Azolla, Anabena azolla forms a symbiotic association. Another

pteridophyte, where a blue green alga (Nostoc spp) has been encountered is in Isoetes coramandaliana,. Symbiotic association of a N_2 fixing blue green alga (Nostoc) is found in *Anthoceros*, a non vasaular plant.

Mechanism of N_2 fixation (Biochemistry of N_2 fixation)

The mechanism of N_2 fixation appears to follow essentially a similar pattern in both symbiotic and non symbiotic microorganisms. In the process, the atmospheric N_2 is reduced first to ammonia by the enzyme nitrogenase. The enzyme nitrogenase is made up of two protein components, one containing iron and molybdenum (molybdoferredoxin- Component- 1) and the other containing only iron called Fe protein or Azoferredoxin (Component ||). Component | has 15-30 atoms of iron. Sulphur and molybdenum with a molecular wt of 200000-300000. It is made up of four sub units. Component || has a molecular wt ranging from 55000- 65000. Besides N_2,nitrogenase can also reduce acetylene.

Symbiotic bacteria

The enzyme (nitrogenase) is extremely sensitive to oxygen. This may not be a problem on anaerobic bacteria. But aerobic microorganism have evolved a mechanism to protect the enzyme from oxygen inactiveation. It is widely believed that in anaerobic bacteria like *Clostridium pastuerianum* the enzyme is the free state and gets inactivated in the presence of O_2,while in aerobic bacteria like Azatobacter, the enzyme is membrane bound (Schneidee et al 1960). In general, in aerobic organisms, the enzyme is protected in two ways

A- Conformational protection

B- Respiratory protection

In conformational protection, the enzyme changes its configuration in the presence of O_2 and becomes immune to it, while in respiratory protection, the enzyme is prevented in coming into contact with O_2,by using it (O_2) in various oxidation reactions.

Work of Viratanen ef al (1947) have revealed that N_2 is first fixed by bacteria into hydroxylamine, which later gets reduced to ammonia. Carboxylic acid of the host (oxaloacetic acid) is used as the acceptor of ammonia to synthesize aspartic acid. According to Wilson and Burris (1953) who used N to trace the course of nitrogen, it is

first reduced to ammonia. The enzyme nitrogenase is protected from O_2 inactivation, by the pigment leghaemoglobin which regulates the O_2 flow and releases O_2 when needed for ATP production.

Studies on cell free N_2 fixation: The work on cell free N_2 fixation (in the presence of the enzyme, but outside the living bacterial cells) was first started by Carnohan et al (1960) who obtained cell free enzyme extracts of nitrogenase from the cultures of Clostridium pastuerianum. Others who have reported successful cell free extracts of the enzyme are- Nicholas and Fisher (1960) from Azatobacter vielandis, Arnon (1961) from *Chromatium spp. Grutt and* wilson (1963) from Bacilluspolymyxa, Schneideri et al (1960) from blue green algae etc. Evens and his associates have reported success in N_2 fixation with cell free *Ritizobium* extracts obtained from the nodules of *Glycine max…*

The enzyme nitrogenase requires certain other specific conditions besides the absence of O_2.A suitable pH (6-5-7), presence of certaun respiratory substrates (pyruvate) to provide energy as well as electrons for reduction of N_2 are necessary. It is believed that N_2 is bound to enzyme surface and will be held there until it is completely reduced.

There are two opinions as to the first step of reduction of N_2. Some opine that the first intermediate product is hydroxylamine, while others argue that it is ammonia. Use of radioisotope of nitrogen (N^{15}) has however supported th "ammonia hypothesis ". However the intermediate product between N_2 and ammonia could be hydroxylamine, hydrazine, diamide or carbamyl phosphate. Since these compounds cannot br delected in the free state it is believed that these are held tight to the enzyme surface and only after reduction into ammonia they are released.

McNary and Burris (1962) proposed a scheme of N_2 fixation involving ATP, a view later supported by Mortenson (1964). Studying the cell free extracts Clostridium pasterianum, Mortenson (1964) found the necessity of ATP in N_2 fixation.

The work of Oliworth (1965) showed that addition of ATP to the reaction medium enhances the rate of N_2 fixation. According to Mortenson (1964), the scheme of N_2 fixation involves ferredoxin, nitrogenase (component | and ||) and ATP. Ferredoxin gets reduced (possible by H and e) by the respiratory substrates and tranfers the electrons to component |, which inturn reduces component ||; component || accepts a molecule of ATP and becomes activated. This activated enzyme accepts N_2 and reduces it to NH_3.

Hardy and his associates (1965) explained N_2 fixation as follows. Reduced ferredoxin transfers its electrons to an unidentified compound X reducing it. This reduced X compound utilizing the energy of ATP forms ammonia from N_2 under the influence of the enzyme nitrogenase.

Burris (1965) proposed a detailed mechanism of N_2 fixation involving ATP and ferredoxin at each step of reduction. In their scheme, pyruvate undergoes decarboxylation to produce acetyl phosphate and CO_2 in the presence of ATP. Dehydrogenation reaction taking place along with carboxylation releases H^+ to the medium, while the electrons are picked by ferredoxin. Acetyl phosphate is converted to acetate. In the process it loses phosphate to transfer its electrons to the enzyme, which also utilizes a molecule of ATP (obtained from dephosphorylation of acetyl phosphate) to reduce N_2 to ammonia.

Role of nif genes in N_2 fixation

Genetic studies conducted on *Klebsiella pneumonieae* have indicated that the bacterium possess genes for regulating the nitrogenase activity. The genes have been named¡¡¡*f* gene(nitogen fixing). Strecher et al (1972) have successfully mapoed the segment of DNA containing the *nif* regions in *Klebsiella pneumonieae.* The *nif* regions are close to the histon region of DNA. So far about 14 *nif* genes have been isolated from Klebsiella(Merrick,1979). These are organised into eight transcriptional units involving two sets of duplications. Studies conducted on other diazotrophic (N_2 fixing) organisms have revealed that the *nif* regions (in all organisms) are closely related, *nif* fragments of DNA from one diazotroph can easily hybridize with those from another diazotroph indicating a close genetic similarity. Bacteriophage (peace 424) mediated *nif* gene transfer has also been attempted successfully between N_2 fixing (*klebsieela)* and non N_2 fixing (Escherichia coli) bacteria. After transduction E. coli hybrid could produce nitrogenase. Currently attempts are being made by biotechnologists to transfer the *nif* genes to other crop plants, so that they possess an inherent N_2 fixing capacity in general, biotechnologists are attempting to achieve the following things.

a) Transfer of *nif genes* from bacteria to plants
b) Expansion of the hosy range of symbiosis to crop plants other than legumes.
c) Increasing the N_2 fixing capacity of symbiotic bacteria.

Regarding the first, there are rapid advances in our knowledge regarding the 12 *nif genes*, their order, gene products and regulation of expression to produce active nitrogenase (Roberts and Brill, 1981).

Nitrogen in soil: Nitrogen is absorbed from the soil either as nitrate (NO_3) or as ammonium (NH_4). The soil usually consists of 0.05% of nitrogen and occurs both in inorganic and organic forms. The chief inorganic forms of nitrogen are nitrates and nitrites of calcium, potassium etc. Various processes take place in soil rendering it (N_2) suitable to be absorbed by plants. These are Denitrification, Ammonification and nitrification.

