Plant Enzymes and Plant Hormones

Prepared by
Prof. Dr. Arafat Abdel Hamed Abdel Latef

Plant Enzymes and Plant Hormones

Prepared by Prof. Dr. Arafat Abdel Hamed Abdel Latef

Plant Enzymes

Enzymology is the study of enzymes, their kinetics, structure, and function, as well as their relation to each other.

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

- ✧ Biocatalysts
- ✧ Biological middlemen
- ✧ Organic catalysts
- ✧ 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 have a complex structure act only up a specific substrate do not change the direction of reactions.
- ✧ Enzymes convert substrates to products without changing themselves

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

Some important dates in early Enzyme History

1836 Berzelius coined the term catalysis (Gk: to dissolve).

1878 Kuhne used the word enzyme (Gk: in yeast) to indicate the catalysis taking place in the biological systems.

1883 Buchner isolated enzyme system from cell-free extract of yeast. He named the active principle as zymase (later found to contain a mixture of enzymes), which could convert sugar to alcohol.

1898 Ducleaux uses suffix "ASE" for enzyme naming.

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**.

Active site: All enzymes possess an area in their molecular organization where substrate materials bind themselves in order to undergo chemical change. This binding site is called the **active site** of an enzyme. An enzyme may have one or more active sites.

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.

An apoenzyme together with its cofactor(s) is called a ***holoenzyme*** (this is the active form). The term "**holoenzyme**" can also be applied to enzymes that contain multiple protein subunits, such as the 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. (**Note: 700 enzymes are known to use the coenzyme NADH**).

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.

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 a 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.

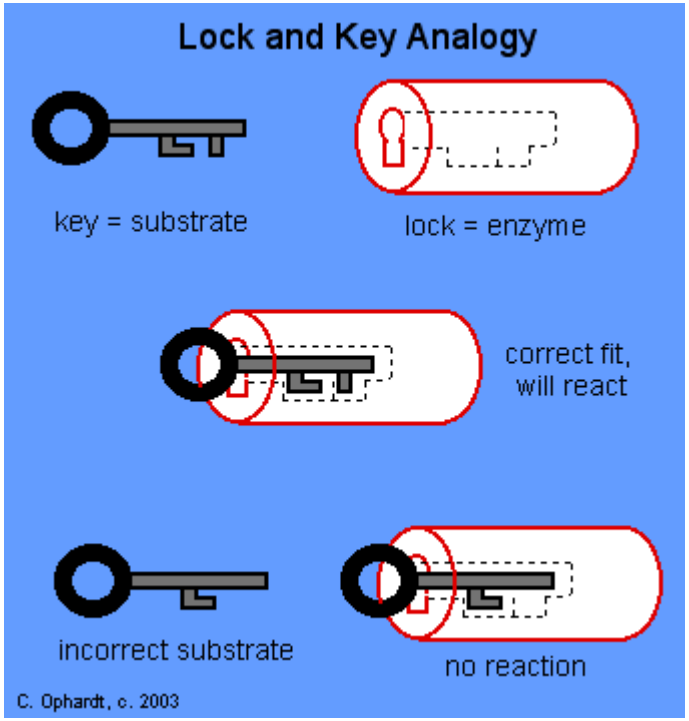
The latest systematic nomenclature system known as the **International Enzyme Commission (IEC)** system is based upon the type of reaction catalyzed.

Specificity

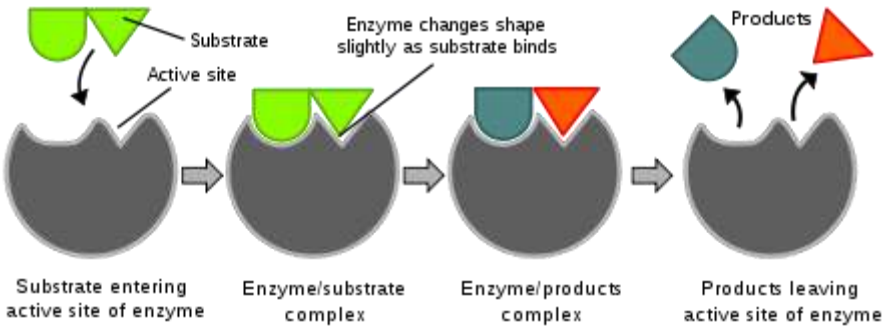
Enzymes are usually very specific as to which reactions they catalyze and the substrates that are involved in these reactions. Complementary shape, charge and hydrophilic/hydrophobic characteristics of enzymes and substrates are responsible for this specificity. Enzymes can also show impressive levels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

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. 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



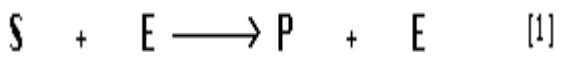
Diagrams to show the induced fit hypothesis of enzyme action.

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 are determined.

Enzyme Kinetics: Basic Enzyme Reactions

Enzymes are catalysts and 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

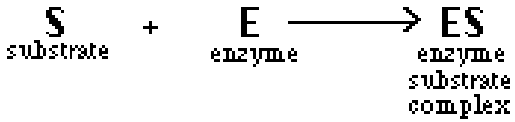


[1]

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

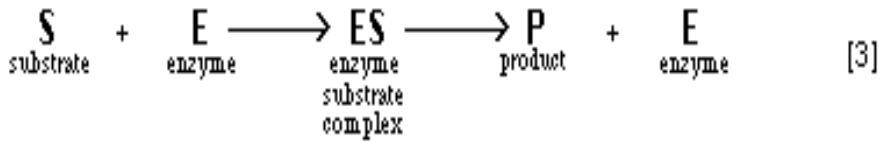
Enzyme Kinetics: The Enzyme Substrate Complex

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]

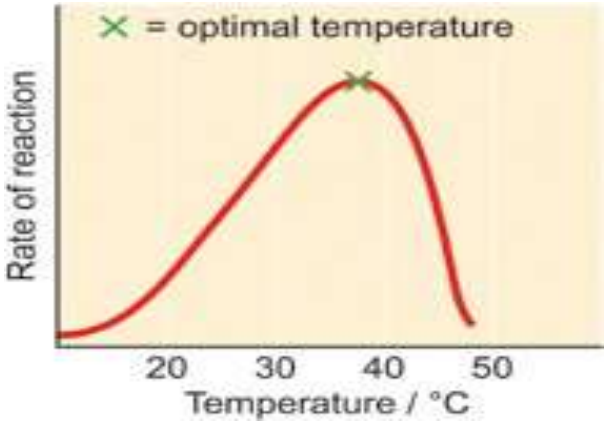
The existence of an intermediate enzyme-substrate complex has been demonstrated in the laboratory, for example, using catalase and a hydrogen peroxide derivative.

