
Part: Plant Metabolism

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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

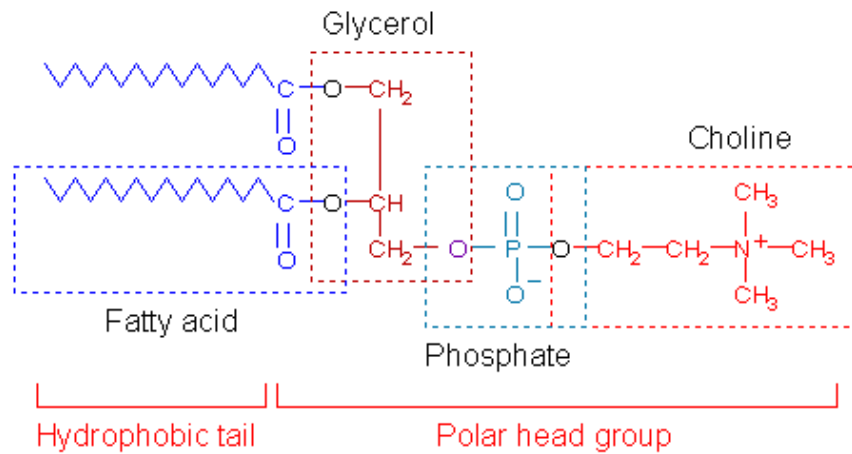
Some cells deposit additional layers inside the primary wall. This occurs after growth stops or when the cells begin to differentiate (specialize). The secondary wall is mainly for support and is comprised primarily of cellulose and lignin.

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 $\times 10^{10}$ 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;

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- Microsomes - a "biochemical" term for the fraction that is obtained from high speed centrifugation of cell homogenates. It includes membrane fragments and ribosomes.
 - Oleosome (sphaerosomes) - 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



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

<u>EC MAJOR CLASSES of Enzymes</u>	
1. <u>Oxidoreductases...</u> [<u>dehydrogenases</u>]	
catalyzes oxidation-reduction reactions, often using coenzyme as NAD⁺/FAD	
<u>Alcohol dehydrogenase</u> [<u>EC 1.1.1.1</u>]	ethanol + NAD⁺ ---> acetaldehyde + NADH
2. <u>Transferases...</u>	catalyze the transfer of the functional group
<u>Hexokinase</u> [<u>EC 2.7.1.2</u>]	D-glu + ATP ---> D-glu-6-P + ADP
3. <u>Hydrolases...</u>	catalyze hydrolytic reactions adds water across C-C bonds
<u>Carboxypeptidase A</u> [<u>EC 3.4.17.1</u>]	[aa-aa]_n + H₂O ---> [aa-aa]_{n-1} + aa
4. <u>Lyases...</u>	cleave C-C, C-O, C-N & other bonds often generating a C=C bond or ring
<u>Pyruvate decarboxylase</u> [<u>EC 4.1.1.1</u>]	pyruvate ---> acetaldehyde + CO₂
5. <u>Isomerases...</u> [mutases]	catalyze isomerizations
<u>Maleate isomerase</u> [<u>EC 5.2.1.1</u>]	maleate ---> fumarate [<u>HFCS</u>]
6. <u>Ligases...</u>	condensation of 2 substrates with the splitting of ATP
<u>Pyruvate Carboxylase</u> [<u>EC 6.4.1.1</u>]	PYR + CO₂ + ATP ---> OAA + ADP + P

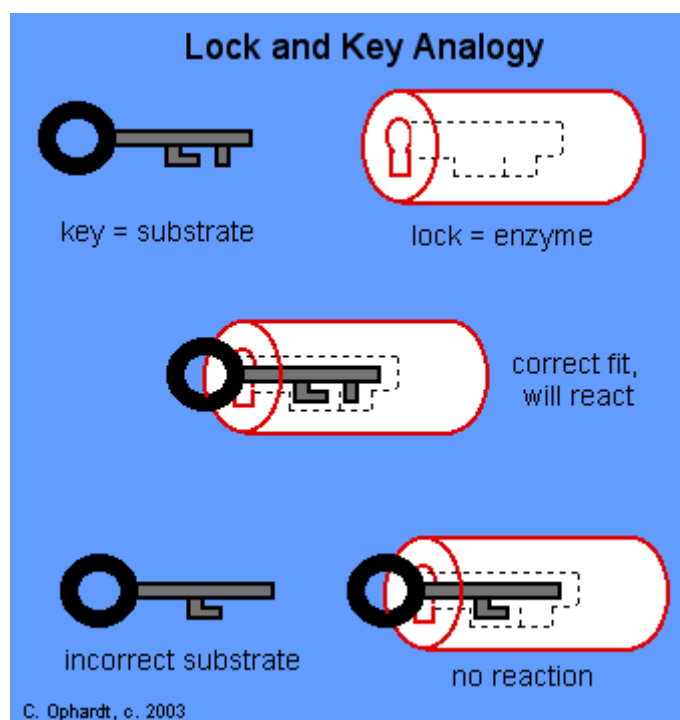
Specificity

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]

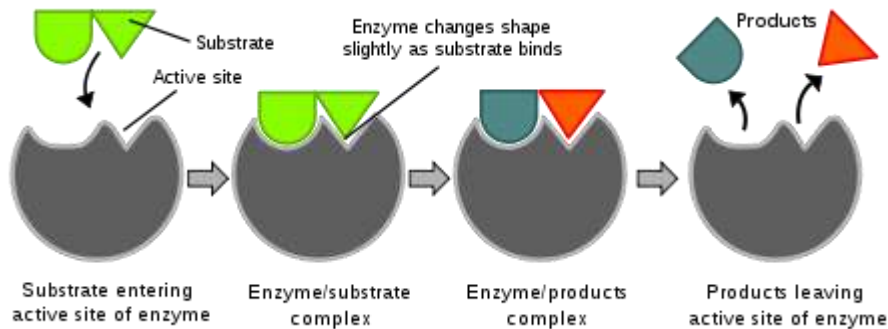
Some enzymes that produce secondary metabolites are described as promiscuous, as they can act on a relatively broad range of different substrates. It has been suggested that this broad substrate specificity is important for the evolution of new biosynthetic pathways.^[25]

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



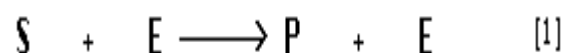
Diagrams to show the induced fit hypothesis of enzyme action.

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.