Denitrification: In this process nitrates and nitrites are converted to ammonia, nitrous oxide and gaseous nitrogen. The soil actually gets depleted of its N_2 contect because these products escape to the atmosphere. As will be explained later this is necessary not only to supply ammonia for the metabolic processes but also to maintain N_2 balance in nature. Denitrification takes place in soil through the activity of *Thliobacillus denitrificans, pseudomonas denitrificans* etc.

Thliobacillus converts nitrates into sulphates and N_2 in the presence of sulpher and water. Some amount of energy is released during the process.

$$6KNO_3 + 5S + 2H_2O. \rightarrow K_2SO_4 + 4KHSO_4 + 3N_2. + \text{Energy}$$

(pot. Nitrate) (pot. Sulphate)+(pot. Hydrogen sulphate)

Ammonification: The process of production of ammonia, from the plante and animal debris, excrets etc by the activity of decomposing bacteria (amonifying bacteria) is called ammonification. The ammonia is then oxidized to produced nitrates. Many ammonifying have been identified in the soil. These are- *Bacillus mycoides, B. ramosus and B. vulgaris.* Many saprophytic fungi are also known to produce ammonia from plant and animal remains.

Nitrification: The process of conversion of ammonia into nitrates is called nitrification This is brought about in soil by the activity of bacteria like *Nitrosomonas and Nitrococcus.* Ammonia first gets converted into nitrites, which later get oxidized to nitrates as follows by the activity of bacteria called Nitrobacter.

$$2NH_3 + 3O_2. \rightarrow 2HNO_2 + H_2O$$

$2HNO_2 + O_2 \rightarrow 2HNO_3$

Nitrogen Cycle: In nature, there is always a perfect balance of different component, chemical compounds and their various forms. For example nitrogen is found in both its organic form and inorganic form. When reactions take place for instance like absorption of nitrogen and its conversion to protein, organic form of nitrogen increases. But this process alone cannot continue for a long time because it increases the organic N_2 content and depletes the inorganic N_2 content. Hence in order to maintain the balance, reactions such as decomposition of organic wasts takes place producing ammonia, N_2 etc adding N_2 to the inorganic side. These various processes are generally mediated by different types of microorganisms. These reactions are cyclic and help to maintain the N_2 balance in nature. N_2 cycle may be defined as the cyclic movement of N_2 in nature in its organic as well as inorganic form, so as to maintain a perfect balance. Reactions like N_2 fixation, nitrification, ammonification etc. Contribute to the maintenance of N_2 cycle in nature.

Nitrogen assimilation: These are two steps at which nitrogen from soil is assimilated in the plant system. These are nitrate reduction, nitrite reduction and reduction of hydroxylamine.

Nitrate reduction: According to Evans and Nason (1954), the enxyme N_2 reductase converts gaseous N_2 to nitrate. This receives hydrogen from NAD, which is used for the reduction of N_2.

Nitrite reduction: Nitrate is further reduced to nitrite, the electrons for this purpose seem to come from photochemical reaction, as seen by the fact, that nitrate is reduced to aminonitrogen readily in illuminated leaves.

$$2HNO_2. + 2H_2O. \rightarrow 2NH_3. + 3O_2$$

An enzyme has been isolated from *Neurospora crassa* which reduces hyponitrite to hydroxylamine in the presence NADH.

Reduction of hydroxylamine: An enzyme hydtoxylamine reductase has been isolated from higher plants as well as *Neurospora,* which reduces hydtoxylamine to ammonia. This has Mn as its cofactor.

$NH_2OH + NADH + H^+ \longrightarrow NH_3. + NADH. + H_2O$

The ammonia released (internally within the plant) will combine with organic acids to form amino acids.

AMINO ACIDS SYNTHESIS

Amino acids are the basic units which combine to build up proteins. In this section biosynthesis of amino acids is discussed uding ammonia and organic acids.

The ammonia released in the cells as a result of nitrogen assimilation, must not remain in the free state as it is harmful to to the plant system. Ammonia is very quickly incorporated into organic acids to produce various amino acids and amides.

Properties of amino acids: Plants are capable of producing 22 types of amino acids which have been found to be part of proteins, besides a few free amino acids (non protein amino acids). All amino acids are basically carboxylic acids, with one amino (NH_2) group. They have the following general formula.

Various types of amino acids are classified based on "R"whichis variable. It may be a simple hydrogen, or non cycluc, cyclic or heterocyclic organic group. Since amino acids have both a Carboxylic group and an amino group, they possess the properties of both acids and bases. Hence they are called *amphoteric compounds.*

Amino acids are synthesized in plants by two biosynthetic pathways:

a) Reductive amination
b) Transamination

Reductive amination: Organic acids get directly converted into amino acids by reacting with ammonia in the presence of $NADH_2$ under the influence of the enzyme dehydrogenase. Though many of the organic acids like oxaloacetic acid, fumaric acid and pyruvic acid can undergo direct amination to produce amuno acids (aspartic acid, alamine etc) the amination of α ketoglutaric acid to produce glutamic acid is of key importance as it provides a link between respiration and protein synthesis in plant metabolism. The synthesis of glutamic acid takes place when ammonia is accepted α ketoglutaric acid in the presence of $NADH_2$ and glutamic dehydrogenase.

The fact that glutamic acid is the primary product of ammonia assimilation may be proved by the rapid assimilation of labelled ammonia (N^{15}) by glutamic acid. Besides the formation of glutamic acid, the other pathways of ammonia assimilation are

a) Oxaloacetic acid + NH_3 ⟶ Aspartic acid

b) Fumaric acid. + NH_3 → Aspartic acid

c) Pyruvic acid. + NH_3 → Alanine

Transamination: Transamination may be defined as the transfer of amino group from an amino acid to a carboxylic acid, and in the process, the amino acid becomes a carboxylic acid and the carboxylic acid becomes an amino acid.

When once the key amino acid, glutamic acid is produced, it produces a number of amino acids by the transamination reaction. According to Wilson et al (1954) all amino acids undergo transamination in which glutamic acid is the key player.

Transamination is catalyzed by the enzyme *transaminase.* The following are examples of transamination.

In transamination reactions, together with transaminase, pyridoxal phosphate or pyridoxamine phosphate are known to be involved as coenzymes.

Amide biosynthesis: Besides amination and transamination, ammonia may be absorbed by amino acids to produce amides. The process of an amino acid acquiring an additional amino group is called amidation. The extra NH_2 group is attached to an acidic group. ATP is required to bring about amidation.

Glutamic acid. + NH_3 + ATP → α Glutamic + ADP

Aspartic acid. + NH_3 + ATP → Aspargine.+ ADP

Glutamine and Aspargine serve as storage for the excess of ammonia, as otherwise it would prove harmful to plants. Amides may also act as a means of translocating NH_2 groups to mobilize storage proteins during growth.

Degradation of amino acids: In manu plants and microorganisms amino acids are the primary source of energy. Amino acid degradation takes place either by *oxidative deamination* or by simple *oxidation.* In oxidative deamination, the amino acids group is removed from the amino acids (Alanine, glutamic acid, glycine or histidine). Oxidation is seen in tryptophan.