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.

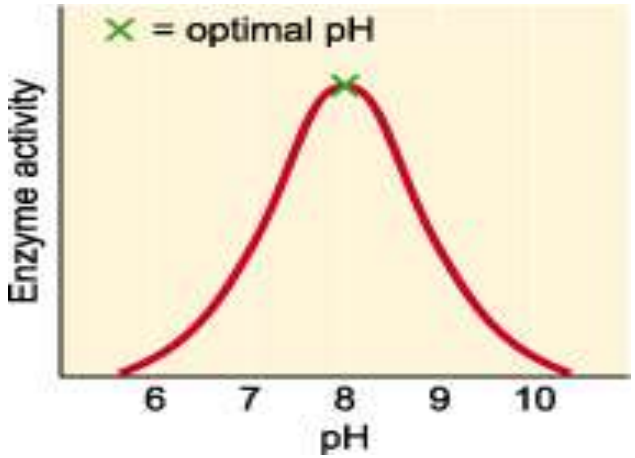
Temperature



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.

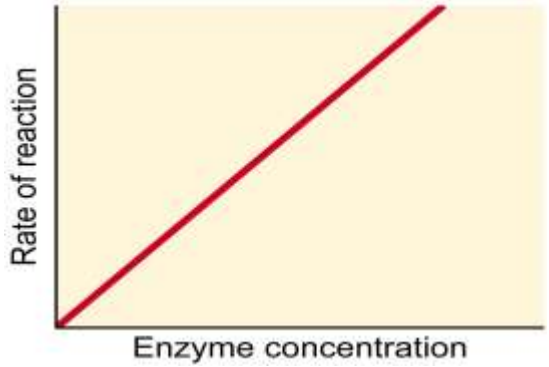
Above this temperature, the enzyme structure begins to break down (**denature**) since at higher temperatures intra- and intermolecular bonds are broken as the enzyme molecules gain even more kinetic energy.

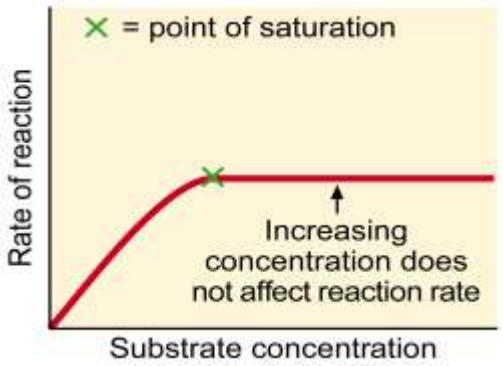
pH



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





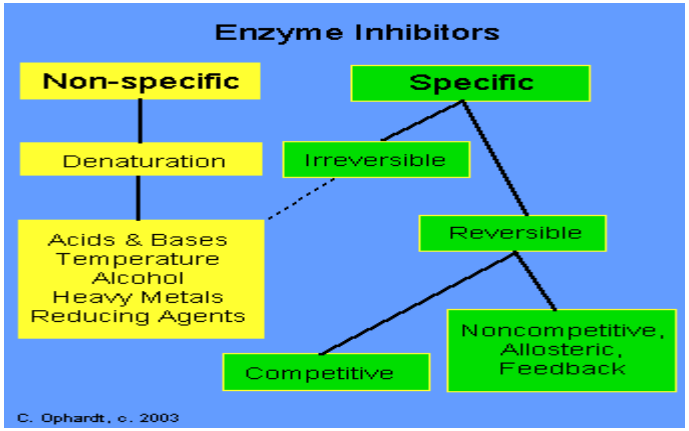
The rate of an enzyme-catalysed reaction depends on the concentrations of enzyme and substrate. As the concentration of either is increased the rate of reaction increases (see graphs).

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.



Competitive Inhibitors:

A competitive inhibitor is any compound which 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.

Non competitive Inhibitors:

A noncompetitive inhibitor is a substance that interacts with the enyzme, but usually not at the active site. The noncompetitive inhibitor reacts either remote from or very close to the active site. The net effect of a non competitive 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. Non competitive inhibitors are usually reversible, but are not influenced by concentrations of the substrate as is the case for a reversible competitive inhibitor.

Classification of Enzymes

Enzymes Are Classified into six functional Classes (EC number Classification) by the International Union of Biochemists (I.U.B.).

on the Basis of the Types of Reactions That They Catalyze

- **EC 1. Oxidoreductases**
- **EC 2. Transferases**
- **EC 3. Hydrolases**
- **EC 4. Lyases**
- **EC 5. Isomerases**
- **EC 6. Ligases**

Principle of the international classification

Each enzyme has **classification number**

consisting of four digits:

Example, **EC:** (**2.7.1.1**) **HEXOKINASE**

- **EC: (2.7.1.1) these components indicate the following groups of enzymes:**

- **2. IS CLASS (TRANSFERASE)**

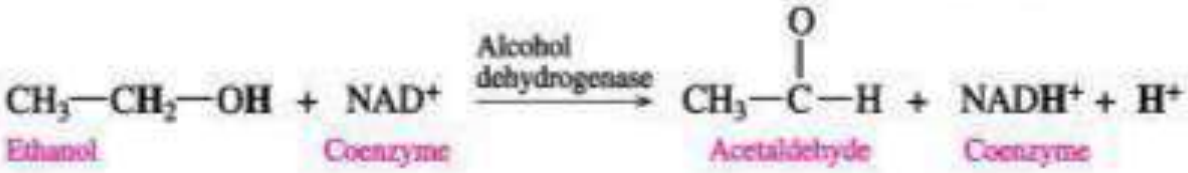
- **7. IS SUBCLASS (TRANSFER OF PHOSPHATE)**

- **1. IS SUB-SUB CLASS (ALCOHOL IS PHOSPHATE ACCEPTOR)**
- **1. SPECIFIC NAME**

  ATP,D-HEXOSE-6-PHOSPHOTRANSFERASE (Hexokinase)