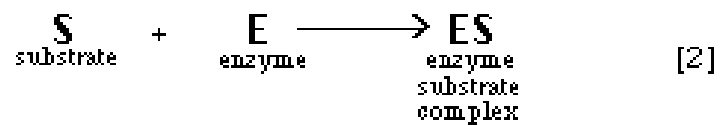
The basic enzymatic reaction can be represented as follows



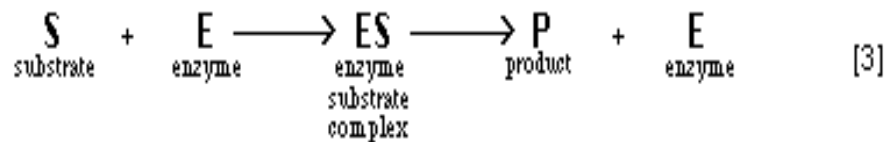
where E represents the enzyme catalyzing the reaction, S the substrate, the substance is changed, and P is the product of the reaction.

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:



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

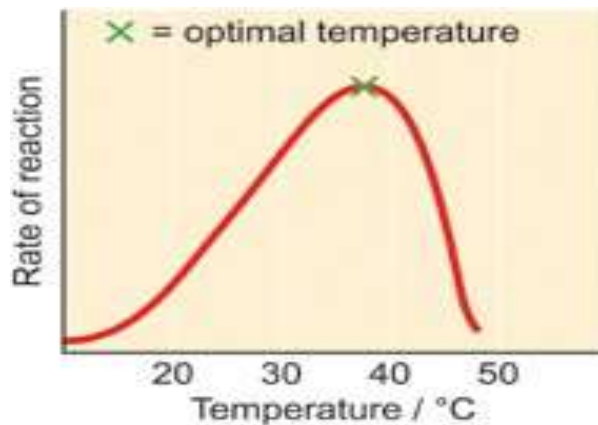


The existence of an intermediate enzyme-substrate complex has been demonstrated in the laboratory, for example, using catalase and a hydrogen peroxide derivative.

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

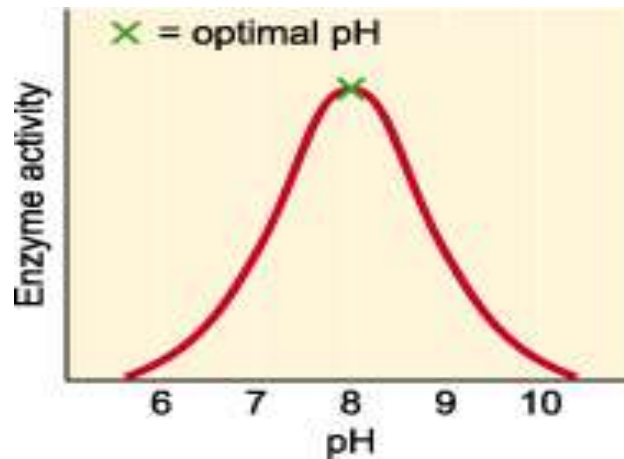


As the temperature rises, reacting molecules have more and more kinetic energy. This increases the chances of a successful collision and so the rate increases. There is a certain temperature at which an enzyme's catalytic activity is at its greatest (see graph). This optimal temperature is usually around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

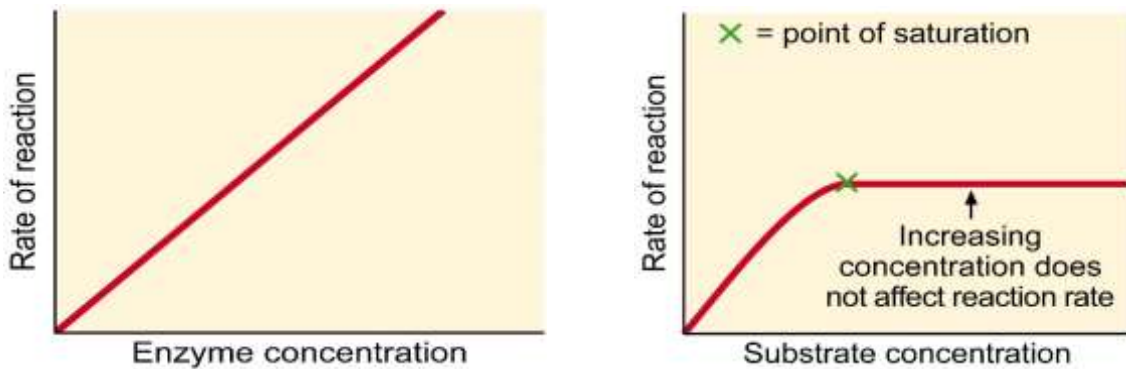
Above this temperature, the enzyme structure begins to break down (**denature**) since at higher temperatures intra- and intermolecular bonds are broken as the enzyme molecules gain even more kinetic energy.

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



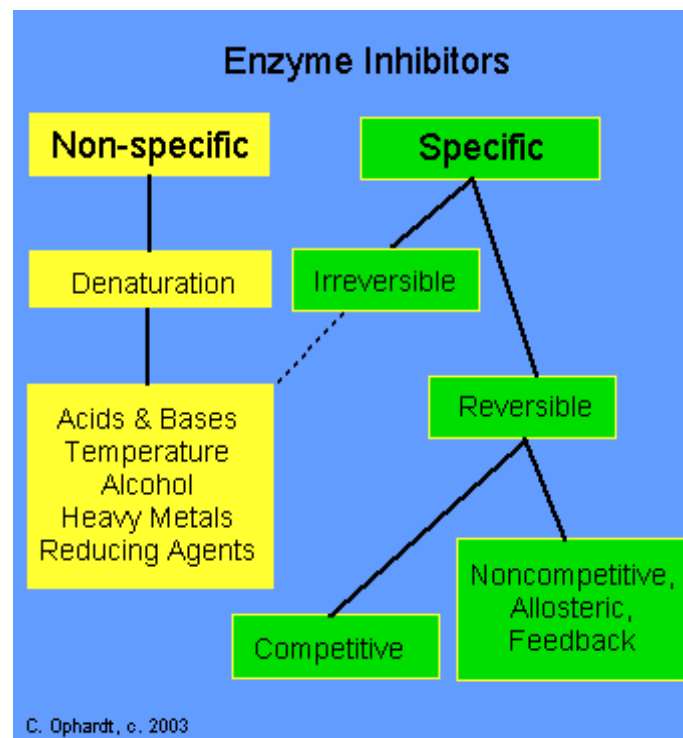
The rate of an enzyme-catalyzed reaction depends on the concentrations of the enzyme and substrate. As the concentration of either is increased the rate of reaction increases (see graphs).