A-Acid \ Oxidase

I. Alanine + O_2 → Pyruvic acid + Ammonia

FAT METABOLISM

Lipids constitute an important group of biomolecules playing a vital role in the storage of reserve food. Basically lipids are insoluble in water but soluble in inorganic solvents like acetone, alcohol, chloroform, benzene, ether etc. Fats are a group of simple lipids which are esters of long chain fatty acids and a trihydric alcohol viz., glycerol. They (fats) can undergo saponification. During esterification all the hydroxyl groups of glycerol react with fatty acids. Like carbohydrates, fats also have C, H and O with less amount of oxygen. The following are some of the important characters of fats.

(i) All fats have glycerols, but fatty acid composition varies.

(ii) Fats are soluble in organic solvents but insoluble in water, as such they are immobile.

(iii) Oxygen content of fats is less in comparison with carbon.

(iv) Each cell synthesizes its fat requirement as they (fats) are immobile.

(v) Fats undergo saponification i.e., when treated with alkali, they release fatty acids and glycerol.

(vi) Due to their low oxygen content, they (store and) release more energy during oxidation. On an average a molecule of fat can yield twice as much energy as from a carbohydrate.

(vii) Fats are generally solids under room temperature, those that remain as liquids under room temperature are called oils.

Distribution of fats in plants: Fats are not generally mobilised for respiration under normal conditions. They are usually the reserve food material found in leaves, stems and roots in small quantities. Major quantity of fats are however stored in seeds, either in endosperm or in cotyledons. E.g. Castor, Coconut, Peanut, Sunflower etc.

Fat metabolism: This involves two processes-synthesis and degradation. Fat synthesis involves the following three steps -

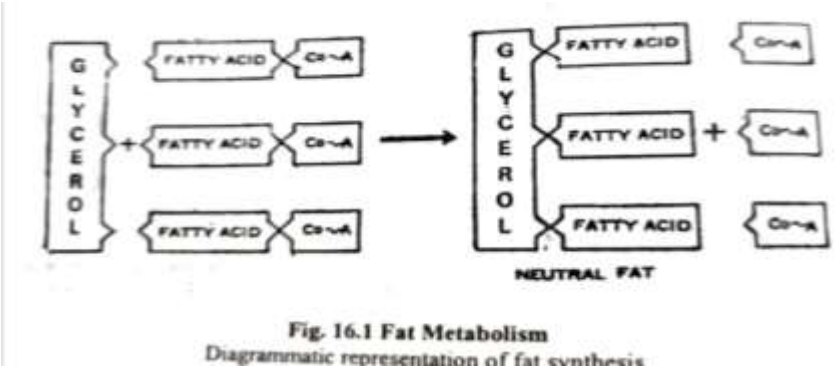


Fig. 16.1 Fat Metabolism
Diagrammatic representation of fat synthesis

(i) Synthesis of fatty acids

(ii) Synthesis of glycerol and

(iii) Condensation of fatty acids and glycerol into fats.

Synthesis of fatty acids: All the naturally occurring fatty acids are long chain molecules having an even number of carbon atoms. The presence of even number of carbon atoms is a salient feature of the fatty acids and number ranges from C6 to C22. The following is a list of some of the common fatty acids.

Lauric CH3 (CH2)10 COOH

Myristic. CH3 (CH2)12 COOH

Palmitic. CH3 (CH2)14 COOH

Stearic. CH3 (CH2)16 COOH

Arachidic CH3 (CH2)18 COOH

Behemic CH3 (CH2)20 COOH

Lignoceric. CH3 (CH2)22 COOH

All fatty acids have basically a long hydrocarbon chain and a terminal carboxyl group. Based on the fact as to whether the hydrocarbon chain has any double bonds, the fatty acids are classified into - Saturated and Unsaturated. In the former the hydrocarbon chain has no double bonds, while the latter has one (monounsaturated) or more than one (polyunsaturated) double bonds. The following list gives the examples of the above two kinds of fatty acids.

Saturated.	**Unsaturated**
Lauric	Palmitoleic

(i) first enzyme complex together with cofactors like ATP, Mn", biotin and CO, participates in the synthesis of an intermediate compound.

(ii) second enzyme complex together with NADP produces the fatty acid and CO_2

Long chain fatty acids are synthesized in a stepwise fashion by the addition of 2 carbon compounds starting with the first 2 carbon compound acetyl CoA. Acetyl Co A which is a key reactant in the respiratory breakdown of carbohydrates first gets converted into malonyl Co A. Malonyl Co A which is a derivative of malonic acid is the key intermediate compound in the synthesis of fatty acids. Malonyl CoA is a 3C compound (Klein, 1957) and is produced from acetyl CoA as follows

Mn(+2)

CH3CO-S-CoA + HCO3 +ATP _____ COOH CH2 CO-S-Co A + ADP + iP

Biotin

(acetyl co A.) (malonyl co A)

The synthesise of malonyl CoA is catalyzed by the enzyme acetyl CoA carboxylase with biotin as coenzyme. Further reactions involved in the synthesis of a fatty acid chain are as follows.

(i) Acetyl Co A gets attached to a protein called acetyl carrier protein (ACP) to form Acetyl CoA ACP complex.

(ii) The complex reacts with malonyl CoA to form acetomalonyl CoA, a5 Ccompound.

H-C-C-SCOA+CH,CO-SCOA ACP

O-C-OH

HOHO

H-C-C-C-C-S-ACP+Coa-SH

HOC-OH (Acetomalonyl CoA)

(iii) Acetomalonyl CoA reacts with acetyl SACP to form a 4 C compound A molecule of CO is released in the process.

Acetomalonyl CoA + Acetyl-S-ACP_____Acetonyl- S- ACP + CO2 + ACP- SH

(iv) The 4 C compound Acetoacety-S-ACP is then converted into a saturated fatty acid-Butyric acid as follows

(0) Acetoacetyl-S-ACP+NADPH+H(+)_____HydroxybutyrylS-ACP +NADP(+)

(b) Hydroxybutyryl-S-ACP. _____Crotonyl S CP +OH(-)

(c) Crotonyl-S-ACP+NADPH+H(+) _____Butyricaid + SACP+NADP(+)

(d) Butyryl-S-ACP+HOH. _____Butryric acid + S- ACP +NADP(+)

The above reaction sequences are repeated to give rise to6C, 8C, 10C units: When the fatty acid chain reaches the required length, the acyl group is

transferred to the SH group of CO A

Synthesis of glycerol: Available evidences indicate that the synthesis of glycerol in plants involves more than one pathway, One of the well understood

mechanisms, involves the 3 carbon compound dihydroxyacetone phosphate (DAP) an intermediate product in the glycolytic breakdown of carbohydrates, In the first step DAP is reduced to glycero phosphate in the presence of NADH The reaction is catalyzed by the enzyme glycerophosphate dehydrogenase.

(i)DAP + NADH2 _____glycerophisphate + NAD (+)

(ii) glycerophosphate is dephosphorylated as follows to give rise to Glycerol.

CH2OH3PO3. CH2OH

CHOH. + H2O_____CHOH. + H3PO4

CH2OH. CH2OH

Glycerol.

