PLANT BIOCHEMISTRY AND PHYSIOLOGY

Course content

i) Metabolism and Biosynthesis of Carbohydrates ✓
ii) Lipids *
iii) Amino acid and proteins ✓
iv) Nitrogen cycle ✓
v) Assimilation of organic & inorganic nitrogen by plants
CAT 1
vi) Nature and function of enzymes ✓
vii) Coenzymes and vitamins.
viii) Plant organic acids
ix) Respiration — Molecular Biology
x) Photosynthesis ✓ — Molecular cell Biology
xi) Exams

Metabolism & Biosynthesis of Carbohydrates

- Glucose is the major form of sugar moiety present in blood and most of the body fluids.
- The digestion of food carbohydrates such as starch, sucrose and lactose produces the monosaccharides, fructose and galactose, which pass into bloodstream.
- The study of synthesis (Anabolism) and degradation (catabolism) of biomolecules in biochemistry is termed as (Metabolism)

Glycolysis => A series of catabolic reactions in which one molecule of glucose (6 carbons) is split into 2 molecules of pyruvate (3 carbons).

Synthesis (Degradation)
- Glucose is the most important carbohydrate.
- Monosaccharides, glucose & galactose are absorbed in liver.
- All monosaccharides are completely absorbed in the small intestines
- Glucose in the circulating blood and tissue fluids is drawn upon by all the cells of the body used for energy production
- Carbohydrates provide half of energy required by the body.

Glycogenesis => Anabolic conversion of glucose to glycogen.
Glycogenolysis => Catabolic conversion of glycogen to glucose
Gluconeogenesis => Anabolic conversion of non-carbohydrate precursors to glucose.
Pentose phosphate pathway => Pathway for conversion of glucose 6-phosphate to ribose 5-phosphate

Carbohydrates as a source of energy (v) Help control blood glucose & insulin metabolism

i) Serve as fuel and get oxidized to provide energy for other metabolic processes.
ii) Metabolic intermediates are used in biosynthesis reactions
iii) A major part of dietary glucose is converted to glycogen for storage in liver.

* Glucose is degraded in the cell by way of a series of phosphorylated intermediates in a pathway; (i) Glycolysis
v) Participate in cholesterol & triglyceride metabolism — tricarboxylic Acid cycle
vi) Help in Fermentation

(i) Glycolysis
- Is the first step in glucose breakdown
- It serves as the foundation for both aerobic & anaerobic cellular respiration.
- Glucose is converted into

Pyruvate.

- Glucose is a six-membered ring molecule found in the blood and is usually a result of the breakdown of carbohydrates into sugars.
- It enters cell through specific transporter proteins that move it from outside the cell into the cell's cytosol.
- All of the glycolytic enzymes are found in the cytosol (liquid medium contained within a cell.

$C_6H_{12}O_6 + 2NAD^+ + 2ADP + 2P_i$

glucose; Nicotinamide Adenine dinucleotide; Adenosine Diphosphate; phosphate

↓

2 pyruvic acid (CH_3)

$2(C_3H_4O_3) + 2H_2O + 2ATP + 2NADH + 2H^+$

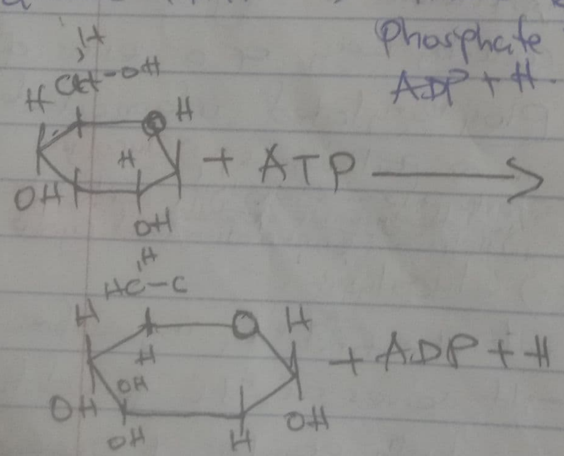
Pyruvate; water; Adenosine Triphosphate; hydrogen ions

Glycolytic steps

Step ONE; Hexokinase.

- Is the first step in glycolysis is the conversion of D-glucose into glucose-6-phosphate (G6P) => by adding a phosphate group.
- The enzyme that catalyses this reaction is hexokinase.

Glucose + ATP ⟶ Glucose-6 Phosphate + ADP + H.



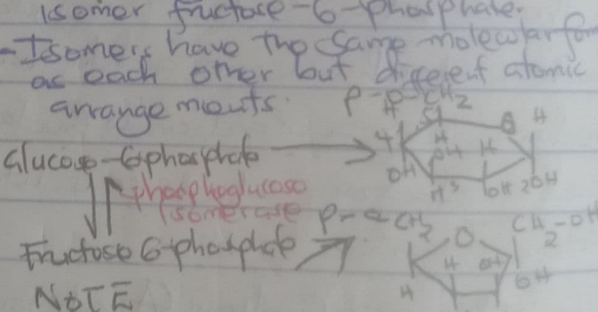
P = phosphate group $-\overset{O}{\underset{O^-}{P}}-O^-$

NOTE — Glucose ring is phosphorylated

- Phosphorylation is the addition of phosphate group to a molecule derived from ATP
- As a result, in glycolysis, 1 molecule of ATP has been consumed.
- Hexokinase catalyzes the phosphorylation of many six membered glucose-like ring structures.
- Atomic Mg is used to shield the negative ions produced from the phosphate group on the ATP molecules.

STEP 2 Phosphoglucose Isomerase

- Rearrangement of G6P into F6P by glucose phosphate isomerase (Phosphoglucose isomerase).
- This enzyme isomerizes G6P into its isomer fructose-6-phosphate.
- Isomers have the same molecular formula as each other but different atomic arrangements.



NOTE

=> It involves the conversion of G6P to F6P

- It occurs with the help of the enzyme phosphoglucose isomerase PI
- It involves isomerisation reaction.

- It involve rearrangement of Carbon–Oxygen bond to transform the six-membered ring into a 5-membered ring

Fructose-6 phosphate (phosphorylation of Fructose-6 phosphate)

step 3: phosphofructokinase

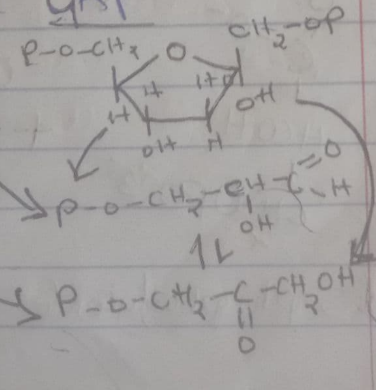
- uses Mg as a cofactor, changes fructose-6 phosphate into fructose 1,6-biphosphate.
- A second molecule of ATP provides a phosphate group that is added to the F6P molecule.
- Mg atom is involved in to help to shield negative charges
- 2 ATP molecules have been used so far.

Fructose-6 phosphate —(phosphofructokinase)→ Fructose-1,6-biphosphate + ADP.

step 4: Aldolase.

- Aldolase splits fructose 1,6 biphosphate (FBP) into 2 sugars that are isomers of each other.
- These 2 sugars are dihydroxyacetone phosphate (DHAP) and glyceraldehyde 3-phosphate GAP.