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:

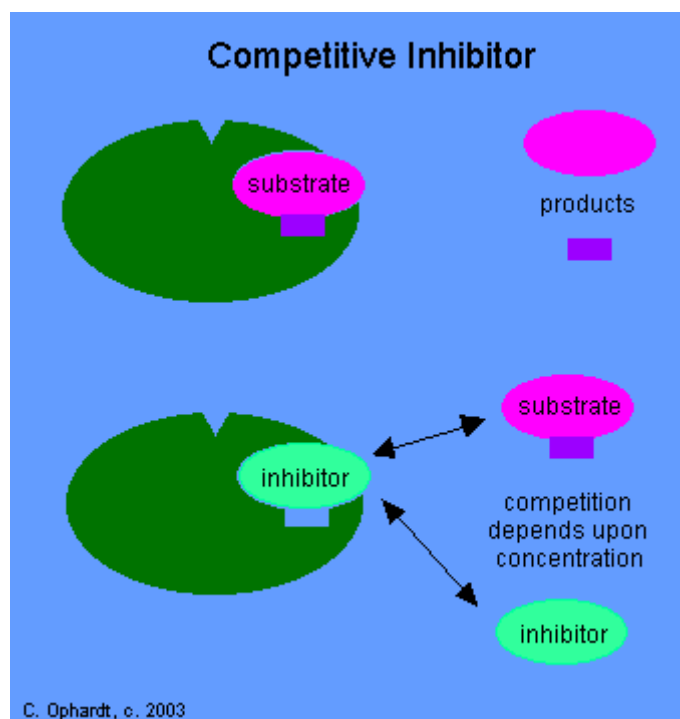
A competitive inhibitor is any compound that closely resembles the chemical structure and molecular geometry of the substrate. The inhibitor competes for the same active site as the substrate molecule. The inhibitor may interact with the enzyme at the active site, but no reaction takes place. The inhibitor 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.

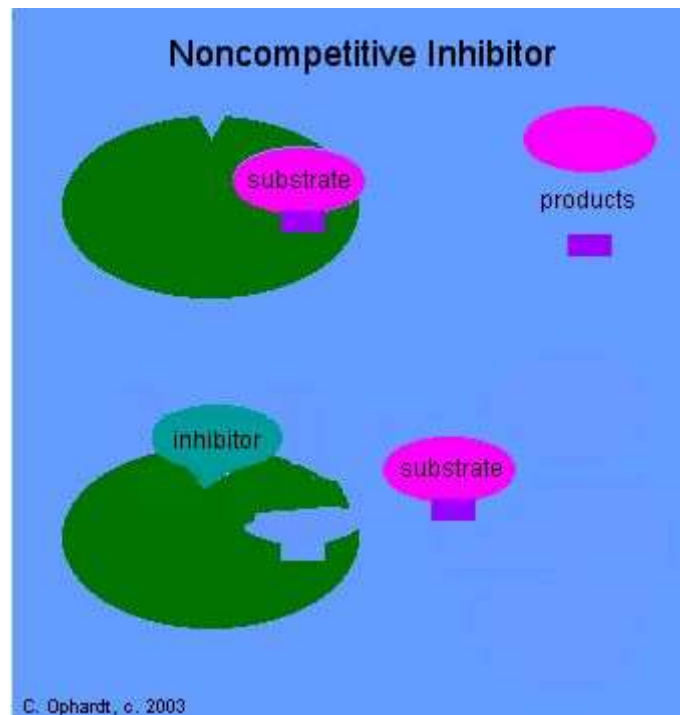
Methanol poisoning occurs because methanol is oxidized to formaldehyde and formic acid which attack the optic nerve causing blindness. Ethanol is given as an antidote for methanol poisoning because ethanol competitively inhibits the oxidation of methanol. Ethanol is oxidized in preference to methanol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



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;

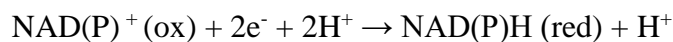
(c) helpful mnemonics to remember: "oil rig" - oxidation is loss, the reduction is gain, or "Leo says grrrr" - loss equals oxidation, gain reduction;

(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.*, $\text{Fe}^{2+} \rightarrow \text{Fe}^{3+}$ 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):



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. CO₂ 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 Krebs cycle intermediate, from the mitochondrion)

c- 2 ALA condense to form a unit of porphyrin

d- 4 porphyrins condense to form porphyrin (tetrapyrrole)

e- Magnesium is inserted

f- A photoreduction step occurs (converts protochlorophyllide \rightarrow 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 (ν). Frequency is inversely related to wavelength $\nu = c/\lambda$ 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 $\text{mol m}^{-2} \text{s}^{-1}$, called the photon fluence rate; or units of energy, $\text{J m}^{-2} \text{s}^{-1}$.

E. What happens when chlorophyll absorbs light?

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

CHL (ground state) \rightarrow 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 $\text{NADP}/\text{H} = -0.32$ 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 (ΔE_m) between water and NADPH:

$$\Delta E_m = E_m (\text{acceptor}) - E_m (\text{donor}). \text{ Or, } \Delta E_m = -0.320 - (0.820) = -1.14 = \text{ca. } -1.2 \text{ eV.}$$

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

$$\Delta G = -n F E_m$$

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:

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

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 = 6.6x10⁻⁵ cm.

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

$$E = h\nu$$

where h = Planck's constant which relates energy to frequency of oscillation and is 6.6255 x 10⁻³⁴ J sec photon⁻¹; and ν = pulses sec⁻¹.