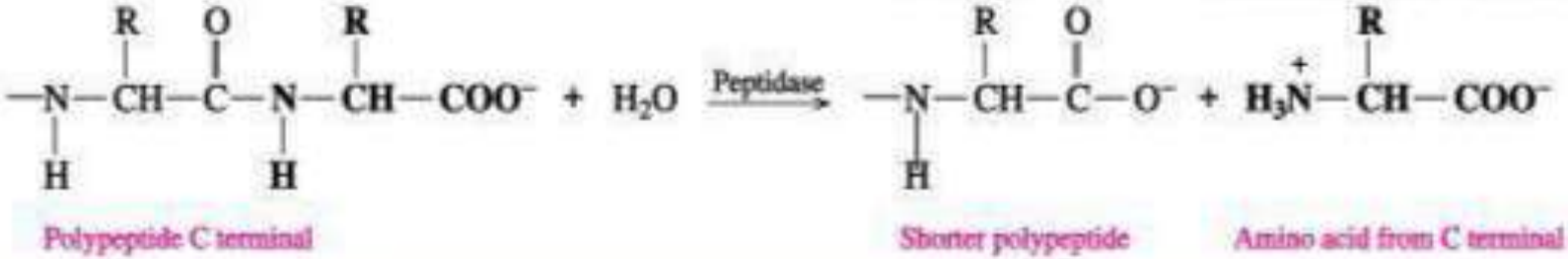
Oxidoreductases, Transferases and Hydrolases

Class	General Reactions Catalyzed	Typical Subclasses	Function
1. Oxidoreductases	Oxidation–reduction reactions	Oxidases Reductases Dehydrogenases	Oxidation Reduction Remove 2H to form double bonds



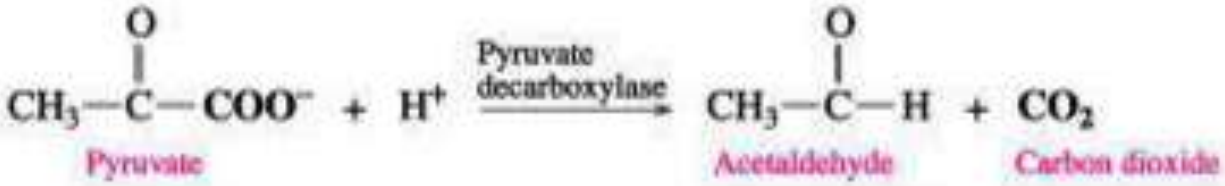
2. Transferases	Transfer of functional groups	Transaminases Kinases	Transfer amino groups Transfer phosphate groups

3. Hydrolases	Hydrolysis reactions	Peptidases Lipases Amylases	Hydrolyze peptide bonds Hydrolyze ester bonds in lipids Hydrolyze 1,4-glycosidic bonds in amylose



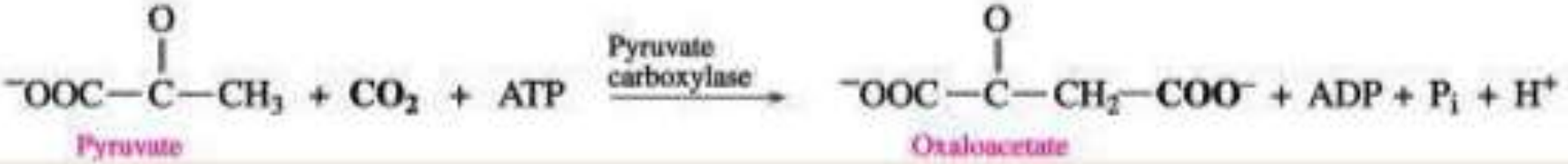
Lyases, Isomerases and Ligases

Class	General Reactions Catalyzed	Typical Subclasses	Function
4. Lyases	Addition of a group to a double bond or removal of a group from a double bond without hydrolysis or oxidation	Decarboxylases Dehydrases Deaminases	Remove CO_2 Remove H_2O Remove NH_3



5. Isomerases	Rearrangement of atoms to form isomers	Isomerases Epimerases	Convert cis and trans Convert D and L isomers

6. Ligases	Bonding of molecules using ATP energy	Synthetases Carboxylases	Combine molecules Add CO_2



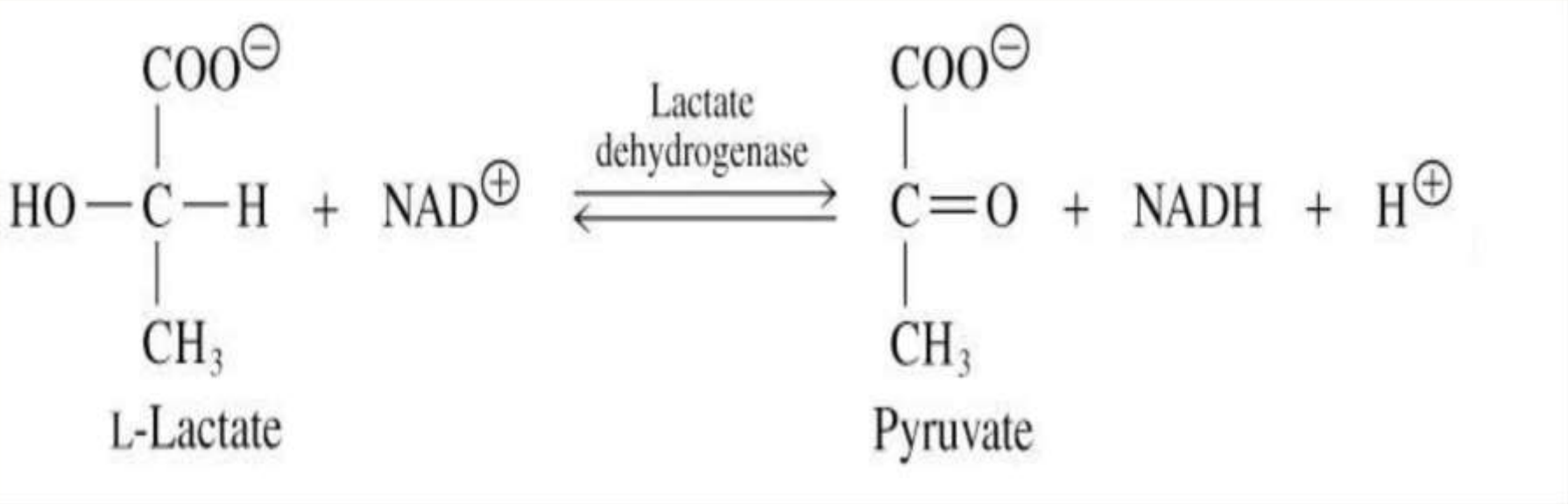
EC 1. Oxidoreductases

- **Biochemical Activity:**
  - **Catalyse Oxidation/Reduction Reactions**

    Act on many chemical groupings to add or remove hydrogen atoms.
- **Examples:**
  - **Lactate dehydrogenase.**
  - **Glucose Oxidase.**
  - **Peroxidase.**
  - **Catalase.**
  - **Phenylalanine hydroxylase.**