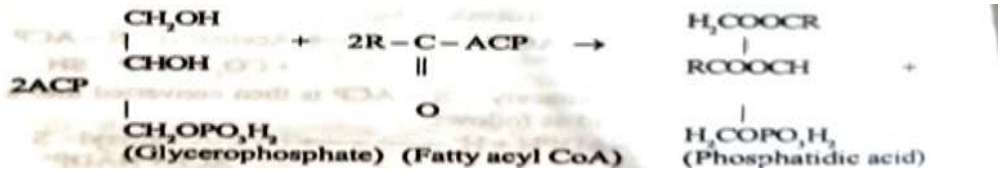
Condensation of fatty acids and glycerol : Fatty acids esterify the three alcoholic groups of glycerol to bring about the synthesis of fats. The three fatty acids reacting with glycerol may be same or different. The sequence of reactions is as follows:

(i) Fatly acids combine with Co A to form fatty acyl Co A complex.

(ii) Glycerol undergoes Phosphorylation to produce glycerophosphate.

The reaction is catalyzed by the enzyme glycerokinase.

(iii) The acyl residues of the fatty acid acyl Co A complex are transfened to the two free hydroxyl groups of the glycerophosphate to form phosphatidic acid



(iv) In the next step, the phosphate residue is removed from the phosphatidic acid molecule to form a diglyceride.

(v) In the last step the free (unesterified) hydroxyl group of the diglyceride reacts with a fatty acyl Co A complex to form a triglyceride as follows:

RCOOCH2. H2COOCR

RCOOCH. + R-CO-ACP _____ HCOOCR. + ACP

H2PO3OCH2. H2COOCR

(Diglyceride) **(Triglyceride)**

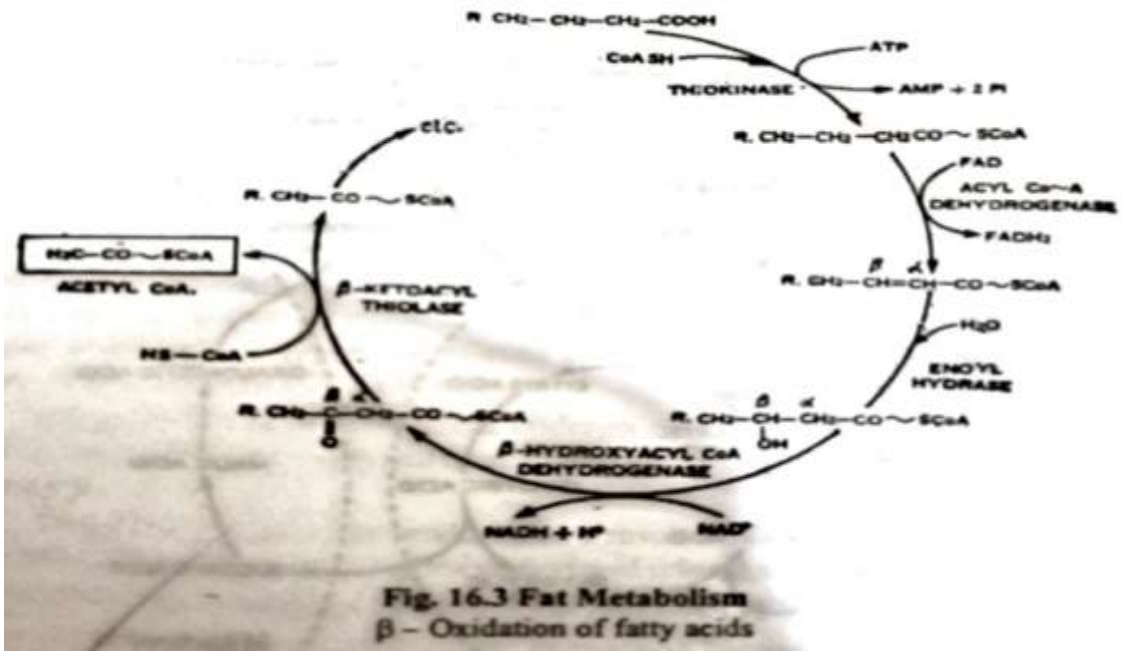


Fig. 16.3 Fat Metabolism
β – Oxidation of fatty acids

Oxidation of fats: Fats are used generally as storage reserve in plants and animals and are utilized as respiratory substrates during non availability of carbohydrates. The enzyme lipase hydrolyses fats into glycerol and fatty acids by breaking the ester bonds. This process is called saponification and is the reverse of condensation. Glycerol and fatty acids are further oxidized as follows

Oxidation of fatty acids: There are two pathways for the oxidation of fatty acids. These are a-oxidation and B-oxidation. The type of oxidation depends on whether ß-carbon atoms or a -carbon atom undergoes oxidation first.

a Oxidation: This process of oxidation is known mostly in plants and is helpful in the oxidation of long chain fatty acids having more than 14 carbon

The enzyme fatty acid peroxidase brings about the oxidative decarboxyladon of fatty acids. HO, is the oxidizing agent and the fatty acids are converted into an aldehyde. The aldehyde will have one carbon less than. the fatty acid.

B Oxidation: This is the principal pathway of fatty acid oxidation. According to F. Knop (1904) who first studied this pathway, at every stage two carbon atoms are removed following the oxidation of carbon atoms. The 2 C fragments are removed in the form of acetyl CoA. The following are the sequential steps of oxidation.

(1**) Activation of fatty acids**: Fatty acids react with Co A and ATP to form Co A complex.

Fatty acid + Co A + ATP→ Acvl Co-A+AMP+2Pi.

(ii) **Dehydrogenation**: Two H Atoms between and carbon atoms are removed from the fatty acyl Co A to form trans unsaturated fatty acyl CoA. The reaction is catalyzed by the enzyme Acyl CoA dehydrogenase.

Fatty Acyl Co-A+FAD→ Trans- fatty acyl Co - A+ FADH

(iii) **Hydration**: A molecule of water is added to trans-fatty Acyl Co-A to form Hydroxyacyl CoA. The enzyme enoyl hydrase catalyzes this reaction

(iv) **Dehydrogenation**: Hydroxyacyl CoA undergoes dehydrogenation to form-Keto Acyl Co-A. The enzyme dehydrogenase catalyzes this reaction

(v) **Thioclastic clevage**: Keto acyl Co A undergoes a clevage reaction with the elimination of a 2 carbon compound acetyl Co A. In the process a molecule of fatty acyl Co A is produced from keto acyl Co A. Fatty acyl CoA now has two carbon atoms less than original. This reenters the cycle once again to undergo clevage. Thioclastic cleavage is catalysed by the enzyme keto acylthiolase.Molecules of fatty acid which undergo oxidation become shorter by 2 carbon atoms for every step and finally get converted into 2 carbon compounds (acetyl CoA). Acetyl CoA molecules produced as a result of oxidation enter the Krebs cycle to undergo further decarboxylation and release energy

CONVERSION OF FATS TO CARBOHYDRATES (Glyoxylate cycle)

Acetyl CoA is the key intermediate compound between carbohydrates and fats: Oxidation of both carbohydrates and fatty acids results in the formation of acetyl CoA. While the interconversion between fatty acids and acetyl CoA is reversible, that between carbohydrates and acetyl CoA is irreversible.