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

$$E = hc/\lambda$$

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

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

multiply by Avogadro's number

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

= 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 Ca²⁺ 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 (Mn²⁺)
- 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

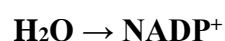
D. ATP synthase/Coupling Factor Complex

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:



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

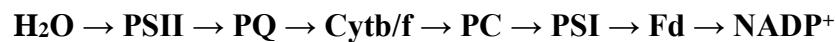
are removed from the water, they are sequentially shuttled from PSII to the cyto b-f complex to PSI and then finally to NADP⁺. Thus:

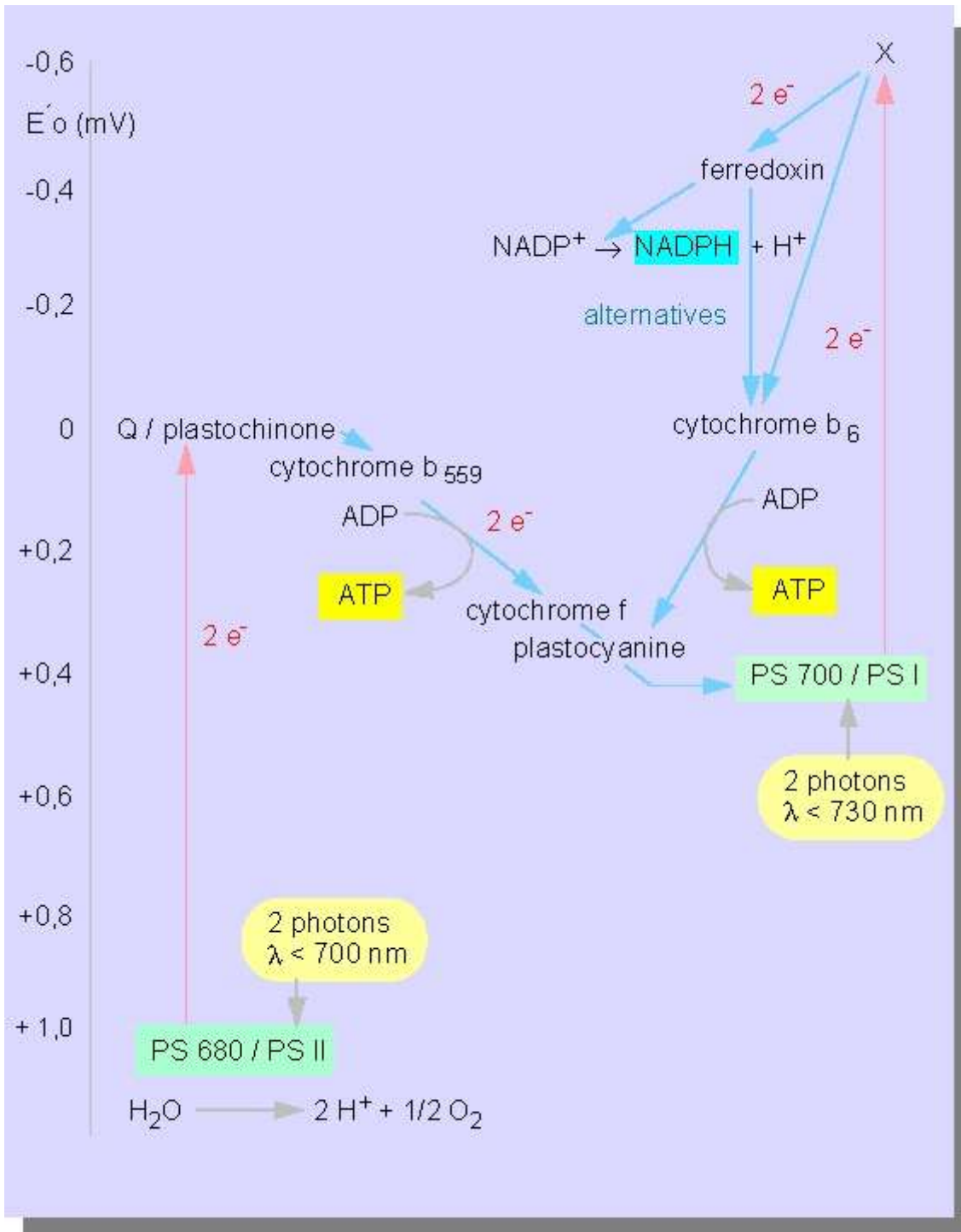


Since PSII, cyt b/f, and PSI are physically separated from one another, there must be a means to transfer electrons between the complexes. A mobile form of plastoquinone (PQ) transfers electrons from PSII to cyt b-f. A copper-containing protein, plastocyanin (PC), transfers electrons from the cytochrome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



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





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,



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 S₀ 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

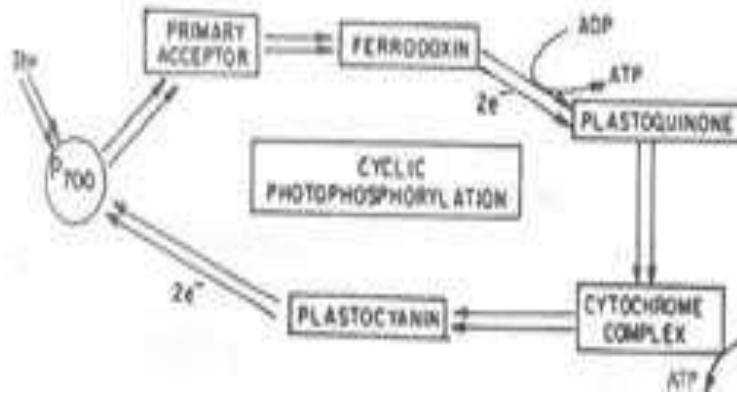
at a useful rate. Living systems use complex macromolecular structures to lower the activation energies of biochemical reactions.

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.

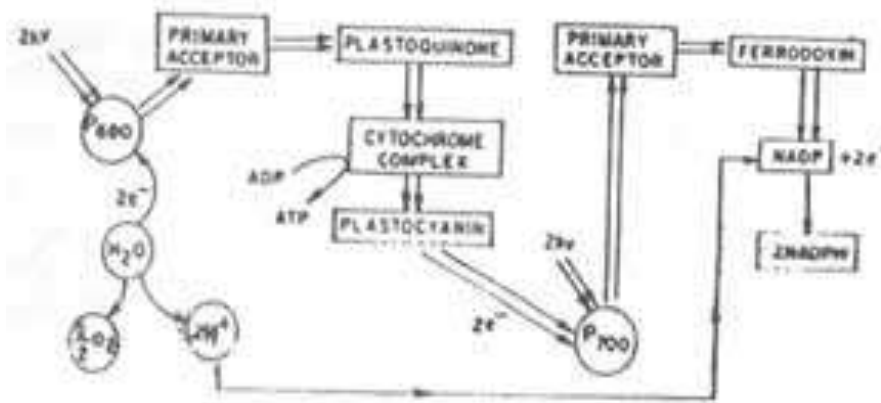


Cyclic Photophosphorylation

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

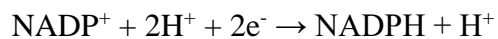
Non-cyclic photophosphorylation

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 $2\text{H}^+ + 1/2 \text{O}_2 + 2\text{e}^-$ by a process called photolysis (or *light-splitting*). The two electrons from the water molecule are kept in photosystem II, while the 2H^+ and $1/2\text{O}_2$ 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 catalyze the reaction (as shown):