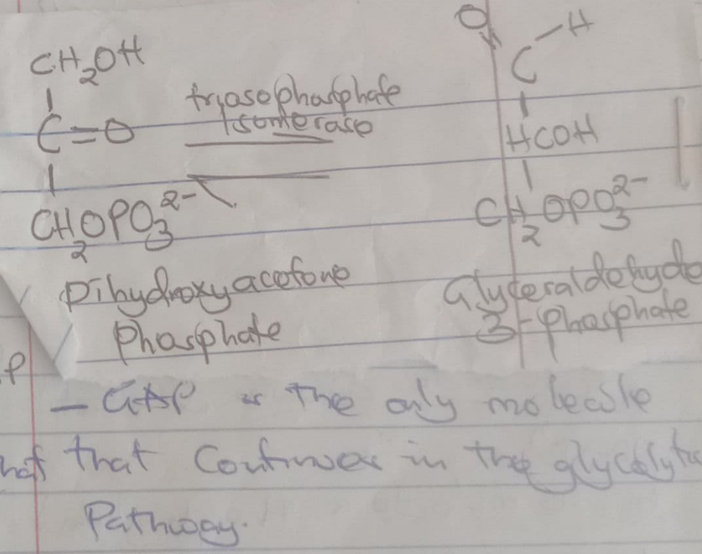
Fructose 1,6 Biphosphate ⇌ Glyceraldehyde-3-phosphate + Dihydroxyacetone phosphate



- This step utilizes the enzyme aldolase, which catalyses the cleavage of FBP to yield two 3-carbon molecules.

step 5; Triosephosphate Isomerase

- Enzyme Triosephosphate Isomerase rapidly inter-converts the molecules dihydroxyacetone phosphate DHAP into GAP.
- GAP is removed/used in the next step of glycolysis



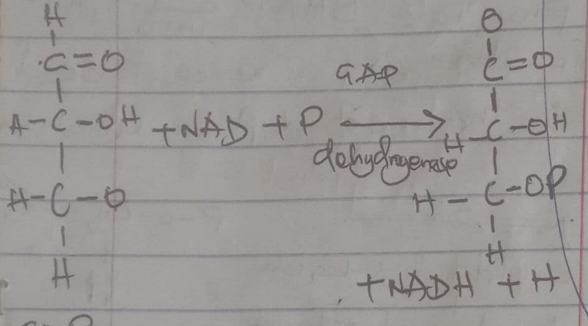
- GAP is the only molecule that continues in the glycolytic pathway.
- As a result, all the DHAP molecules produced are further acted on by the enzyme Triosephosphate isomerase (TIM), which re-organizes the DHAP into GAP so it can continue in glycolysis

- At this point, in the glycolytic pathway, we have two, 3-carbon molecules but have not yet fully converted glucose into pyruvate.

(GAPDH)

Step 6; Glyceraldehyde-3-Phosphate Dehydrogenase

- G3PDH dehydrogenates and add an inorganic phosphate to glyceraldehyde-3-phosphate producing 1,3 biphosphoglycerate



GAP.

NOTE a) glyceraldehyde-3-phosphate (GAP) is oxidized by the enzyme nicotinamide adenine dinucleotide (NAD)

b) The molecule is phosphorylated by the addition of a free phosphate group.

⇒ (i) G3PDH dehydrogenates GAP by transferring one of its H^+ molecules to the oxidizing agent nicotinamide adenine dinucleotide NAD^+ to form NADH + H^+

(ii) It also adds a phosphate from the cytosol to the oxidized GAP to form 1,3 biphosphoglycerate.

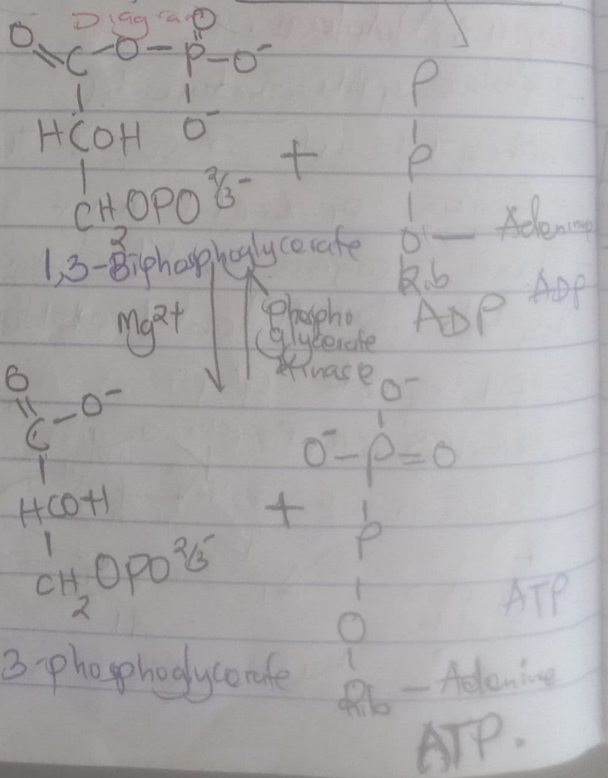
Step 7; Phosphoglycerate Kinase

- It transfers a phosphate group from 1,3 biphosphoglycerate to ADP to form ATP and phosphoglycerate.

Since there are 2 molecules of 1,3 biphosphoglycerate, 2 molecules of ATP are synthesized at this step.

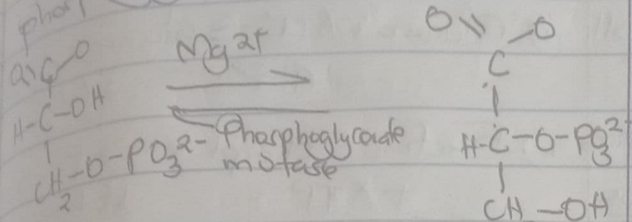
- 2 molecules of ATP already used are cancelled leaving a net of zero ATP molecules up to this stage of glycolysis.
- Mg is involved to shield the negative charges on the phosphate groups of the ATP molecule.
- The phosphate is transferred to a molecule of ADP that yields our first molecule of ATP.

Diagram



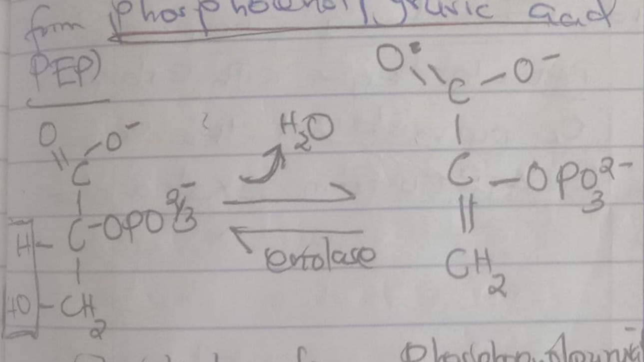
STEP 8 Phosphoglycerate Mutase
- The enzyme relocates the P from 3-phosphoglycerate from the 3rd carbon to the 2nd carbon to form 2-phosphoglycerate.