(1) carbohydrates → pyruvic acid → Acetyl co A

(1)Fatty acids →Acetyl CoA

The above reactions show that while fats can be synthesized by carbohydrates via pyruvic acid and acetycl Co A, the reverse is not possible because acetyl CoA cannot form pyruvic acid (the reaction is irreversible). In order to solve this problem, Komberg and Krebs (1957) proposed a new pathway for the synthesis of carbohydrates from fats.

This pathway is known as the Glyoxylate cycle. Glyoxylate pathway was first demonstrated in microorganisms and subsequently in germinating seeds of Angiosperms when the reserve food breaks down.

Basically the Glyoxylate cycle is nothing but Krebs cycle with some modifications. The essential features of this cycle are as follows

(i) Fatty acids get converted into fragments of acetyl CoA by oxidation, while glycerol enters into glycolysis cycle.

(ii) Acetyl CoA combines with oxaloacetic acid of the Krebs cycle to form citric acid

(iii) Citric acid gets converted to cis aconitic acid and then to isocitric acid due to dehydration and rehydration.

(iv) Isocitric acid breaks up into succinic acid and Glyoxylic acid under the influence of the enzyme isocitrase.
Isocitric acid →Succinic acid + Glyoxylic acid

(v) Succinic acid continues further in the Krebs cycle by getting converted into fumaric acid, malic acid and oxaloacetic acid.

(vi) Malic acid either undergoes oxidative decarboxylation (Ochoa reaction)

or reductive decarboxylation (Wood/Werkman reaction) to finally form pyruvic acid.

(a) Malic acid + NADP→ Pyruvic acid + NADPH2, +CO2

(b) Malic acid+NAD→ Oxaloacetic acid + NADH2

, (c) Oxaloacetic acid→→ Pyruvic acid +CO_2

Pyruvic acid gets converted into PEP (Phosphoenol pyruvic acid) which form glucose and fructose by reverse reactions of glycolysis. Glucose and fructose combine to form sucrose.

Oxidation and glyoxylate pathway occurs in certain membrane bound particles called glyoxysomes, while the conversion of succinic to oxaloacetate (Krebs Cycle) occurs in mitochondria. The site for conversion of fats to carbohydrates is therefore glyoxysomes, mitochondria and cytoplasm (from pyruvic acid to carbohydrates).

PROTEIN SYNTHESIS

Of the two important macromolecules that mastermind all the activities of the living systems, proteins are one, the nucleic acids being the other. Proteins are present all over the cell and are important both structurally and functionally The name protein was first suggested by Berzelius (1838). Protein in Greek means "First rank. Proteins are highly complex organic molecules with molecular weight ranging upto many millions. Chemically proteins are madeup of C, H.O and N. In addition to these major elements, some proteins may also haave

sulphur, phosphorous, iron zinc, iodine etc as components. The ratio of major

elements in proteins are C-50-55%, O-20.23%, N-15-18% and S-0-4%

Proteins are made up of building blocks called amino acids (ie. on hydrolysis,

proteins yield these units). The idea that proteins are built up of amino acids was first proposed by Emil Fischer. Amino acids are carboxylic acids which have atleast one COOH group and one NH, group. Other functional groups may also be present.

There are atleast 2 different types of amino acids encountered in plants. The amino acids are bound to one another in a protein chain by means of linkages or bonds called peptide linkages. Peptide linkages are formed between the COOH group of one amino acid and the NH, group of the other, with the elimination of a molecule of water. Several amino acids combine together with the peptide bond and form a peptide chain. Several of these peptides constitute a polypeptide chain. The polypeptide chain folds and refolds in a characteristic fashion to form a protein molecule.

Peptides

These are made up of amino acids linked with CONH (peptide) bonds Peptides are intermediate compounds produced during the synthesis or degradation of proteins

.Amino acids ---------- peptides ---------- proteins

Proteins

These are the final productsin the N, metabolism, and are made up of folded

peptide chains which have many additional linkages (depends upon the point

of folding) in addition to the CONH bond. Proteins have a three dimensional configuration and havevery high molecular weight reaching upto 10-10

Although proteins contain not more than 20 amino acids, they are of immense variety and complexity due to variations in combination of amino acids. In general the three following aspects pertaining to the amino acids

decide the type of protein. These are the quality, quantity and sequence of amino acids the peptide chains.

In other words, the type of amino acid, its quantity and the sequence in which these acids are arranged forms the basis for the categorization of proteins. proteins may differ in the number of peptide chains per molecule, type of secondary linkages (SH) etc.

Structure of proteins

Modern biochemists regard four structural levels in the protein molecule which accounts for its complexity. These levels are-primary. Secondary, tertiary and quaternary structure, in the order of increasing complexity.

Primary structure

This is the simplest and the most basic structure and refers to the linear peptide cham Besides the CONH bond, disulphide bonds (-S-S-) are found or among the peptide chain linkage in the sulphur containing amino acids.

Secondary structure

The primary polypeptide chain spirally coils forming the L. helical chain. The characteristic bonds of the secondary structures are hydrogen bonds between the carbonyl and amino groups of peptide links, salt linkages and Vander Wall's forces which help maintain the helical structure.

Tertiary structure

Folding and superfolding of the helical chain in a three dimensional urientation constitutes the tertiary structure. Interaction between the amino

acid residues situated far apart in the chain brings about the folding.

Quaternary structure

This consists of more than one identical sub units (poly peptide chain) which associate with one another to form a functional unit. Individually each subunit is non functional. Such complicated structures are usually seen in enzymes. For example the enzyme phosphorylase has two sub units, which are inactive individually, but as dimers, they are active. A protein with identical sub units in the quaternary structure is known to be homogenous or if the subunits are dissimilar it is known as heterogenous. A protein with more than one sub Unit (monomer) is referred to as an oligomeric protein.

Classification of Proteins.

As the proteins possess, a very complex strucuture, their classification also a problem as many categorizations are possible based on structure, function etc. The following is a generalized classification. The major groups are recognized in proteins based on their solubility and chemical properties

I Simple proteins

II Conjugated proteins and

III. Derived proteins.

I. SIMPLE PROTEINS

Chemically these have only amino acids. No additional compounds are present in the molecule Simple proteins are of the folowing kinds

1. Albumins

These are soluble in water, dilute salts, acids and alkalies. Heat denatures the protem structure by coagulation. Some of the commonly occuring albumins are Legumelin (from legume seeds). Amylase (from barely), Leucous(from cereals)etc.

2. Globulins

less soluble in water than albumins but soluble in less concentrated salt solutions. Heat coagulation is seen in globulins also. Eg. Legumin (pears), Tuberin (potato) etc

3. Glutelins

Insoluble in water and salt solutions, but soluble in weak acids and alkalies. Glutelins are generally found in plants and are heat immune. Seed proteins found in cereals like Wheat (glutenin) and rice, oryzenin) are best examples glutelins.

4.Prolamines

These proteins are water insoluble, but dissolve in 70-80% alcohol Prolamines have a high concentration of the amino acid proline and other amides. Eg. Gliadin (Wheat), Hordein (barley) and Zein (maize)

5. Scleroproteins

These are fibrous proteins found as an integral part of the structural framework of plants. These proteins are insoluble in most of the solvents.