1. Oxidoreductases

- Catalyze oxidation-reduction reactions



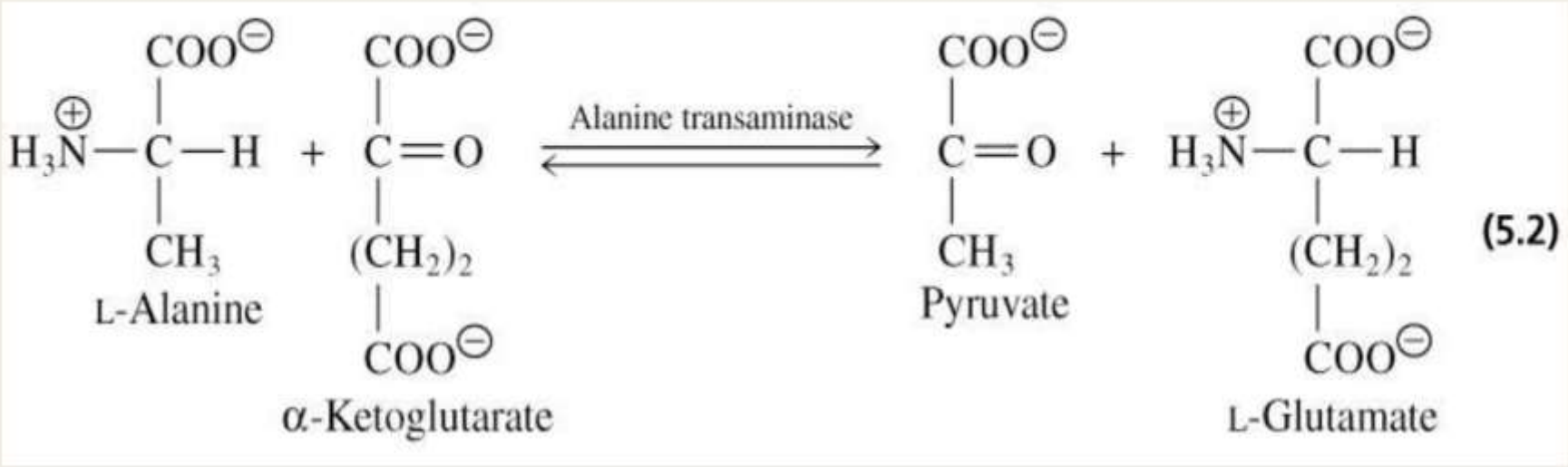
- oxidases
- peroxidases
- dehydrogenases

EC 2. Transferases

- **Biochemical Activity:**
  - **Transfer a functional groups (e.g. methyl or phosphate) between donor and acceptor molecules.**
- **Examples:**
  - **Transaminases (ALT & AST).**
  - **Phosphotransferases (Kinases).**
  - **Transmethylases.**
  - **Transpeptidases.**
  - **Transacylases.**

2. Transferases

- Catalyze group transfer reactions

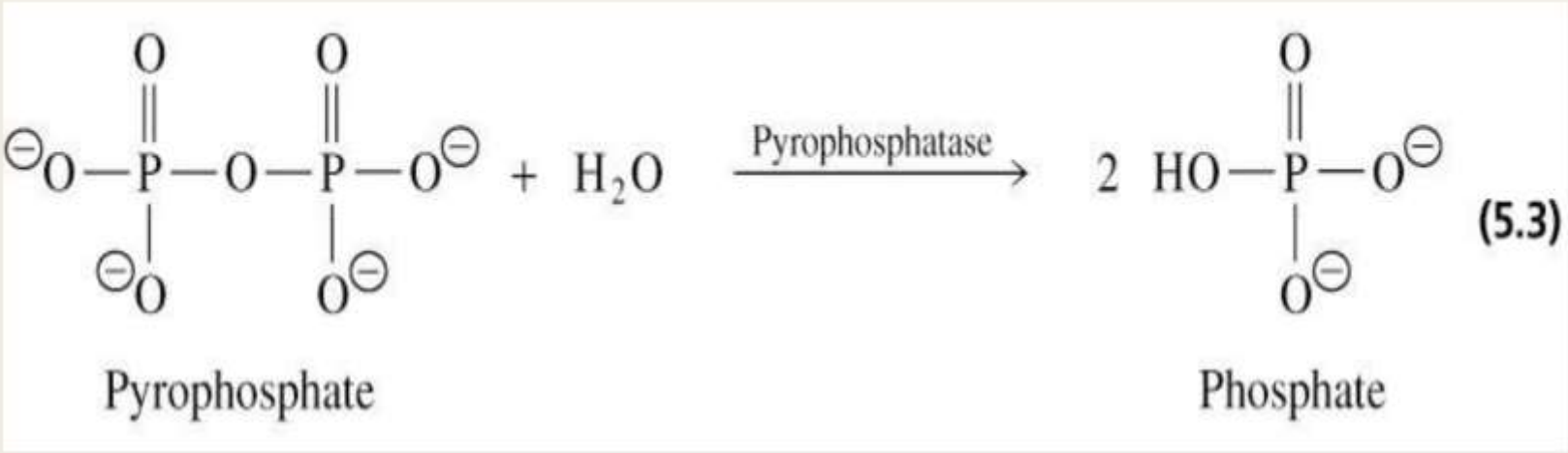


EC 3. Hydrolases

- **Biochemical Activity:**
  - **Catalyse the hydrolysis of various bonds Add water across a bond.**
- **Examples:**
  - **Protein hydrolyzing enzymes (Peptidases).**
  - **Carbohydrases (Amylase, Maltase, Lactase).**
  - **Lipid hydrolyzing enzymes (Lipase).**
  - **Deaminases.**
  - **Phosphatases.**

3. Hydrolases

- Catalyze hydrolysis reactions where water is the acceptor of the transferred group