- Simple rearrangement of the position of the phosphate group on the 3 phosphoglycerate mutase (PGM)



3-phosphoglycerate   2-phosphoglycerate

step 9: Enolase - Dehydration Reaction
- Enolase removes a molecule of water from 2-phosphoglycerate to form phosphoenolpyruvic acid (PEP)



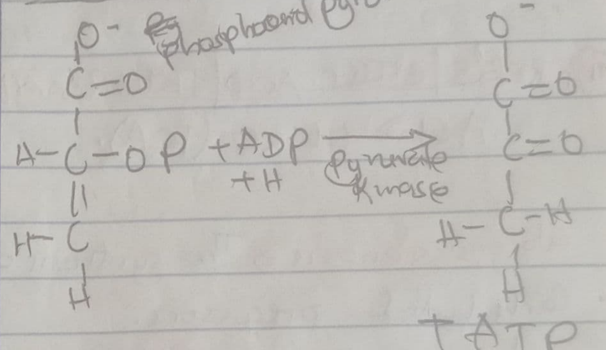
2-phosphoglycerate   phosphoenolpyruvate (PEP)

step 10: Pyruvate Kinase
- Transfers a P_i (phosphoryl group) from PEP to ADP to form pyruvic acid and produces ATP.
- PEP is converted into pyruvate with the help of the enzyme Pyruvate Kinase

- phosphate group attached to the 2 carbon of the PEP is transferred to a molecule of ADP, yielding ATP
- Since there are 2 molecules of PEP, 2 ATP molecules are generated

Question: Account for the 2 ATP molecules produced in glycolysis

diagram



Summary
step 1 & 3 = -2ATP
step 7 & 10 = +4ATP
Net Visible ATP produced = 2.

NOTE
- After glycolysis, cell must continue respiration in either aerobic or anaerobic
- A cell that can perform respiration and which finds itself in presence of O_2 will continue to the aerobic citric acid cycle in the Mitochondria

- If a cell able to perform aerobic respiration, is in a situation where there is no O_2 such as muscles under extreme exertion, it will move into a type of anaerobic respiration called homolactic fermentation
- Certain cells such as yeast are unable to carry out aerobic respiration and will automatically move into a type of anaerobic respiration called alcoholic fermentation.

(ii) KREB'S / CITRIC ACID / TRICARBOXYLIC ACID CYCLE (TCA).

Gluconeogenesis ⇒ The synthesis of non carbohydrate precursors.
- Liver is the site for gluconeogenesis
- Occurs when carbohydrates in the diet is insufficient to meet the demand in the body, with the intake of protein rich diet at the time of starvation, when tissue proteins are broken down to amino acids.

Gluconeogenesis & Glycolysis
- Are both opposing metabolic pathways and share a no of enzymes.
- In glycolysis, glucose is converted into pyruvate and in gluconeogenesis pyruvate is converted to glucose.
- However, gluconeogenesis is not exact reversal of glycolysis.

⇒ There are 3 essentially irreversible steps in glycolysis which are bypassed in gluconeogenesis

(i) Glucose + ATP → Glucose 6 phosphate + ADP (glucokinase)

ii) Fructose 6 phosphate + ATP ⇒ Fructose 1,6 biphosphate + ATP

iii) Phosphopyruvate + ATP → Pyruvate + ATP

⇒ In gluconeogenesis

Reactions of Gluconeogenesis

(i) Formation of phosphoenol (PEP) pyruvate begins with carboxylation of pyruvate at the expense of ATP to form oxaloacetate

- Oxaloacetate is converted to phosphoenolpyruvate by phosphorylation with GTP, accompanied by a simultaneous decarboxylation

(ii) Fructose-6 Phosphate is formed from fructose 1,6-diphosphate by hydrolysis and the enzyme fructose 1,6-diphosphatase catalyses the reaction.

(iii) Glucose is formed by hydrolysis of glucose 6-phosphate catalysed by glucose-6-phosphatase

Gluconeogenesis of Amino acids

- Amino acids which could be converted to glucose are called glucogenic amino acids.
- Most of the glucogenic amino acids converted to the intermediates of citric acid cycle either by transamination or deamination

Gluconeogenesis of propionate.

- Propionate is a major source of glucose in ruminants, and enters the main gluconeogenic pathway via the citric acid cycle after conversion of Succinyl CoA.

Gluconeogenesis of Glycerol.

- At the time of starvation glycerol can also undergo gluconeogenesis.
- Further metabolism of glycerol does not take place in the adipose tissue because of the lack of glycerol kinase necessary to phosphorylate it.

Importance to note about Gluconeogenesis (3 marks)

① Purpose

i) Is crucial for maintaining blood glucose level.

ii) Metabolites e.g. lactate created by muscles & RBCs and glycerol produced by adipose tissue are removed from the circulation through gluconeogenesis cycle.

iii) Hence a constant supply of glucose to the brain, red blood cells and other tissues that rely on glucose as the primary energy source

② Substrates

- Amino acids, glycerols and lactate (produced from lactase during intense exercise or anaerobic respiration.
- The amino acids supported are alanine and glutamine

③ Enzymes

- Pyruvate Carboxylase
- Phosphoenolpyruvate Carboxykinase (PECK)
- Fructose 1,6 biphosphatase
- Glucose-6-Phosphatase

⇒ These Catalyse the Conversion of substrates into glucose.

④ Regulation

- is tightly regulated to prevent wasteful of energy and ensure that glucose is synthesized when necessary
- Hormones like glucagon and cortisol promote gluconeogenesis, while insulin inhibits it.
- This hormonal regulation helps maintain blood glucose homeostasis

⑤ Location

- Takes place in the liver which is the primary organ in homeostasis

⑥ Energy cost

- Is energetically expensive process requiring input of ATP & GTP (both high-energy molecules at various steps

-

⑦ Interplay with glycolysis

- It is a reverse of Glycolysis
- It is not a perfect reversal due to several energetically unfavourable reaction in glycolysis.

Summary

Gluconeogenesis is a crucial metabolic pathway that allows the body to produce glucose from non-carbohydrate sources when dietary sources are inadequate or during periods of high demand for glucose.

- This pathway helps maintain blood glucose levels within a narrow range ensuring the proper functioning of various tissues, especially the brain, which relies heavily on glucose as an energy source.

Practical 1

Extract protein from plants

- proteins are insoluble
- Homogization
- Quantification of protein amount in sample

- use of drop spectrophotometer
- UV spectrophotometer. Can scan samples of absorb light from 200-400nm
- Infrared
- Visible region of the light spectrum.
- Subject any substance to monochromatic light, an increase of conc of that sub will increase | An increase in pathlength increases the transmittance but reduces absorbance

$A = ECL$ (L – pathlength, C – concentration)

Bar lamplard

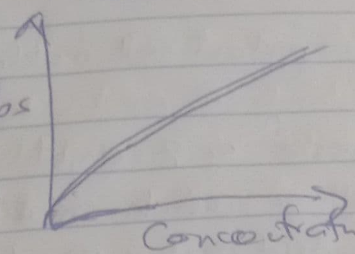
Cuvet.