6. Histones

Most of the nucleoproteins, hemoglobin and globins are histones. They are insoluble in water but insoluble in dilute ammonia. Histones have a high concentration of lysine and arginine.

7. Protamines

These are soluble in water, dilute acids and ammonia and do not undergo heat coagulation. Basic amino acids are rich in protamines, while tryptophan and tyrosine are completely absent. Eg. Nucleoproteins of sperms in fish.

II. CONJUGATED PROTEINS

In these proteins, non amino acid components are associated with the molecule. The non amino components are called prosthetic groups There are seven major categories of conjugated proteins.

1. **Nucleoproteins**

Proteins are found in combination with nucleic acids. Hydrolysis can separate nucleic acids and simple proteins. Nucleoproteins are water soluble

2. **Glycoproteins**

Also called mucoids, these are conjugated with carbohydrates like macromolecular polysaccharides, sugars, sugar phosphate etc. Glycoproteins are found in cell membranes.

3. **Chromoproteins**

These constitute the pigments and are coloured, the coloured appearance is due the presence of metals such as Cu, Fe, Mg etc. Chlorophylls

flavoproteins, carotenoids, cytochromes etc are typical examples of chromoproteins

4. **Lipoproteins**

Proteins in conjugation with lipids are called lipoproteins. Lecithin, Cephalin etc are the lipids found in combination with proteins. Lipoproteins are an integral part of all cell membranes.

5. **Metalloproteins**

Certain types of enzymes which require metals as activators belong to this group. Many of the respiratory enzymes belong to this group.

6. Phosphoproteins

These are proteins with phosphoric acid as the prosthetic group.

Phosphoproteins are insoluble in water, but soluble in alkalies. E.g. milk and egg protein (casein and viteline).

7. Lecithoprotein

Proteins with lecithin as the prosthetic group (Eg. white of egg) belong to this group.

III. DERIVED PROTEINS

These are degradation products of proteins. Primary proteins on hydrolysis with acids or alkalies yield derived proteins.

(A) Primary derived proteins and

(B) Secondary derived proteins

A Primary derived Proteins

There are two categories of primary derived proteins. These are metaproteins and Coagulated proteins.

(i) *Metaproteins*: These are insoluble in water but dissolve in dilute salt solutions, acids and alkalies. Hydrolysis of natural proteins on long treatment with alkalies or acids yield metaproteins

(ii) *Coagulated proteins*: Treatment with heat or alcohol of natural

proteins yields these proteins.

B. Secondary derived proteins

Three types of secondary derived proteins have been identified. These are

poses, peptones and peptides.

(i) *Proteoses*: Prolonged hydrolysis of metaproteins yield proteoses Albuminoses from albumin). These are water soluble but immuneng heat treatment

(i) *peptones*: Pepsoner are obtained by continued hydrolysis of proses

Like proteoses, these are also water soluble and heat insenative.

(ii)*Peptides*: Prolonged hydrolysis of natural proteins yields peptides. These are also water soluble and not coagulated by heat.

Functions of proteins

Most of the vital activities of the cell are controlled by the two master

molecules namely proteins and nucleic acids. Proteins are important both from the point of view of structure and function of the cell. Structurally proteins constitute in integral part of membranes, pigments etc. Functionally in the form of enzymes, proteins play such a vital role in cell physiology, that without proteins (enzymes) no metabolic reaction is possible. Even the synthesis of DNA is regulated by proteins. In the form of Chromoproteins (Chlorophyll, Cytochromes etc), proteins mediate all energy transformation reaction. It is because proteins are so important that the nucleic acid directly regulates the protein synthesis.

BIOSYNTHESIS OF PROTEINS

Protein structure is highly complex (as has been pointed out earlier) as well as specific. The primary structure is determined by the quality, quantity and the sequence of amino acids. Even an alteration of the position of one amino acid would alter the structure of the protein, and if it happens to be an enzyme regulating an important reaction, the entire reaction is disturbed leading to several abnormalities. Hence it is but natural that there is direct control of DNA in the synthesis of proteins. DNA, RNA and ribosomes of the cell are involved in the synthesis of proteins.

The central dogma: The basic mechanism of protein synthesis is that the nuclear DNA regulates the assembly of ampno acids in the ribosomes to produce a polypeptide chain. Since DNA cannot move out of the nucleus it sends a messager -mRNA which carries the message in the form of sequence of nucleotides. This mRNA forms a template on the ribosomes. Another type of RNA, IRNA found in cytoplasm (whose nucleotide sequence is complemen to that of mRNA), picks up the amino acids and assembles them on the nos to produce a peptide chair

This may be briefly expressed as follows:

DNA Replication DNA Transcription → RNA Translation translation Transcription is the copying of a complementary messenger RNA strand on a DNA strand. In translation, the genetic information presente mRNA (m the form of sequence of nucleotides) directs the sequence of acids to be assembled. We shall study these events in detail.

Transcription

Copying of a complementary strand of mRNA requires the following a-template, activated precursors, a divalent metal ion and RNA polymerase.

DNA molecules serves as a template for the production of mRNA. Of the two strands of DNA only one strand called the 'sense' or the 'coding' strand transcribes mRNA. Single stranded DNA also can serve as a template.

The activated precursors required for mRNA synthesis are - Adenine triphosphate, Guanine triphosphate, Uracil triphosphate and Cytosine triphosphate (ATP, GTP, UTP and CTP)

Mg" or Mn" are the divalent metal ions required for mRNA synthesis.

The enzyme RNA polymerase necessary for transcription. The nucleotides of mRNA are assembled together with the help of RNA polymerase. The enzyme consists of two parts - a core enzyme and a sigma factor. The sigma factor initiates transcription of mRNA on the DNA template and the core enzyme continues transcription.The DNA base sequencearethus transcibed into mRNA base sequence.

tRNA (transfer RNA): This is present freely in cytoplasm. Also called RNA (soluble RNA) is the smallest among the various types of RNA molecules found in the cell. Although the tRNA is single stranded like other types of RNA, it appears double stranded because of coiling. Complementary base pairs of the same strand form pseudo bondings of hydrogen due to folding There are two regions which are free without bonding-one at the acceptor and which always has CCA base pair sequence and the other in the middle region of the molecule called the anticodon region, where there bases turn out of the folded portion and remain free. The types of tRNA is based on the types of these free bases. tRNA has the following functions.

(i) Attach to a particular amino acid at the acceptor and (CCA) with adenine nucleotide. The choice of amino acid however depends on the type of free bases present at the anticodon end.

(ii) to attach itself at a specific site on the mRNA and

(iii) to free the amino acid after it gets attached to another amino acid by means of peptide linkage.

The wobble hypothesis

The triplet code or codon is degenerate i.e., there are many more codons than there are amino acids. Only 20 amino acids are involved in protein synthesis, while there are 64 possibilities or types of codons (43). Hence more than one codon can code for an amino acid. It is interesting to find out as to why the codes are degenerate? There could have been only so many codons as there are amino acids. But there is problem here. If there were to be only one code for an amino acid, what happens if there is a mutation at that site in DNA? Obviously the code changes and the amino acid cannot be incorporated in the protein leading to abnormalities. On the other hand, if there are alternate codes, they act as a protection against mutation in a specific base. Hence degeneracy of codes is a must.