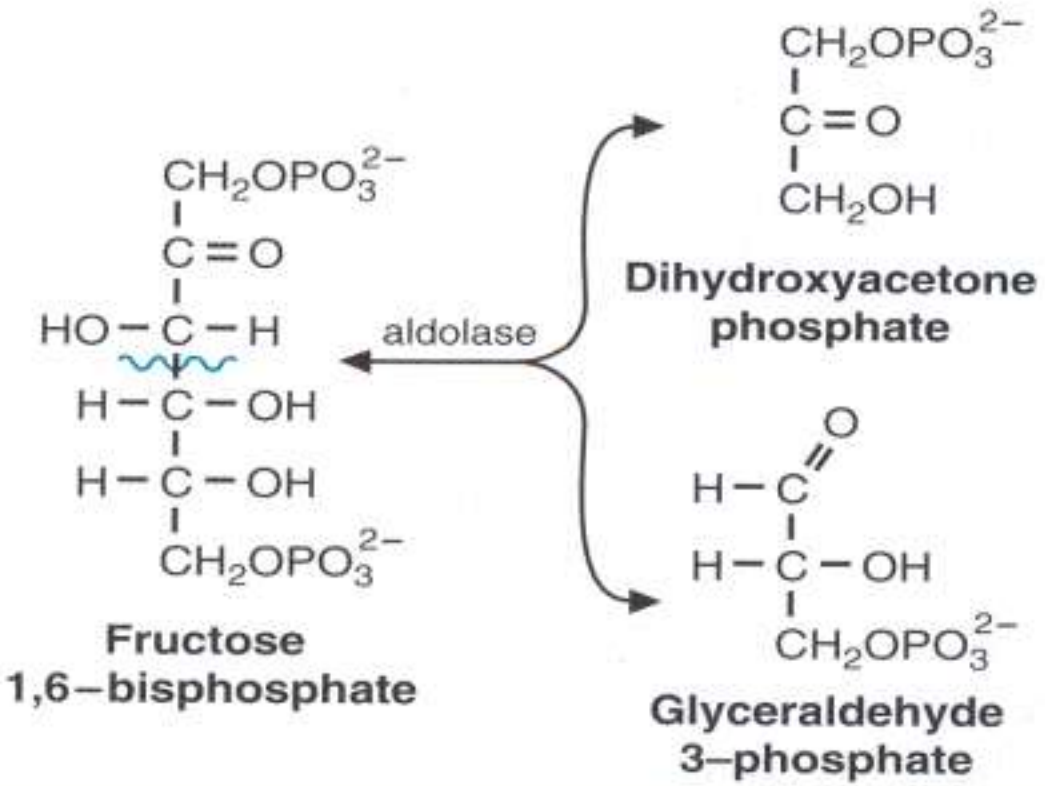
(5.3)

- esterases
- peptidases
- glycosidases

EC 4. Lyases

- **Biochemical Activity:**
  - **Cleave various bonds by means other than hydrolysis and oxidation.**
  - **Add Water, Ammonia or Carbon dioxide across double bonds, or remove these elements to produce double bonds.**
- **Examples:**
  - **Fumarase.**
  - **Carbonic anhydrase.**

4. Lyases

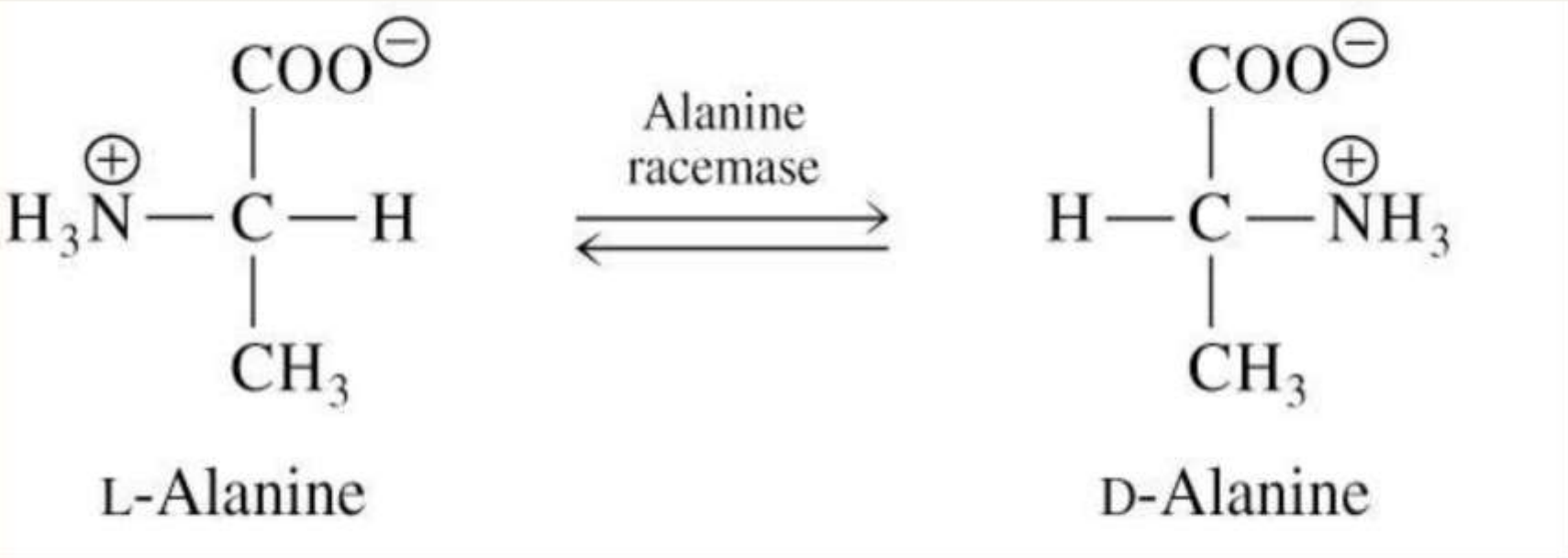

CH2OPO3 2−
C=O
HO−C−H
H−C−OH
H−C−OH
CH2OPO3 2−
Fructose
1,6−bisphosphate
aldolase
CH2OPO3 2−
C=O
CH2OH
Dihydroxyacetone
phosphate
O
H−C
H−C−OH
CH2OPO3 2−
Glyceraldehyde
3−phosphate

EC 5. Isomerases

- **Biochemical Activity:**
  - **Catalyse isomerization changes within a single molecule.**
  - **Carry out many kinds of isomerization:**
    - **L to D isomerizations.**
    - **Mutase reactions (Shifts of chemical groups).**
- **Examples:**
  - **Isomerase.**
  - **Mutase.**