Molar Absorbtivity

Pathlength

Absorbance => UV spectroscopy

Prepare standards with known concentration



Regression coefficient => Right concentration is near to 1 between 0.97 to 1

1 Afasor

$y = mx + c$ (x – concentration)

$A = ECL$

Q. 2mg/ml $C_1V_1 = C_2V_2$

$2mg/ml \times V_1 = 1mg/ml \times 4$

$2V_1 = 4$

$V_1 = 2ml$

U

2mg 10+5

Tricarboxylic Acid / Krebs or Citric acid cycle

- It is an important cell metabolic hub.
- It is composed of 8 eight enzymes, all of which are within the mitochondrial matrix except the ~~outer~~ outlier Succinate dehydrogenase, which is related to the respiratory chain on the inner mitochondrial membrane.
- The cycle serves a gateway for aerobic metabolism for molecules that can convert to an acetyl group or dicarboxylic acid.
- Regulation of TCA cycle occurs at three distinct points that include the following enzymes;
  (i) Citrate Synthesis
  (ii) Isocitrate dehydrogenase
  iii) alpha-ketoglutarate dehydrogenase.
- The cycle also plays a role in replenishing precursors for the storage form of fuels such as amino acids and cholesterol.
- The Krebs cycle does not require presence of oxygen, this element is necessary for the last stage of aerobic cellular respiration i.e oxidative phosphorylation.

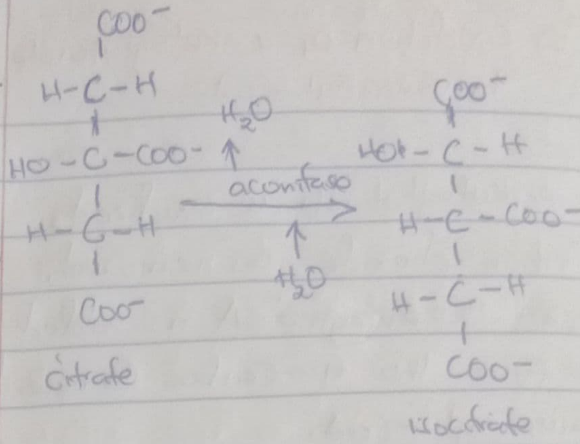
- Organic molecules endowed with energy (carbohydrates, lipids, proteins) are split in previous reactions. Before entering the Krebs cycle, they transform into acetyl-CoA, a molecule formed by an acetyl group (CH_3CO)- and by an acyl transporter called Coenzyme A.
- However, the preferred source of acetyl-CoA remains glycolysis.
- The acetyl group is then oxidized, and the energy obtained is used for the synthesis of ATP in cooperation with oxidative phosphorylation.
- In Eukaryotes, the Krebs cycle reactions take place in the mitochondrial matrix, a dense solution that surrounds the mitochondria crests; in addition to water, the matrix contains all the enzymes necessary for the biochemical reactions of the cycle, coenzymes and phosphates.
- The Krebs cycle is controlled and regulated by the availability of the NAD+ and FAD substrates, while high concentrations of NADH inhibit it.

Net Equation.

Acetyl CoA + 3NAD + FAD + ADP + HPO_4^{2-}

↓

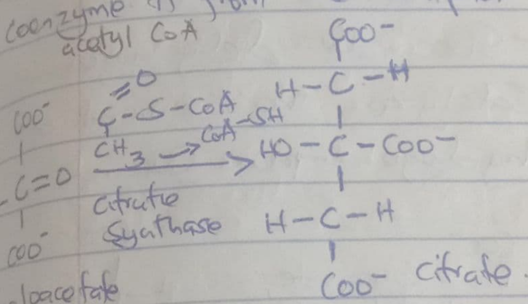
$2CO_2$ + CoA + 3NADH$^+$ + FADH$^+$ + ATP



citrate → isocitrate (aconitase)

① Formation of Citrate

- Condensation of acetyl-CoA with oxaloacetate to form citrate, catalyzed by citrate synthase.
- Water molecule attacks the acetyl leading to release of coenzyme A from the complex.



acetyl CoA + oxaloacetate → citrate (citrate synthase)

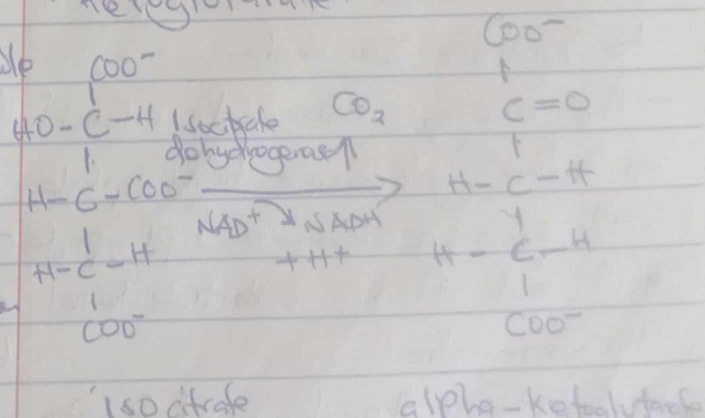
② Formation of Isocitrate

- The citrate is rearranged to form an isomeric form, Isocitrate by an enzyme acontinase.
- In this reaction, a water molecule is removed from the citric acid then put back on in another location.
- Overall effect of this conversation is that the -OH group is moved from 3' to the 4' position on the molecule.
- This transformation yields the molecule isocitrate.

③ Oxidation of Isocitrate to α-Ketoglutarate.

- Isocitrate dehydrogenase Catalyzes oxidative decarboxylation of isocitrate to form α-ketoglutarate.
- In the reaction, generation of NAD from NAD is seen.

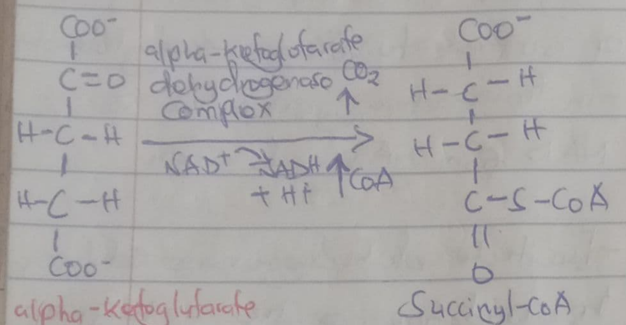
- Isocitrate dehydrogenase catalyses the oxidation of the -OH group at the 4' position of isocitrate to yield an intermediate which has a Carbon dioxide molecule removed from it to yield alpha-ketoglutarate.



Isocitrate → alpha-ketoglutarate (Isocitrate dehydrogenase; NAD^+ → NADH + H^+; CO_2)

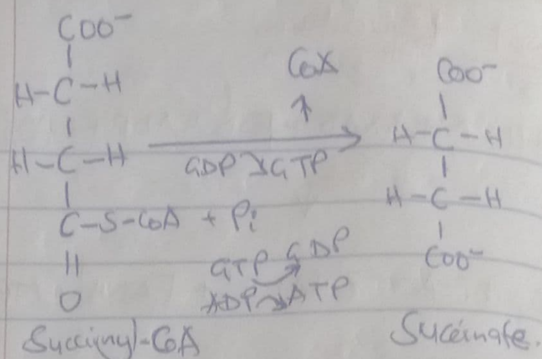
④ Oxidation of α-Ketoglutarate to Succinyl-CoA.

- α-Ketoglutarate is oxidized, Carbon dioxide is removed, and Coenzyme A is added to form the 4-Carbon Compound Succinyl-CoA.
- During This Oxidation, NAD^+ reduced to $NADH + H^+$
- The reaction is catalysed by alpha-ketoglutarate dehydrogenase.