Regarding tRNA present in cytoplasm, since they have to bind themselves to a specific codon, there should have been as many tRNA's as there are codons. But the number of tRNA is only as many as there are amino acids. This means, the anticodons of the tRNA's must be able to 'read' more than one codon of mRNA. How is this reading possible when bonding between base pairs is highly specific?

Crick (1966) proposed "the Wobble hypthesis' in order to solve this apparent dilemma. According to this hypothesis, only the first two bases of the codon have a precise pairing with the bases of the anticodon while the third one may Wobble (non specific). The pairing in the third base is ambigous. Thus a single tRNA can pair (bind) with more than one mRNA codon differing in only the third base. For example the anticodon UCG of tRNA can read codons AGC and AGU of mRNA. The pairing between UGC and AGU is a perfect pairing as per the Crick and Watson pattern. But in the second case i.e., between UGC and AGU, the pairing between the first two bases is normal, while between G and U is against the normal pairing pattern. This unsual bonding G and U is called Wobble pairing.

The degeneracy of the code is not totally randon. Multiple codons for an amino acid always carry the same bases in the first two positions in a triplet with only the third Wobbling.

For example all the four codons for Valine have the first two bases-GU and for alanine it is GC.

Valine-GUU, GUC, GUA and GUG

Alanine-GCU, GCC, GCA and GCG.

Translation

The process of translation consists of activation of amino acids, transfer of activated amino acid to tRNA, initiation of synthesis of polypeptide chan chain elongation and chain termination.

Activation of amino acids

Before amino acids (20 of them) get themselves attached to the specifie type of tRNA they have to be activated (energized). The activation is carried out by the ATP molecule in the presence of the specific enzyme called aminoacyl synthetase with Mg as the cofactor. The reaction produces aminoacyl adenylate (AAA) or aminoacyl AMP. Pyrophosphates arereleased in the process.

Amino acid + ATP + Enzyme-Enzyme AA>AMP complex +Ppi

Transfer of activated amino acids to tRNA

The activated amino acid (aminoacyl AMP) is transfered on to a specific tRNA, during which AMP and the enzyme (aminoacyl synthetase) are released.

Enzyme AA.AMP +tRNA _____AA.tRNA + AMP + Enzyme

Initiation of Polypeptide chain synthesis

This involves two steps viz., preparation of the site of protein synthesis and initiation of the polypeptide chain.

(i) *Preparation of the site of synthesis*

Ribosomes in cytoplasm are the actual sites of protein synthesis. These are usually separated into their subunits (50s and 30s), when they are not participating in protein synthesis. The mRNA usually attaches to the 30s subunit through its 5' end carrying the AUG codon. In the process of attachment certain substances called initiation factors (IF) help. These factors are of three types in prokaryotes (IF1, IF2, IF3) and are of six types in eukaryotes (elF2, elF2al, elF2a2, elF2a3, elFa4 and elF3)

The attachment of the mRNA to the 30s submit requires the help of IF3 and Mg". The IF3 probably unwinds the rRNA (ribosomal RNA), so that it can bind to mRNA. After the attachment of mRNA, an mRNA-30s complex is formed. The starting amino acid in a polypeptide is methionine (met) in the case of eukaryotes and N-formly methionine (f-met) in the case of prokaryotes. The amino acid (met or f-met) tRNA complex attaches to the initiation codon (AUG) on the mRNA 30-s complex through its anticodon (UAC). This forms the 30s initiation complex and requires the participation of IF2, IF3 and GTP (energy donor).

(i) 30s+mRNA-_____ mRNA-30s complex

(ii) mRNA-30s complex + met tRNA-_____70s-mRNA-met- t RNA

During the formation of the initiation complex the 50s subunit combines with the 30s subunit to form the 70s unit. This union is facilitated by the participation of IFI.With the formation of the 70s ribosome) the initiation of the polypeptide chaingegins.

(ii) Initiation of the polypeptide chain

Each ribosome has two cavities P site site) and Asite (aminoacyl sites). AA tRNA complex occupies the A site, while the met-tRNA (tRNA with the initiator aminoacid methionine) will be the P site.This occupation requires a molecule of GTP and the participation of IF, The energy of GTP is utilized for the formation of the peptide linkage.

Peptide linkage occurs when the carboxyl group of peptidyl tRNA (met. tRNA) present at the P site reacts with the amino group (NH) of AA tRNA at the A site. The enzyme peptidyl transfers catalyses the formation of peptide bond. Later the AA+tRNA moves from A site to the P site in the presence of GTP and IF2

Formation of the polypeptide chain

A specific type of tRNA (which has bases complementary to the codon of mRNA) linked to a particular amino acid gets attached to a specific site o mRNA when its anticodons can form a pairing with the first codon of the mRNA. Soon after a second tRNA with its amino acid joins the second se (triplet) on mRNA. The first and the second amino acids attached to different tRNA form a peptide linkage as both are linked to mRNA via the anticodons of tRNA. A molecule of water is released during the formation of the peptide linkage

Peptide bond

COOH +NH2_____ CONH + H2O

As soon as the peptide linkage is formed the tRNA is made free and comes back to cytoplasm. This whole process in which a specific tRNA carries its amino acid to a specific site is called translation i.e. the code (triplet) is translated into an amino acid. Here the sequence of bases in tRNA will be identical with the base sequence of that part of DNA coding for mRNA.

Chain elongation

Elongation of the chain requires the participation of certain factors called elongation factors (EF). These are EF-Tu, EF-TS and EF-G in prokaryotes and EF-1 and EF-2 in eukaryotes. After the first amino acid tRNA complex has moved from the A site of the ribosome to the P site, a second AA. tRNA complex now occupies the A site. The codons are recognized enzymatically.

This requires the participation of Ef-Tu, GTP and Mg". Peptide linkage takes place between the two amino acids and the AA. tRNA complex migrates to the P site rendering the A site vacant. Now a third amino acid tRNA complex occupies the A site and theprocess continues. The shifting of th AA. tRNA complex from A site (releasing site) is called translocation. At the P site the tRNA is released from the ribosome while A site has a peptide chain liked to a tRNA which has brought in the last amino acid of the peptide chain. During translocation, the ribosome moves relative to the mRNA in the 5'-3' direction.

Chain termination

The elongation of the chain continues by the alternate occupation and vacancy at the A site by the AA. tRNA complexes until a termination codon (UAA, UAG or UGA) reaches the ribosomes. The termination codon sends signals to the ribosomes to attach release factors (RF-1, RF-2 and RF-3 in prokaryotes and RF in eukaryotes).

The release factors interact with the enzyme peptidyl transferase (which catalyses the formation of peptide bond) resulting in the hydrolysis of the bond between the polypeptide chain and the tRNA (linked to the last amino acid of the peptide chain at the site) and the former is released from the ribosome

Other events that occur at the termination of the polypeptide chain are

- (i) Release factors get disassociated from the ribosomes due to the hydrolysis of GTP.

(ii) tRNA is also released.

(ii) Ribosomal sub units (30s and 50s) get disunited.

(iv) The polypeptide cham is processed by the removal of the methionine residue from the chain. Depending upon the circumstances the chain may get cleaved into two or more pieces.