This consumes the H⁺ ions produced by the splitting of water, leading to a net production of 1/2O₂, 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 biphosphate; C₅) to form 2 molecules of PGA (C₃)
- ◆ first product of carbon fixation is PGA (Calvin's experiments)
- ◆ catalyzed by the enzyme ribulose biphosphate 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

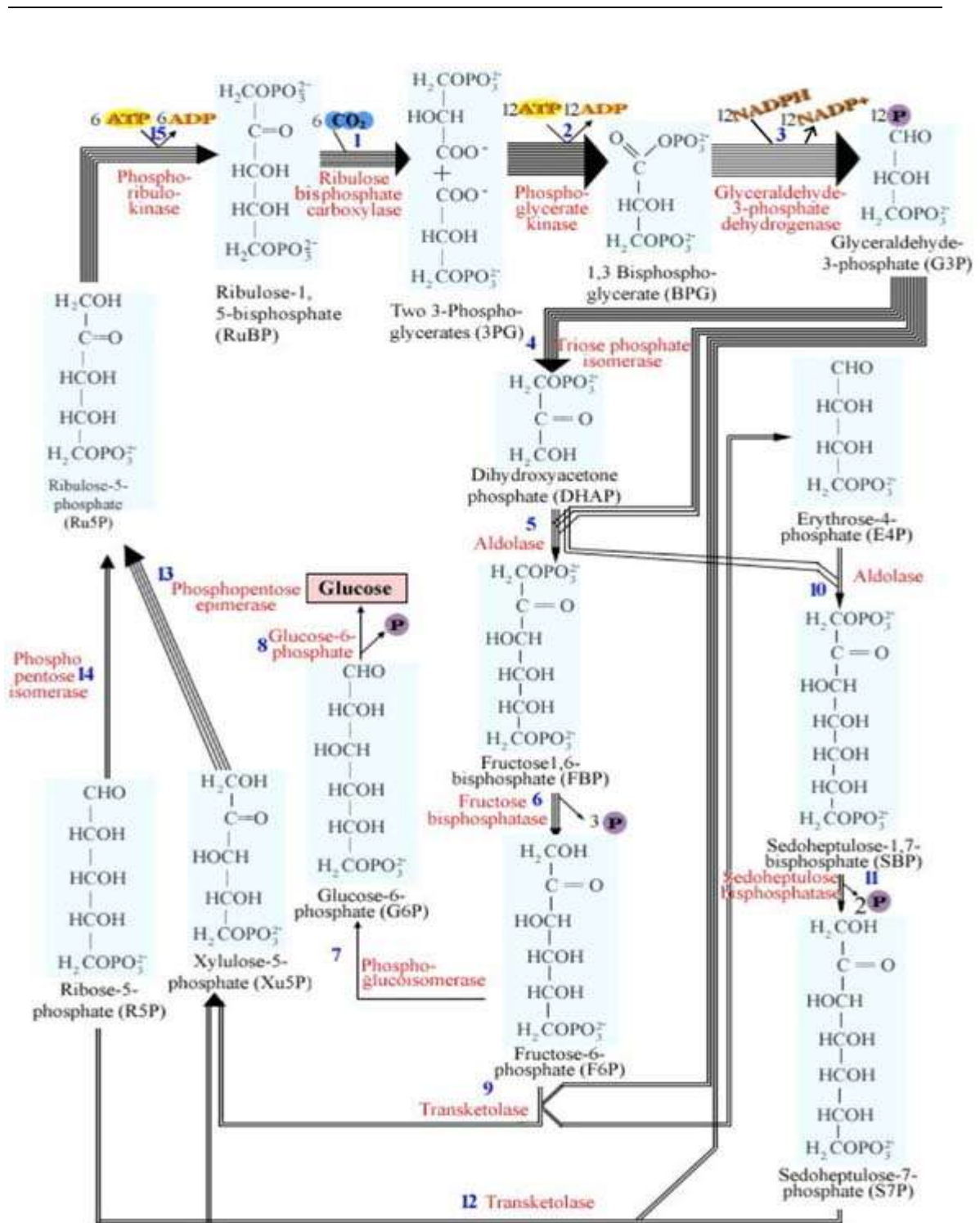
Complex series of reactions (rearrangement) that result in the net removal of a C₃ carbohydrate from the cycle (release) and the production of the precursor to the starting material (recharging):

- ✧ 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 CO₂ 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

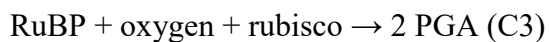
Unlike most enzymes, rubisco is not substrate specific - it also has an oxygenase function. In addition to its normal substrate (carbon dioxide), rubisco also binds oxygen to RuBP. Although rubisco has a higher affinity for binding carbon dioxide ($K_m = 9 \mu\text{M}$), if enough oxygen is present,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

C. The reaction catalyzed by ribulose biphosphate 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:



Compare this to the normal reaction:



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 $[\text{carbon dioxide}]/[\text{oxygen}]$ ultimately determines the product of the rubisco reaction.

if $[\text{carbon dioxide}/\text{oxygen}] = \text{high}$; then it favors normal Calvin cycle

if $[\text{carbon dioxide}/\text{oxygen}] = \text{low}$; 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 NADPH, 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

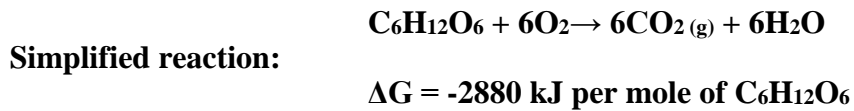
This is the initial enzyme that fixes carbon dioxide. The product is ultimately malate which accumulates in the vacuole during the night (hence the "acid" term).

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 FADH₂.



The reducing potential of NADH and FADH₂ 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.