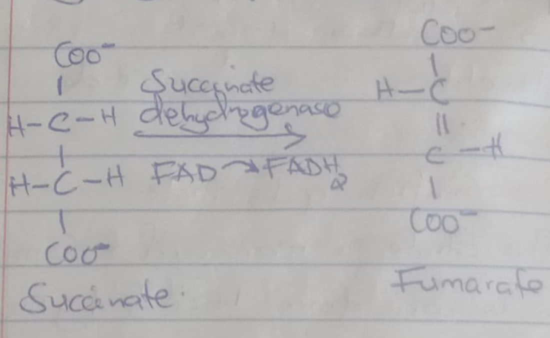
⑤ Conversion of Succinyl-CoA to Succinate.

- CoA is removed from Succinyl-CoA to produce Succinate.
- The energy released is used to make guanosine triphosphate (GTP) from guanosine diphosphate (GDP) and Pi by substrate-level Phosphorylation.
- GTP can then be used to make ATP.
- The enzyme Succinyl-CoA Synthase Catalyzes this reaction of the citric acid cycle.



⑥ Oxidation of Succinate to Fumarate.

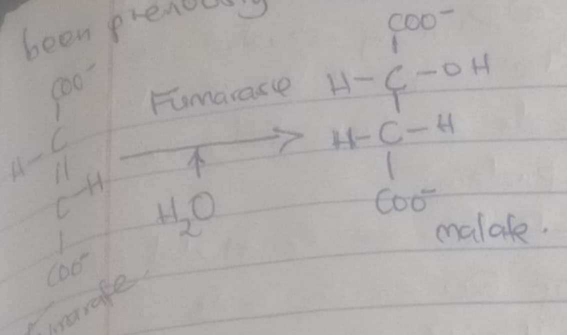
- Succinate is oxidized to fumarate.
- During this oxidation, FAD is reduced to $FADH_2$
- Succinate dehydrogenase Catalyzes the removal of two hydrogens from Succinate.



⑦ Hydration of Fumerate to Malate.

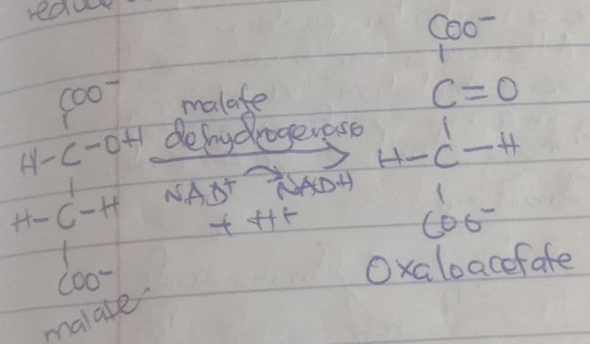
- The reversible hydration of fumarate to L-malate is Catalysed by fumarase (fumarate hydratase)
- Fumarase Continues the rearrangement process by adding H and O back into the substrate that ha

been previously removed



(8) Oxidation of Malate to oxaloacetate.

- Malate is oxidized to produce oxaloacetate, the starting compound of the citric acid cycle by malate dehydrogenase
- During this oxidation, NAD^+ is reduced to NADH+ H^+



ATP GENERATION

1. 3NAD+ = 9ATP
2. 1 FAD = 2ATP
3. 1ATP = 1ATP

12

Total ATP = 12ATP

⇒ Krebs cycle primary transforms the acetyl group and water, into carbon dioxide and energized forms of the other reactants.

Significance of TCA

1. Intermediate compounds formed during Kreb's cycle are used for the synthesis of biomolecules like amino acids, nucleotides chlorophyll, cytochromes & fats.
2. Intermediate like Succinyl CoA takes part in the formation of chlorophyll.
3. Amino acids are formed from α-Ketoglutaric acid, pyruvic acid and oxaloacetic acid
4. TCA releases plenty of energy (ATP) required for various metabolic activities of cell.
5. By this cycle, carbon skeleton are got, which are used in process of growth and for maintaining the cells.

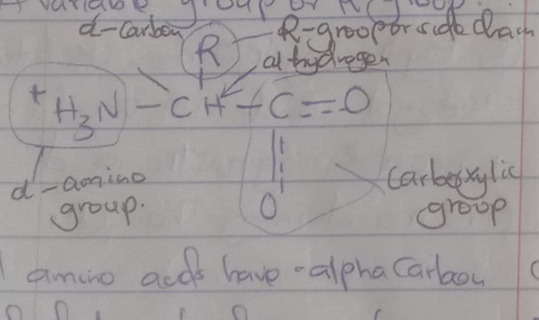
Amino Acids and Proteins

① PROTEINS

- Are biological polymers composed of amino acids linked together by peptide bonds.
- Proteins are organised into 4 levels which are based on the complexity of the chain & the fact that they contain different amino acid structures

Structure of Amino Acid

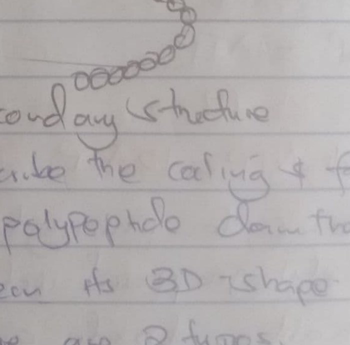
- All amino acids have the following structural properties
(i) A Carbon (the alpha carbon)
ii) A hydrogen atom (H)
iii) A carboxyl group (−COOH)
iv) An amino group (−NH_2)
⇒ Structural properties of Amino acids are;
(v) A variable group or 'R' group.



- All amino acids have alpha Carbon bonded to a hydrogen atom, Carboxyl group and amino group
- The 'R' group varies among amino acids and determines the differences between these protein monomers
- Amino acid sequence of a protein is determined by the information found in the cellular genetic code

Protein Structural Levels

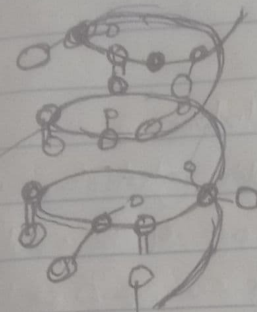
a) Primary structure ⇒ Describes the unique order in which amino acids are linked together to form protein.
- Proteins are constructed from a set of 20 amino acids.
- The 'R' groups varies among amino acids and determine the difference between protein monomers
- The amino acid sequence of a protein is determined by the information found in the cellular genetic code.
- The order of amino acids in a polypeptide chain is unique and specific to a particular protein.
- Altering a single amino acids causes a gene mutation which most results in a non-functioning protein.
Primary structure of a Protein (Drawing)



(b) Secondary structure
- Describe the coiling & folding of a polypeptide chain that gives protein its 3D shape
- There are 2 types;
(i) Alpha (α) helix structure ⇒ In an alpha helix, the protein chain is coiled like a loosely coiled spring

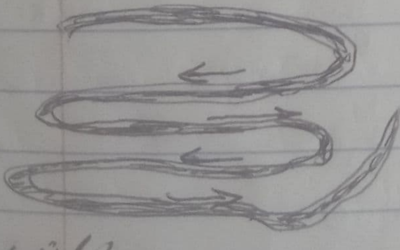
Alpha means, if you look down the length of the spring, the coiling is happening in a clockwise direction as it goes away from you.