In eukaryotes, the polypeptide chain passes through a tunnel between the two subunits of the ribosome into the cavity of the cisterna of the endoplasmic reticulum.

Rate of Protein synthesis

The rate of transcription in Escherichia coli DNA for the enzyme controlling trypotophan synthesis is 28 nucleotides per second at 39°C. The speed of translation is 7 amino acids per second. For the haemoglobin molecule, the rate

GENETIC REGULATION OF PROTEIN SYNTHESIS IN PROKARYOTES

All the activities of an organism, be it unicellular or multicellular, are ultimately controlled by the genes. In multicellular organisms, where cells differ widely in structure and function all of them carry the same genes, since all of them are traceable to a single cell viz, zygote. This poses an interesting question as to the function of genes. How do similar genes function differently? The answer to the question lies in differential gene action. In other words, not all genes are active at all times. The genes are switched on and switched off at different times. This differential gene action is also responsible for the synthesis of different types of proteins (enzymes) at different times. Not all enzymes development (seed germination) may not be required at later stages (flowering

process) and vice versa. The metabolic products produced may induce or repress the enzyme synthesis. This is called the Inducing system and the Repressing system. When the supply of a particular enhances the enzyme (protein) synthesis it is called the inducing system and when the supply of a metabolic product suppresses the enzyme synthesis it is called the repressing system (end product inhibition).

Jacob and Monod (1961) proposed a genetic hypothesis to explain the mechanism of induction and repression. The scheme of gene action proposed by Jacob and Monod is called the Operon concept (operon model). Originally (as suggested by them), the operon mechanism consisted of an operator gene and a number of structural genes. As per current understanding however, the operon system consists of (a) regulator gene, (b) promoter gene, (c) operator gene, (d) structural genes, (e) repressor, (f) corepressor and (g) inducer.

Structural genes: There are many structural genes associated in an oper system. These direct the synthesis of the mRNA and govern the sequence of amino acids in a protein molecule. Each structural gene might produce a particular

kind of protein or all structural genes might regulate the production of a single protein. The activities of the structural gene (s) are controlled by the promoter and operator site of the operon system. The most well studied structural genes (zy and a) are those of the lac operon system in Escherichia coli.

Operator gene: The operator gene is situated adjacent to the first structural gene. It switches on or switches off the functioning of the structural gene (protein synthesis). In case a structural gene has to be suppressed, a repressor attached itself to the operator to form an Operator-repressor complex. In the case of protein synthesis, the operator repressor complex prevents the transcription by blocking the movement of RNA polymerase.

Promoter gene: The promoter gene is continuous with the operator gene and is believed to lie left to it. It is suggested that RNA polymerase binds to thepromoter site during transcription. Three regions have been recognised in the promoter site. These are (a) recognition site, initial binding site and the mRNA initiation site (operator site).

(i) Recognition site: Also called the cga site (catabolic gene activator site), it consists of certain palindromic sequences of DNA. These symmetrical sections of DNA are recognised by proteins having symmetrically placed sub units. This site, also called the CRp site (cyclic AMP receptor protein site) binds a CR protein to the promoter gene and thus facilitates the binding of the enzyme RNA polymerase. It has been found that in E.coli CRP combines with CAMP (cyclic adenosine monophosphate) forming a CRP-CAMP complex which binds to the promoter enhancing the binding of RNA polymerase and activates transcription. This regulation is called positive control.

(ii) Initial binding site: This consists of seven bases (DNA) to which the RNA polymerase binds.

(iii) *RNA initiation site*: The site where transcription begins is called initiation site. This is the region overlapping with the operator region.

Regulator gene: The regulator gene directs the activity of the operator gene by producing inhibitor proteins called repressors. This repressor protein binds to the operator gene and blocks the path of RNA polymerase, thus preventing transcription. If an inducer is present in the system, it binds to the repressor which undergoes conformational change and becomes inactive. As the inactive repressor cannot bind to the promoter, the structural genes get activated and protein synthesis continues.

Repressor, inducer and corepressor: This is a protein whose function is to attach to the operator gene to prevent the process of transcription. In an inducible system (an inducer is a metabolic product which promotes protein synthesis), if an inducer is absent, the repressor blocks protein synthesis by itself to the operator gene. When an inducer is present it (inducer) binds to the repressor rendering it inactive and transcription continues

In a repressible system, if only a repressor is present, it is inactive by itself and cannot attach to the operator gene and hence transcription continues However when another protein called corepressor is present it attaches to the repressor forming a repressor-corepressor complex, which blocks the operator gene thus preventing transcription.

GENETIC REGULATION IN EUKARYOTES

The mechanism of gene regulation of protein synthesis explained above illustrates the phenomenon in prokaryotes, whereby a repressor protein transcribed by regulator genes may activate or prevent the protein synthesis depending on the presence of inducer or corepressor.

In eukaryotes, genetic regulation takes place by a slightly different mechanism. Two types of proteins associated with the DNA of the chromosomes possibly play a role in gene regulation. These are histones and non histone chromosomal proteins (NHC proteins). Since histones of different rganisms have similar amino acid composition it is unlikely that they can selectively repress gene action. At best, histones may probably bring about a general (non specific) repression. It is believed that histones under certain situations bring about supercoiling of DNA preventing the movement of RNA polymerase and thus blocking transcription. This is shown by the fact that RNA precursors are incorporated only in those regions where chromatin in diffuse (no super coiling)

Three models have been proposed by various scientists to account for gene regulation in eukaryotes. These are 1. Frenster's model, 2. Non histone depressor model and Britten and Davidson's model. A. Non specific repression

by histones B. Specific binding of NHC proteins to histones

C. Removal of histone by NHC proteins

D. De repression of DNA leading to transcription

Britten Davidson Model: Also known as operon operator model, this is somewhat different from the other two models As per this model, four types of genes are involved in the regulation process. These are sensor genes, producer genes, integrator genes and receptor genes.

1. Sensor genes: Comparable to the promoter genes these are sensitive to the metabolic status of the cell. The sensor gene is stimulated by various metabolic substances.

2. Producer genes: These regulate the output of metabolism and control the formation of enzymes, cell organelle, membranes etc.

3. Integrator gene: As the name suggests, the gene integrates the activities of other genes. The integrator gene sends specific molecular signals to other genes as to whether the sensor gene is activated or not. Each sensor gene may be associated with a single or a group of integrator genes. In the latter case, a sensor gene may send a variety of molecular signals to other genes through its various integrator genes.

4. Receptor gene: This acts as an intermediate between the integrators and the producers, When the molecular signal reaches the receptor gene, inturn activates the producer gene. A producer may have one or a group of it receptor genes associated with it in the same way as sensors have several integrators. This helps, not only in the variety of origin of molecular signals. (from sensors) but also variety in reception of these signals (by receptors) Perhaps multiintegrator gene complex and multireceptor gene complex may have a specific multichannel relationship.

Part: Practical Plant Physiology

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Experiments

1- Vander Blank's theory

2- Determination of glucose

3- Determination of scurcose

4- Determination of starch

5- Determination of Enzymes activity

6- Factors affecting enzymes activity

7- Determination of pigments

8- Determination of proteins

9- Determination of amino acids

10- Determination of fats

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