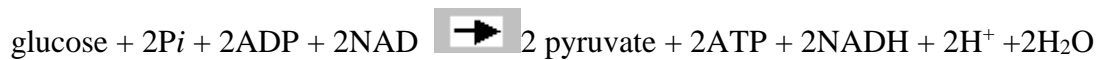
Glycolysis

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:



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 $2\text{NADH} + 2\text{H}^+$

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 H₂O and becomes PEP

Step Ten

2PEP to 2 Pyruvates

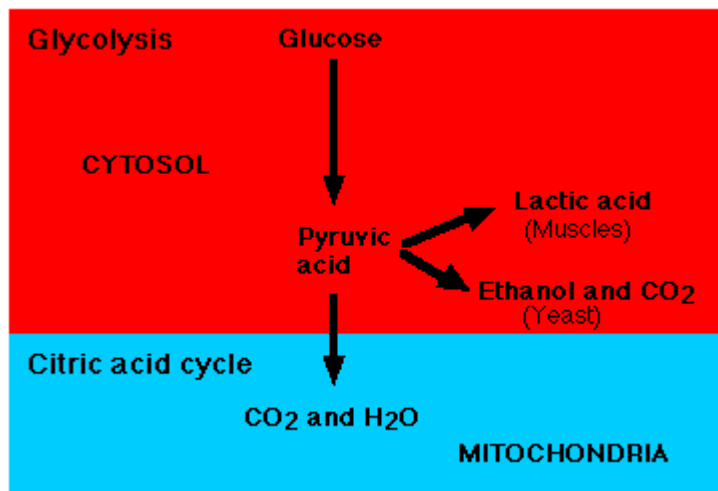
This step is facilitated by the enzyme pyruvate kinase

Two PEP transfer their P to ADP.

Two ATP molecules are made.

Without O₂: NADH + H⁺ is used in fermentation.

With O₂: 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**.

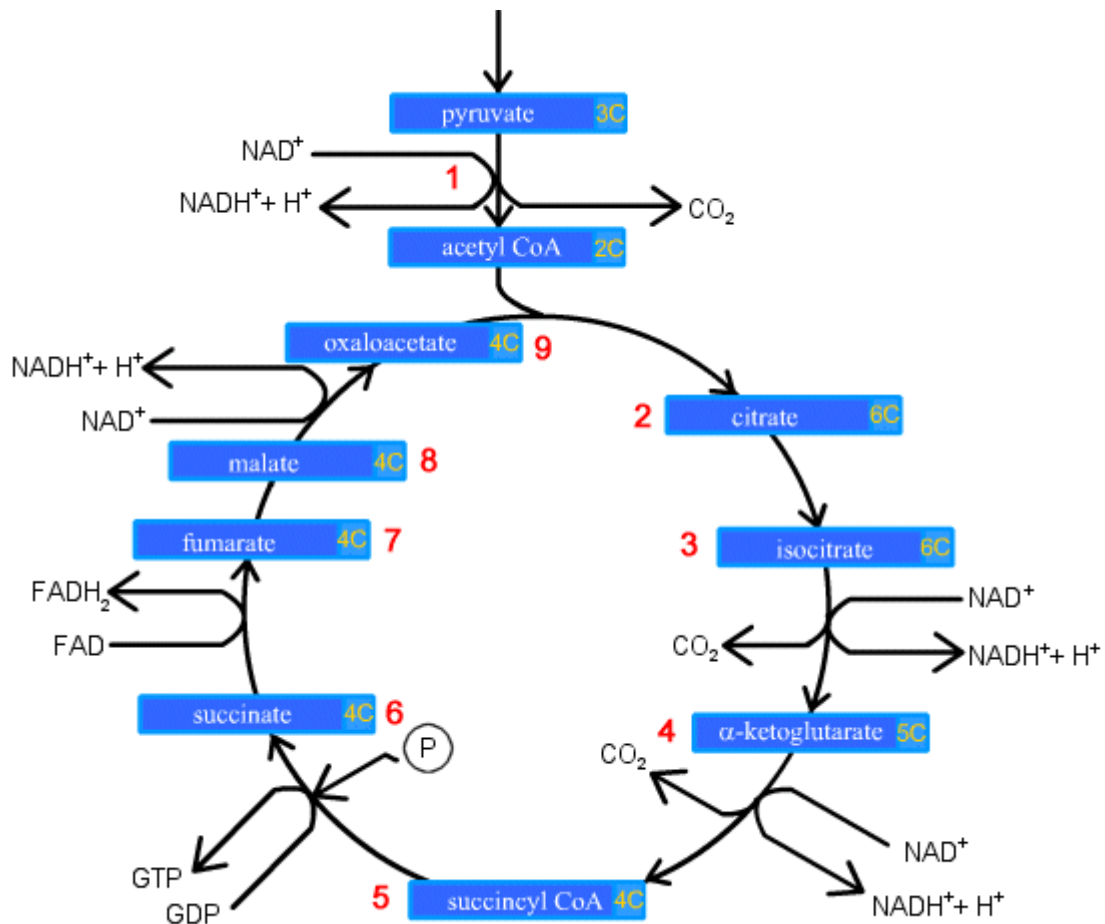
Pyruvate Oxidation

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 Krebs 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 CO₂ 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.

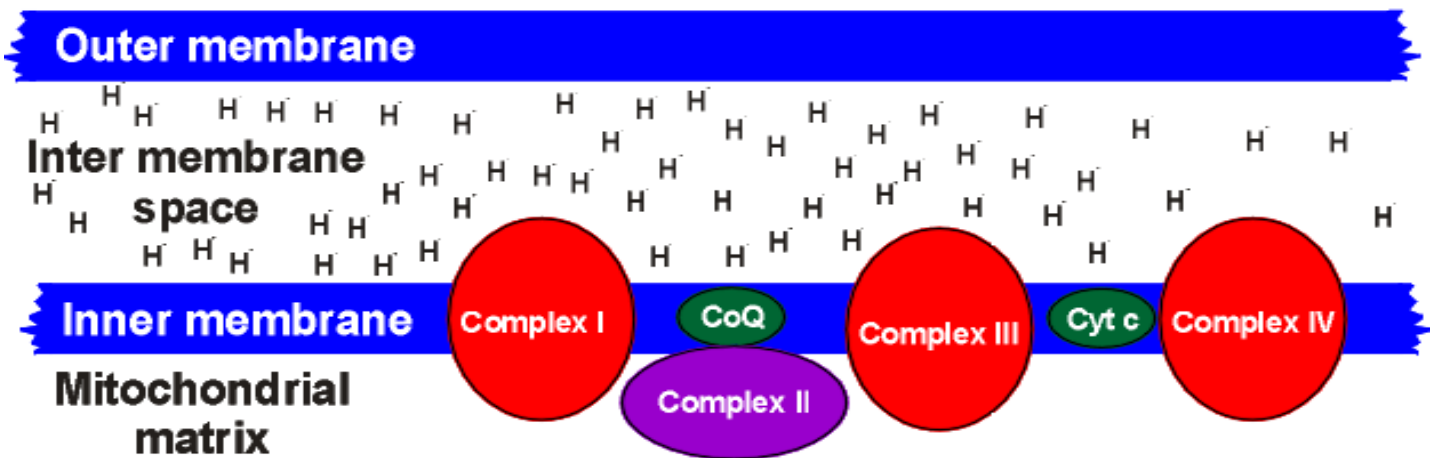
Electron Transport Chain

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)