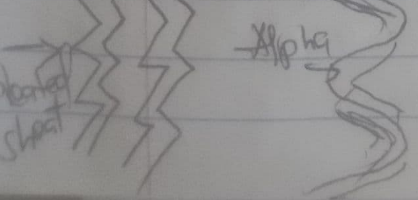
- The structure remains a coiled string and is secured by hydrogen bonding in a polypeptide chain.



ii) Beta (B) Pleated sheet.

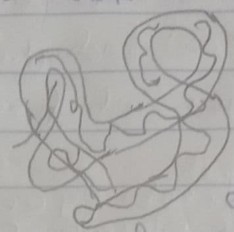
- The chains are folded such that they lie alongside each other.
- The structure appears to be folded or pleated and is held together by hydrogen bonding between polypeptide units of the chain that lie adjacent to one another.
- The folded chains are again held together by hydrogen bonds involving exactly the same group as in the alpha helix.
- The diagram below shows what is reffered to as anti-parallel sheets.





(c) Tertiary structure

- The final 3D structure of a protein is its tertiary structure, which pertains to the shaping of the secondary structure
- Involve coiling, looping & folding often with straight chains of amino acids in between.



- The tertiary structure of a protein is a description of the way the whole chain folds itself to its final 3-dimensional shape.

Is held by different bonds

(i) **Disulphide bonds** ⇒ where 2 Cysteine amino acids are found together, a strong double bond (S=S) is formed between Sulphur atoms within the Cysteine monomers

ii) **Ionic Bonds** ⇒ If 2 oppositely charged 'R' groups (+ve & -ve) are found close to each other, and ionic bonds form between them.

iii) **Hydrogen Bonds** ⇒ found in the polypeptide chain and between amino and 'R' groups.

iv) **Hydrophobic & Hydrophilic interaction** ⇒ Some amino acids may be hydrophobic while others are hydrophilic.

(v) Van der Waals Forces ⇒ also assist in stabilization of protein structure.

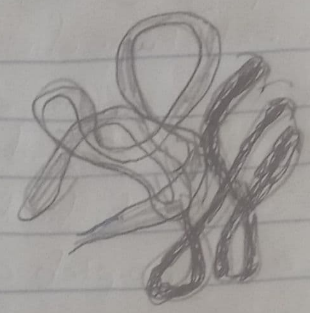
3D structure fall into 2 types

① Globular Proteins ⇒ Form ball-like structures where hydrophobic parts are towards the centre and hydrophilic are towards the edges which make them water soluble.
- They have metabolic roles eg enzymes in all organisms, plasma proteins and antibiotics in mammals.

② Fibrous Proteins
⇒ These proteins form long fibres and mostly consist of repeated sequences of amino acids which are insoluble in water.
- Usually have structural roles eg Collagen in bone & Cartillage, Keratin in fingernails and hair

(d) Quaternary structure
- Formed by interaction between multiple polypeptides
- Some proteins are made up of multiple Polypeptide chains Sometimes with an inorganic Component e.g a haem group in HB called a prosthetic Group

- These Proteins will only be able to function if all sub-units are present.



- Example;
① Haemoglobin
- water soluble globular protein composed of 2 α polypeptide chains, an 2 B polypeptide chain and inorganic prosthetic haem group

- Carries Oxygen around in the blood and it is facilitated in doing so by the presence of the haem group which Contains a Fe^{2+} ion, onto which The Oxygen molecules Can bond.
- Haemoglobin is globular and has a tertiary structure
- It is Conjugated - contains haem groups eg prosthetic group

(2) Collagen

- Consist of 3 polypeptide chains wound around each other.
- Each of the chains is a coil itself.
- H bonds form between these chains which are around 1000 amino acids in length, which gives the structure strength.
- This is important given collagens role as structural proteins.
- The strength is increased by the fact that collagen molecules form further chains with other collagen molecules and form covalent cross links with each other, which are staggered along the molecules to further increase stability.

Functions of Collagen

(i) Forms the structure of bones
(ii) Makes up cartilage & connective tissues
(iii) Main components of tendons, which connect skeletal muscles to bones
(iv) Prevents blood that is being pumped at high pressure from bursting the walls of arteries

⇒ Collagen molecules wrapped around each other form collagen fibrils which themselves form collagen fibres.

Differences Between Haemoglobin & Collagen:

(i) Shape, solubility, composition of amino acid, & Prosthetic structure, Tertiary structure,

Haemoglobin	Collagen
- Globular in shape	Fibrous
- Soluble in water	- Insoluble
- Contain wide range of amino acids	- 35% of its structure is made of glycine
- Contains a haem prosthetic group.	- Doesn't posses a prosthetic group.
- Much of it is wounded into α helices	- Much of it is made up of left handed helix structures.
- Conjugated	- Not conjugated
- Has a functional role (carries O_2)	- Has a structural role.

ENZYMES

- Are biological catalysts that tremendously accelerate the rate and efficiency of a chemical reaction in living organisms.
- During such reaction, an enzyme acts upon substrates and converts them into different molecules referred to as products.

2 types of enzymes

(a) The exoenzymes (extracellular enzymes) are those enzymes whole activities and functions occur outside the cell and they retain their ability even when they are removed from the cell

(b) The endoenzymes (intracellular enzymes) are those enzymes that function inside the cell and lose their ability when extracted from the cell

Characteristics of Enzymes

(i) Enzyme is a protein and therefore gets destroyed by heating.

ii) It is easily influenced by environmental changes - Eg Temp, pH & conc of substrate & enzyme. Thus enzymes have optimal conditions in which they can work at peak efficiency.

iii) Acts as a Catalyst - Enzymes speeds up the rate of a reaction by lowering the activation energy.

iv) Functions specifically - It acts only on one kind of substance called the substrate so, it is very specific. (Are substrate-specific)

v) Function in reverse - It means enzymes does not determine the direction of reaction but it only function in accelerating reaction rate until it reaches equilibrium. The enzyme also functions in substance synthesis and substance breaking down reaction.

vi) Required in Small amounts
- Because enzymes are not consumed in the reactions they catalyze and can be used over and over again, only a very small quantity of an enzyme is need to catalyze a reaction.

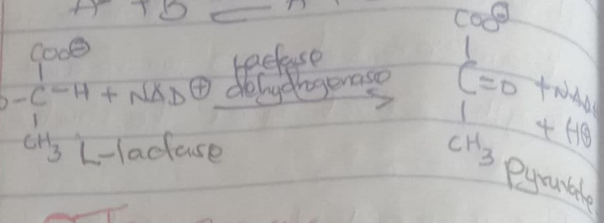
Classification of Enzymes

- Enzymes are classified on the based of reaction they catalyse.
- This distinguishes enzyme from one another
- Enzymes speed up the reaction by lowering activation energy.