5. Isomerases

- Catalyze isomerization reactions



EC 6. Ligases

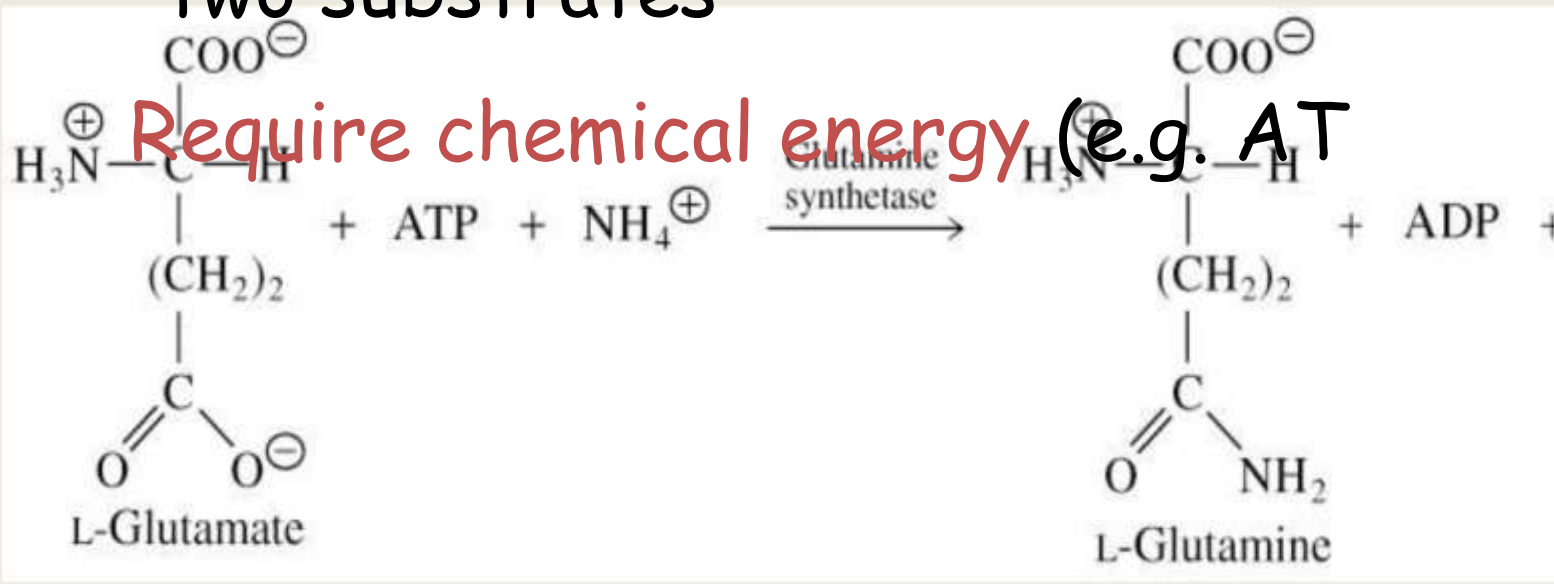
- **Biochemical Activity:**
  - **Join two molecules with covalent bonds**

    Catalyse reactions in which two chemical groups are joined (or ligated) with the use of energy from ATP.
- **Examples:**
  - **Acetyl~CoA Carboxylase.**
  - **Glutamine synthetase**

6. Ligases (synthetases)

- Catalyze ligation, or joining of two substrates

Require chemical energy (e.g. AT



Enzymatic Component of Antioxidants System

Superoxide Dismutase (SOD-E.C.1.15.1.1)

SODs are the representative of metalloproteins that catalyze the dismutation of superoxide radicle into O_2 and H_2O_2 under stress conditions; hence regarded as the first line of defense. Based upon specific location and affinity to bind with metal cofactor, they are typically classified as Fe-SOD (chloroplast), CuZn-SOD (plastid and cytosol) and Mn-SOD (mitochondria) isoform. The elevated level of SOD slows the rate of conversion of superoxide radicle (O_2^-) into caustic hydroxyl radicle

Catalase (CAT-E.C.1.11.1.6)

CAT is first identified and characterized encoded by the nuclear gene. It is a heme-containing tetrameric protein responsible for the cellular level of H_2O_2 into O_2 and H_2O by dismutation reaction especially in peroxisome and glycosomes due to the presence of oxidase enzyme. These cellular compartments are the main hub to carry out energetic-metabolic pathways (photorespiration and β-oxidation of fatty acids) and generate H_2O_2a higher rate. High turnover rate and not essentiality of reducing elements represented CAT as an effective detoxifying agent of H_2O_2 in an energy-efficient way.

Ascorbate Peroxidase (APX-E.C.1.1.11.1)

APX is also a heme-containing enzyme and exists in different isoforms based mainly depend upon location *viz*; sAPX (stroma of chloroplasts), tAPX (thylakoid), gmAPX (membrane of glyoxisome), cAPX (cytosol). APX catalyzes the reduction of hydrogen peroxide into water molecules using ascorbate as a reducing agent; the first step of the Ascorbate-Glutathione cycle. CAT and APX act simultaneously in different locations of the cellular compartment, but widely distributed APX are considered as a more efficient scavenger in stress conditions than CAT due to high affinity toward H_2O_2.

Dehydroascorbate Reductase (DHAR-EC.1.8.5.1)

DHAR, chemically a monomeric enzyme with thiol in side-chain reduces dehydroascorbate to regenerates ascorbate. The reduction reaction is initiated by accepting an electron from reduced glutathione as a reducing substrate. Oxidation-reduction of redox biology facilitates the excessive accumulation of ascorbate in the apoplast and symplast of cells, consequently provide stress tolerance by maintaining redox homeostasis.

Monodehydroascorbate Reductase (MDHAR-E.C.1.6.5.4)

Flavin adenine dinucleotide is a major constituent of enzyme MDHAR usually dispersed in the chloroplast as well as cytosol. These enzymes are highly specific for monodehydroascorbate (MDHA) and catalyze reversible reaction to regenerate ascorbate using NADPH as the electron donor to increase the pool size of ascorbate. Direct and indirect, MDHAR interconnection with APX is important in scavenging of H_2O_2 along with AsA level and thus maintaining redox state under oxidative stress.

Guaiacol Peroxidase (GPX-E.C.1.11.1.7)

GPX is a heme-containing monomeric enzyme located at intracellular or/and extracellular to restrict H_2O_2 formation. It assists many processes such as cell wall lignification, ethylene biosynthesis and also helps in wound healing in the plant; hence regarded as a "Stress Enzyme". In such a process, GPX exploits H_2O_2 to oxidize the substrate by using guaiacol/pyrogallol as a reducing substrate and consume a left-over portion of peroxide in a constructive manner.

Glutathione Reductase (GR-E.C.1.6.4.2)

GR belongs to the class oxidoreductase that transfers electron transfers electrons from NADPH to glutathione disulfide (GSSG) to generate reduced glutathione (GSH). In a series of the reaction catalyzed by MDHAR, DHAR and APX, GSH is

paid to remove hydrogen peroxide. In a plant cell, a high ratio of GSH/GSSG is crucial for providing tolerance under stress. The other important enzymatic antioxidants include Glutathione S-Transferases (GST), Methionine Sulfoxide Reductase (MSR), Glutaredoxin (GRX), Thioredoxin (TRX) and Peroxiredoxins (PRXs).