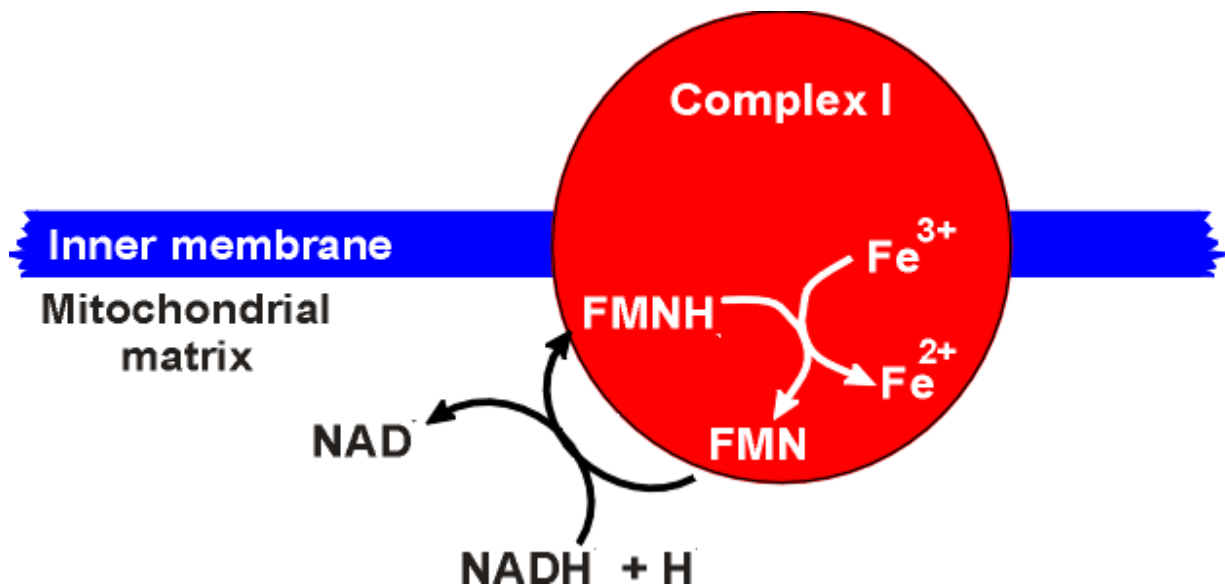
Cytoplasm



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.

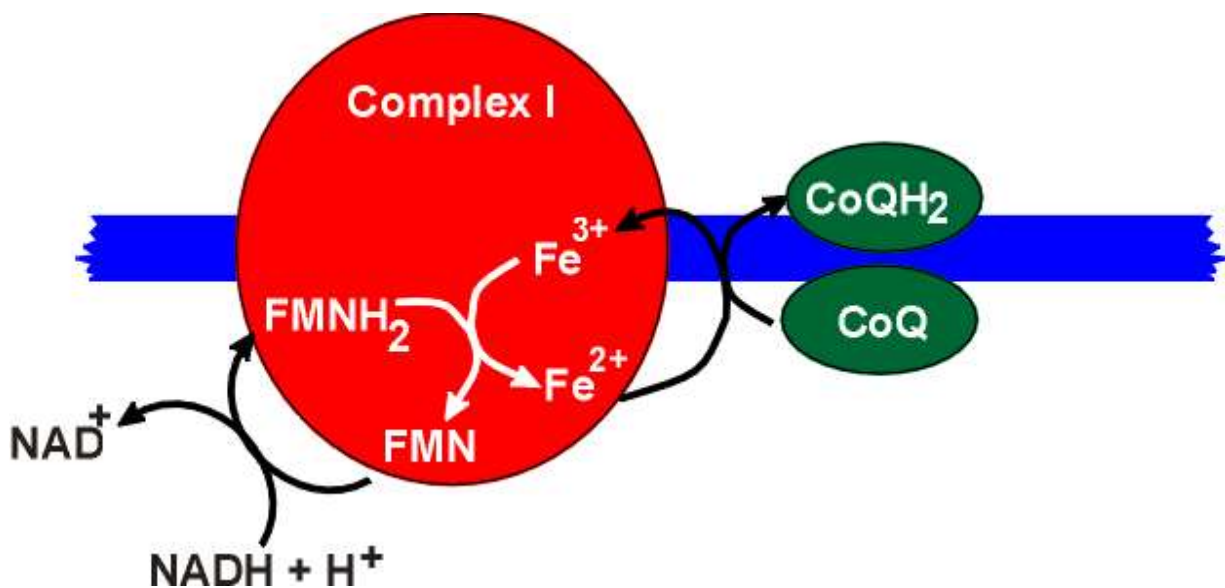
The electrons are then passed to iron-sulfur proteins (FeS) in complex I (this is non-heme iron). The electron is accepted by Fe^{3+} which is reduced to Fe^{2+} :



(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 CoQH₂ (ubiquinol) while the FeS proteins are oxidized back to Fe³⁺ state.



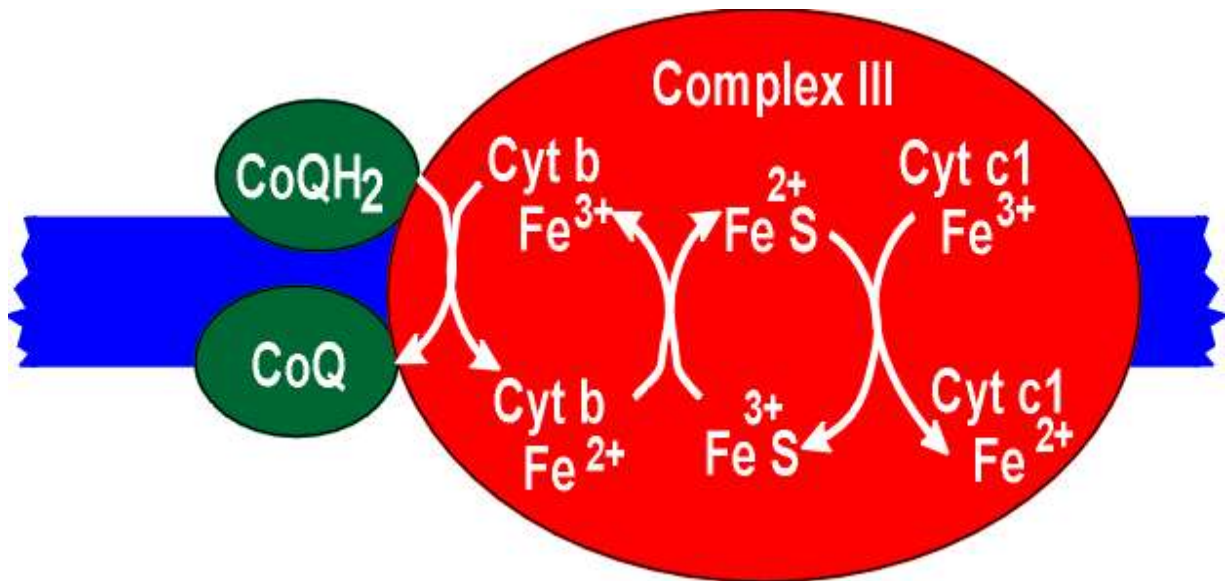
CoQ is small and lipid soluble so it is mobile in the mitochondrial membrane. It diffuses easily and shuttles the electrons to complex III (we will talk about complex II later).