Classes of Enzymes

① Oxidoreductases / Dehydrogenases
- Catalyse oxidation - reduction reactions
- Involves the transfer of electrons from one species to another.
- Commonly called dehydrogenases

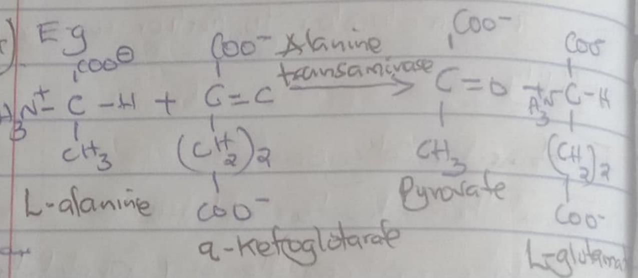
$$A^- + B \rightleftharpoons A + B^-$$



② Transferases
- Catalyse the transfer of a functional group. May require the presence of co-enzymes.
- Co-enzymes are organic compounds other than amino acids required for catalysis.

$$A-B + C \rightleftharpoons A + B-C$$

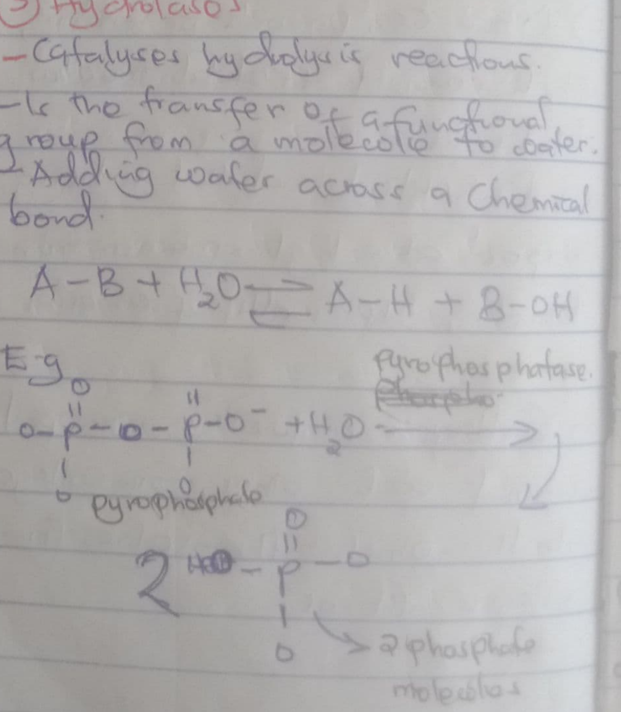
Eg



③ Hydrolases
- Catalyses hydrolysis reactions.
- Is the transfer of a functional group from a molecule to water.
- Adding water across a chemical bond

$$A-B + H_2O \rightleftharpoons A-H + B-OH$$

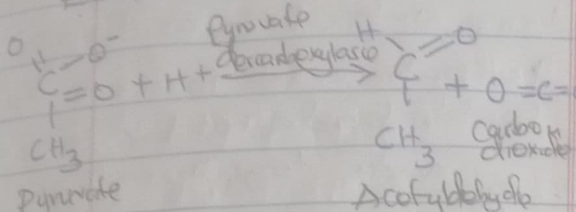
Eg



④ Ligases
- Catalyse addition or removal of a functional group to form a double bond. Include non-hydrolytic, non-oxidative, elimination reactions.

Eg A-B ⇌ X=B + X-Y
 | |
 X Y

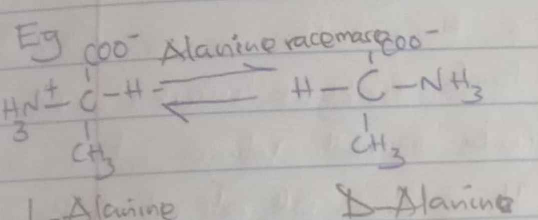
Example



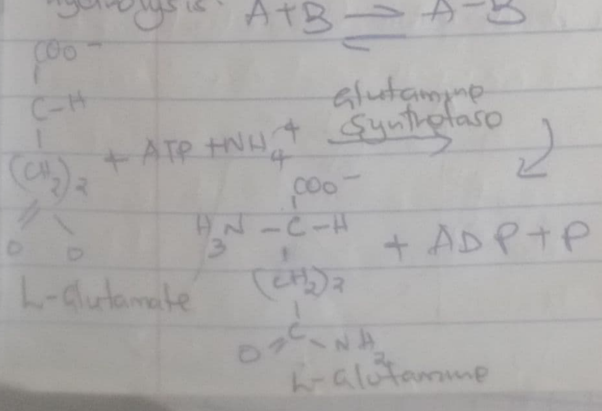
⑤ Isomerases
- Catalyse isomerisation reactions.
- Is a structural change within a single molecule.
- Intramolecular group transfer.

A-B ⇌ A-B
| | | |
X Y Y X

Eg



⑥ Ligases
- Catalyse ligation reactions.
- This is the chemical joining of 2 substrates at the expense of ATP hydrolysis. A+B ⇌ A-B



Mechanism of Enzyme Action
- Enzymes action occurs when substrate is avaible and the side of enzyme is empty.
- Into this condition, substrate contains the active site. The active site then will have shape transformation by encoding the substrate and form weak chemical bonding in the form of enzyme-substrate complex.
- In the active site, substrate will be converted into final form known as product.
- The product is released from the enzyme bond and the enzyme is free to bond with other structure.

E + S ⟶ ES ⟶ E + P.

Enzyme Mechanism
a) Substrate enters active site of enzyme.
b) It forms enzyme substrate complex
c) Product leaves the enzymes

Kinds of hypotheses explaining enzyme Mechanism

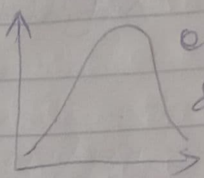
a) Lock and key hypothesis
- Was postulated by Emil Fisher in 1898
- According to this hypothesis, like a lock can be opened by its key only, a substance processing

specific Composition only can combine with the specific active site found in the specific enzyme's surface.
- The molecules on which the activities of the enzymes ~~that~~ take place are called Substrates
- The substrates processing accurate geometric configuration can fit within the enzymes active site.
- Enzymes' active site is highly specific to the surface of the substrate.
- Sub of Enz fit like a lock and key adaptation providing a lock and key enzyme model.

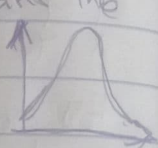
b) Induced - Fit Model
- Enzyme can change its shape to fit the substrate shape.
- It states that a substrate binds to an active site and both change shape slightly, creating an ideal fit for catalysts.
*. In the induced fit model, both the substrate · enzyme's active site undergoes conformational changes up until the substrate is fully attached to the enzyme
- At this point, the final shape and charge are established. This prompts the enzyme to start acting catalytically. Initially, the enzyme's active site and substrate are not exactly completely.

Factors affecting Catalytic activity of Enzymes

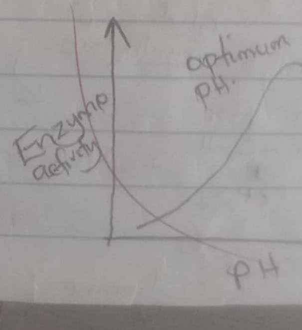
(i) Temperature
- Increase in temp, molecules increase in collision
- Enzyme best works at an optimum temperature - As you increase in temp, rate increases, temp above the optimum interferes with the peptide bonds that hold amino acids together thus, enzyme reaction is denatured



ii) pH
- Changes in pH breaks the intermolecular bonds

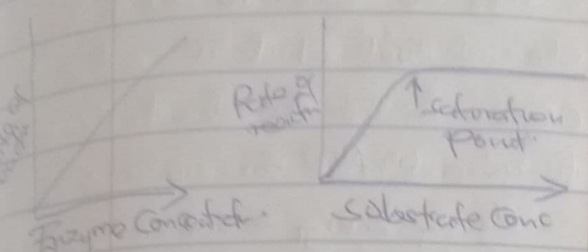


- Each enzyme works within quite a small range.
- There is a pH at which its activity is greatest (the optimal pH)
- This is because changes in pH can make and break intra and intermolecular bonds, and changing the shape of the enzyme and therefore its effectiveness



iii) Concentration of Enzyme & Substrate.