Industrial use of enzymes

Enzymes are used in the food, agricultural, cosmetic, and pharmaceutical industries to control and speed up reactions in order to quickly and accurately obtain a valuable final product**.** Enzymes are crucial to making cheese, brewing beer, baking bread, extracting fruit juice, tanning leather, and much more. The industrial uses of enzymes are also increasing since they are being used in the production of biofuels and biopolymers. The enzymes can be harvested from microbial sources or can be made synthetically. Yeast and E. coli are commonly engineered to overexpress an enzyme of interest. This type of enzyme engineering is a powerful way to obtain large amounts of enzyme for biocatalysis in order to replace traditional chemical processes.

Industrial uses of enzymes: Examples

Breweries wouldn't be able to brew our beer without enzymes and the yeast that contain them. One of the first steps of the brewing process involves sprouting grain and breaking that starch into maltose and glucose sugar molecules via amylase enzymes. Yeast then consume these simple sugars and produce alcohol and carbon dioxide via glycolysis and alcoholic fermentation. These processes together require a whopping 12 enzymes! Using the whole yeast organism is much more efficient that trying to recreate this process with synthetic enzymes**.** The alcoholic fermentation process takes two pyruvate molecules from glycolysis and converts them to ethanol via pyruvate dehydrogenase and alcohol dehydrogenase. The production of cheese follows a similar process, but instead requires bacteria to perform glycolysis to convert the sugars in milk to the lactic acid that gives cheese and yogurt its exceptional flavor.

Enzymes are transforming the non-food industrial sectors to improve processes and decrease energy usage. For example acrylamide is made from acrylonitrile using nitrile hydratase. The organism *Rhodococcus rhodochrous* J1 was directed to overexpress the enzyme nitrile hydratase. This enzyme efficiently converts acrylonitrile into acrylamide under mild conditions and offers an improvement over more traditional techniques.

The conventional method of producing glycolic acid involved reacing formaldehyde with carbon monoxide over an acid catalyst at high temperature and pressure. Enzymes have offered a more mild alternative. E. Coli can be made to overexpress nitrilase, which, when combined with other enzymes such as lactoaldehyde reductase and lactoaldehyde dehydrogenase in a chain reaction provides an easier method for glycolic acid production.

Other industrial application of enzymes in industry

Other industrial application of enzymes in industry include lipase, polyphenol oxidases, lignin peroxidase, horseradish peroxidase, amylase, nitrite reductase**,** and urease. Many of these enzymes are used for biosensors because of the specific affinity between a substrate and its enzyme. Others, such as horseradish peroxidase, are used for chemical detection of biomarkers in tissue.

Of course, we can't overlook the importance of enzymes in the food industry. Purified enzymes are essential for brewing beer, baking bread, making cheese, and extracting fruit juice. Cheesemaking is an age old tradition that requires surprisingly few ingredients: milk, bacteria, rennet, and salt. The bacterial culture is the source of flavor and texture. Rennet is an enzyme that breaks down the milk protein casein to form the cheese curd. The enzyme is naturally found in the stomach's of milk drinking/producing animals, but fermentation-produced chymosin is sourced from plant, fungal, and microbial sources for industrial cheese-making purposes.

The quest for green technology is driving innovation in both the production of specific enzymes and in the use of enzymes already available. Whether it is in the form of using enzymes to make a new use of an old renewable energy source, or simply eliminating the need for extreme temperatures and pressures to synthesize a product, enzymes are an increasingly important component of green energy technologies. Our ability to create designer enzymes will push these molecules to the forefront of many industrial processes including food, drugs, cosmetics, plastics, and much more in the immediate future.

Plant Hormones

Plant growth regulators

Plant growth rgulators are defined as small, simple chemicals produced naturally by plants to regulate their growth and development.

Characteristics

Plant growth regulators can be of a diverse chemical composition such as gases (ethylene), terpenes (gibberellic acid) or carotenoid derivates (abscisic acid). They are also referred to as plant growth substances, phytohormones or plant hormones. Based on their action, they are broadly classified as follows:

- **Plant Growth Promoters** – They promote cell division, cell enlargement, flowering, fruiting and seed formation. Examples are auxins, gibberellins and cytokinins.
- Plant Growth Inhibitors – These chemicals inhibit growth and promote dormancy and abscission in plants. An example is an abscisic acid.

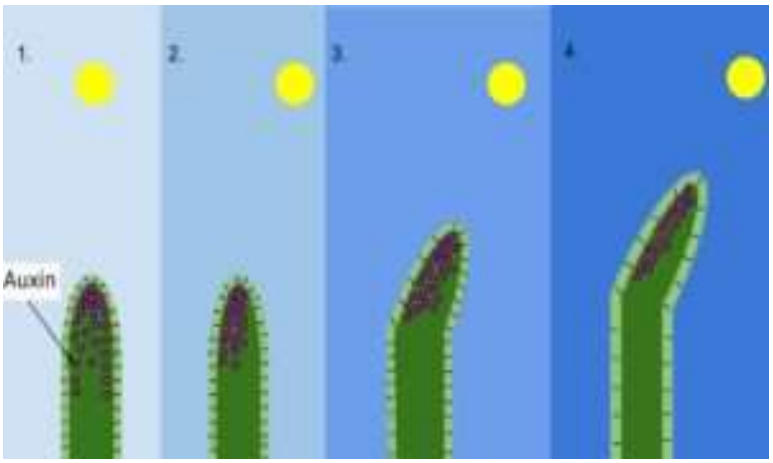
Note: Ethylene can be a promoter or an inhibitor, but is largely a Plant growth inhibitor.

All plant growth regulators were discovered accidentally. Let’s take a detailed look at each regulator and learn about it more closely:

Auxins

Discovery

Auxins were the first growth hormone to be discovered. They were discovered due to the observations of Charles Darwin and his son, Francis Darwin. The Darwins observed that the coleoptile (protective sheath) in canary grass grows and bends towards the source of light. This phenomenon is 'phototropism'. In addition, their experiments showed that the coleoptile tip was the site responsible for the bending. Finally, this led to the isolation of the first auxin by F. W. Went from the coleoptile tip of oat seedlings.



Types

First isolated from human urine, auxin is a term applied to natural and synthetic compounds that have growth regulating properties. Plants produce natural auxins such as Indole-3-acetic acid (IAA) and Indole butyric acid (IBA). Natural auxins are found in growing stems and roots from where they migrate to their site of action. Naphthalene acetic acid (NAA) and 2, 4-dichlorophenoxyacetic (2, 4-D) are examples of synthetic auxins.