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

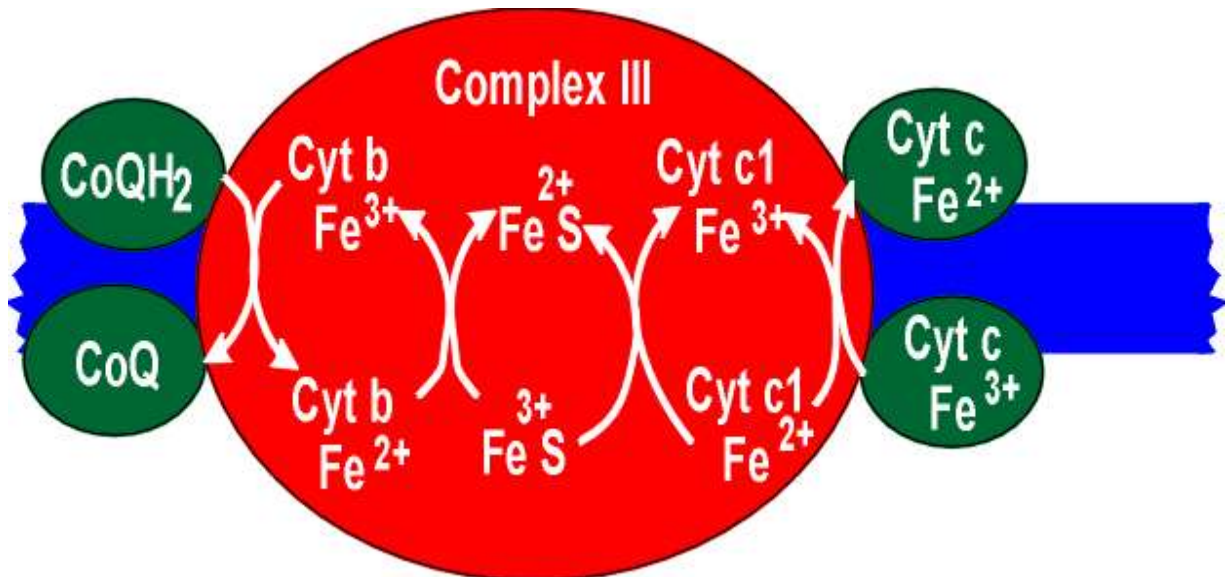
oxidation states as they pass on the electrons.

CoQH₂ passes 2 electrons to cyt b causing the Fe³⁺ to be reduced to Fe²⁺. The electrons are passed to the FeS protein and then to cyt c1.



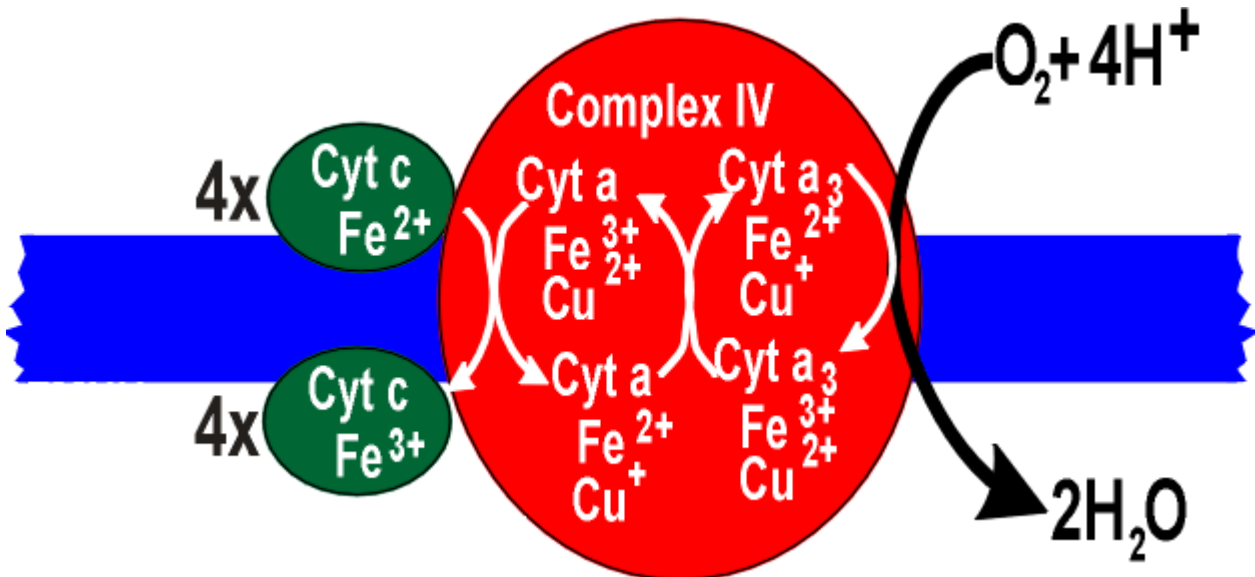
Step 4

Cyt c is another small mobile protein. It accepts electrons from complex III (Fe³⁺ is reduced to Fe²⁺) and shuttles them to the last electron transport protein in the chain (complex IV).



Step 5

Complex IV contains cytochrome a and cytochrome a₃ (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 O₂ 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 O₂ (it doesn't balance with respect to electrons).

Part: Practical Plant Metabolism

Prepared by Prof. Dr. Arafat Abdel Hamed Abdel Latef

Experiments

1- Vander Blank's theory

2- Determination of glucose

3- Determination of sucrose

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