Effects

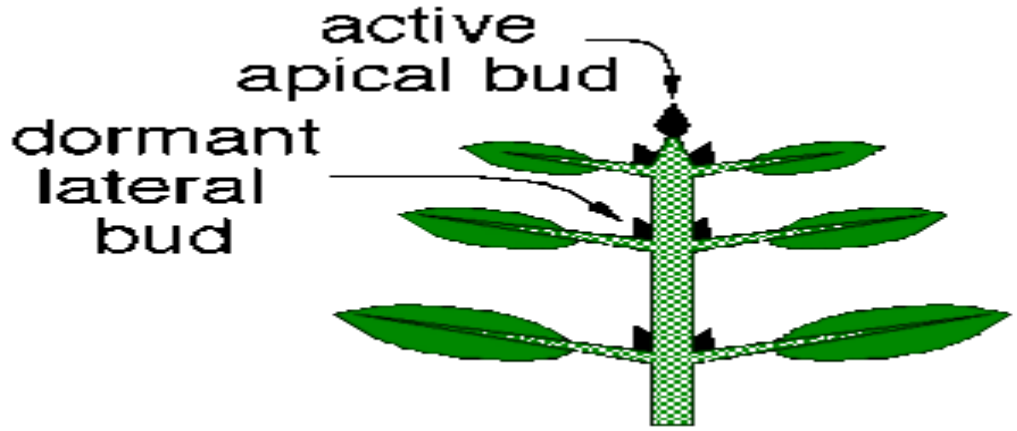
- Promote flowering in plants like pineapple.
- Help to initiate rooting in stem cuttings.

- Prevent dropping of fruits and leaves too early.
- Promote natural detachment (abscission) of older leaves and fruits.
- Control xylem differentiation and help in cell division.

Applications

- Used for plant propagation.
- To induce parthenocarpy i.e. the production of fruit without prior fertilization.
- 2, 4-D is widely used as a herbicide to kill dicotyledonous weeds.
- Used by gardeners to keep lawns weed-free.

Note: The growing apical bud in higher plants inhibits the growth of the lateral buds. This phenomenon is '**Apical Dominance**'. Removal of the apical bud allows the lateral buds to grow. This technique is commonly used in tea plantations and hedge-making.



Gibberellins

Discovery

It is the component responsible for the 'bakane' disease of rice seedlings. The disease is caused by the fungal pathogen *Gibberella fujikuroi*. E. Kurosawa treated uninfected rice seedlings with sterile filtrates of the fungus and reported the appearance of disease symptoms. Finally, the active substance causing the disease was identified as gibberellic acid.

Types

There exist more than 100 gibberellins obtained from a variety of organisms from fungi to higher plants. They are all acidic and are denoted as follows – GA_1, GA_2, GA_3 etc. GA_3 (Gibberellic acid) is the most noteworthy since it was the first to be discovered and is the most studied.

Effects

- Increase the axis length in plants such as grape stalks.
- Delay senescence (i.e. ageing) in fruits. As a result, their market period is extended.
- Help fruits like apples to elongate and improve their shape.

Applications

- The brewing industry uses GA_3 to speed the malting process.
- Spraying gibberellins increase sugarcane yield by lengthening the stem.
- Used to hasten the maturity period in young conifers and promote early seed production.
- Help to promote bolting (i.e. sudden growth of a plant just before flowering) in cabbages and beet.

Cytokinins

Discovery

F. Skoog and his co-workers observed a mass of cells called ‘callus’ in tobacco plants. These cells proliferated only when the nutrient medium contained auxins along with yeast extract or extracts of vascular tissue. Skoog and Miller later identified the active substance responsible for proliferation and called it kinetin.

Types

Cytokinins were discovered as kinetin. Kinetin does not occur naturally but scientists later discovered several natural (example – zeatin) and synthetic cytokinins. Natural cytokinins exist in root apices and developing shoot buds – areas where rapid cell division takes place.

Effects

- Help in the formation of new leaves and chloroplast.
- Promote lateral shoot growth and adventitious shoot formation.
- Help overcome apical dominance.
- Promote nutrient mobilisation which in turn helps delay leaf senescence.

Abscisic Acid

Three independent researchers reported the purification and characterization of three different inhibitors – Inhibitor B, Abscission II and Dormin. Later, it was found that all three inhibitors were chemically identical and were, therefore, together were given the name abscisic acid. Abscisic acid mostly acts as an antagonist to Gibberellic acid.

Effects

- Regulate abscission and dormancy.
- Inhibit plant growth, metabolism and seed germination.
- Stimulates closure of stomata in the epidermis.
- It increases the tolerance of plants to different kinds of stress and is, therefore, called ‘stress hormone’.
- Important for seed development and maturation.
- It induces dormancy in seeds and helps them withstand desiccation and other unfavourable growth factors.

Ethylene

Discovery

A group of cousins showed that a gaseous substance released from ripe oranges hastens the ripening of unripe oranges. Consequently, they found that the substance was ethylene – a simple gaseous Plant Growth Regulator. Ripening fruits and tissues undergoing senescence produce ethylene in large amounts.

Effects

- Affects horizontal growth of seedlings and swelling of the axis in dicot seedlings.
- Promotes abscission and senescence, especially of leaves and flowers.
- Enhances respiration rate during ripening of fruits. This phenomenon is 'respiratory climactic'.
- Increases root growth and root hair formation, therefore helping plants to increase their absorption surface area.

Application

Ethylene regulates many physiological processes and is, therefore, widely used in agriculture. The most commonly used source of ethylene is Ethephon. Plants can easily absorb and transport an aqueous solution of ethephon and release ethylene slowly.

- Used to break seed and bud dormancy and initiate germination in peanut seeds.
- To promote sprouting of potato tubers.
- Used to boost rapid petiole elongation in deep water rice plants.
- To initiate flowering and synchronising fruit-set in pineapples.
- To induce flowering in mango.

- Ethephon hastens fruit ripening in apples and tomatoes and increases yield by promoting female flowering in cucumbers. It also accelerates abscission in cherry, walnut and cotton.

Practical Part

1- Determination of sucrose using invertase.

2- Determination of starch using diastase.

3- Effect of substrate concentration on enzyme Activity.

4- Effect of enzyme concentration on enzyme Activity.

5- Effect of temperature on enzyme activity.

6- Effect of pH on enzyme activity.

7- Determination of SOD activity.

8- Determination of CAT activity.

9- Determination of POD activity.

10- Determination of APX activity.

11- Determination of GR activity.

12- Effect of auxin on seed geremination.

13- Effect of Gibberellin on seed germination.

14- Effect of cytokinin on seed germination.

15- Effect of Abscisic Acid on seed germination.

16- Effect of ethylene on seed germination.