- Increase in enzymes increases more substrate formation due to increase in substrate binding.
- If all enzyme active sites are occupied, there will be no substrate to bind.



- 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.

iv) Presence of Co-enzymes & Co-factors.

- Enzymes require Co-enzymes and Co-factors in order to function properly.
- These small molecules often assist in the catalytic process by participating in electron transfers or carrying chemical groups.
- The presence or absence of these coenzymes and cofactors can significantly impact enzyme activity.

v) Inhibitors

- Are molecules that reduce or actually shut down enzymatic reactions; we have competitive & non-competitive inhibitors, allosteric inhibitors.

vi) Activators

- Some compounds can enhance enzyme activities
- Allosteric activators e.g, can bind to the enzymes at regulatory sites and increase its catalytic activity.

vii) Enzyme structure.

- Changes in the 3-dimensional structure of an enzyme either due to denaturation or conformational changes can affect its activity
- Denaturation is often irreversible and cause by high temperatures or extreme pH.

viii) Ionic strength
- The concentration of ions in the reaction environment can influence enzyme activity.
- High salt concentrations can disrupt enzyme-substrate interactions by shielding charges on the enzyme and substrates

xii) Time
- Enzyme activity may change over time due to factors like enzyme stability and degradation.
- Some enzymes naturally degrade after certain time or with repeated uses.

PHOTOSYNTHESIS

- Photosynthesis is the process by which organisms convert light energy into chemical energy in the form of reducing power (as NADPH or NADH) and ATP and use these chemicals to drive carbon dioxide fixation and reduction to produce sugars.
- In oxygenic photosynthetic organisms, including higher plants, the source of reducing equivalents is H_2O, releasing O_2 as a by-product.
- The overall reaction of oxygen photosynthesis can be represented as

$$CO_2 + 2H_2O \longrightarrow (CH_2O) + H_2O + O_2$$

- This process is responsible for producing virtually all the O_2 in the atmosphere and for fixing about 1011 tons of carbon from CO_2 into organic compounds annually.
- The sugars produced by the photosynthetic fixation of CO_2 provide raw material for the biosynthesis of all organic molecules found in plants.
- They are also the source of the chemical fuel which is oxidized by oxygen in the mitochondria in order to generate ATP for use in a wide variety of energy-consuming processes in the plant such as biosynthesis, active transport of ions and metabolites across membranes, and intracellular movement of organelles.
- Photosynthesis occurs primarily in leaf cells in organelles called chloroplasts, which are about 5µm long and bound by two membrane envelopes.
- Number of chloroplasts per leaf cell varies from 1 to over 100, depending on cell type, species and growth conditions.
- The utilisation of light energy to drive the synthesis of NADPH and ATP takes place in a complex system of membrane-enclosed sacs in the chloroplast referred to as thylakoid membrane, and the reactions involved in CO_2

fixation and reduction to sugar are catalysed by soluble enzymes in the chloroplast matrix refered to as the stroma.

- An overall equation for photosynthesis in green plant is;

$$nCO_2 + nH_2O \xrightarrow[\text{chlorophyll}]{\text{light}} (CH_2O)_n + nO_2$$

Carbon dioxide, Water, Carbohydrate, Oxygen

- Hexose sugars and starch are commonly formed, so the following equation is often used;

$$6CO_2 + 6H_2O \longrightarrow C_6H_{12}O_6 + 6O_2$$

Carbondioxide, Water, Carbohydrate, Oxygen

- Two sets of reactions are involved. These are light dependent reaction, for which light energy is necessary, and the light-dependent reactions for which light energy is not needed.
- The light dependant reactions only take place in the presence of suitable pigments ~~bod~~ that absorb ~~ab~~ certain wavelengths of light.
- Light energy is necessary for the splitting (photolysis) of water into hydrogen and oxygen; Oxygen is a waste product.
- Light energy is also needed to provide chemical energy, in form of ATP, for the reduction of carbon dioxide to carbohydrate in the light independent reactions.
- The photosynthetic pigments involved fall into two categories; primary pigments and accessory pigments.
- The pigments are arranged in light-harvesting clusters called photosystems of which there are two types I and II.
- In photosystem, several hundred accessory pigment molecules surround a primary pigment molecule, and the energy of the light absorbed by the different pigment is passed to the primary pigment.
- The primary pigments are two forms of chlorophyll.
- These primary pigments are said to act as reaction centres.

(i) Light Reaction (Light Dependent)

- This takes place in the thylakoid membranes inside the chloroplast an organelle found inside the cells of green tissues.
- Light ~~products~~ provides energy for chlorophyll molecule that release electrons
- These split water into oxygen and hydrogen

$$2H_2O \longrightarrow 2H_2 + O_2$$

- The hydrogen then moves into the next stage.

(ii) Dark reaction (Light Dependent)

- This takes place in the watery stroma of the chloroplast.
- Here the hydrogen is combined with carbon dioxide by the Calvin cycle to give carbohydrate and water.

$$2H_2 + CO_2 \rightarrow CH_2O + H_2O$$

NB ⇒ Carbohydrates are simple sugars, which can be moved through the vascular system of the plant in solution to wherever they are needed.

- This process not only provides the basis for all food production but it also supplies the oxygen which animals and plants need for respiration.
- The simple carbohydrates such as glucose, may be built up to form starch for storage purposes or as cellulose for building cell walls.
- Fats and oils (lipids) are formed from carbohydrates by a process of esterification which produces mostly triglycerides.
- These are usually found in seeds and are a form of concentrated energy.

- protein material, which is an essential part of all living cells, is made from carbohydrates and nitrogen compounds and also frequently contains sulphur.
- These form amino acids which are held together in proteins by peptide bonds.

Chloroplast structure and Function.

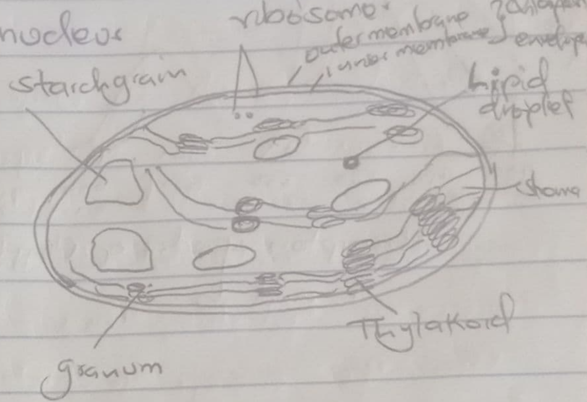
- In Eukaryotic organisms, the photosynthetic organelle is the chloroplast.
- In dicots, chloroplasts can be seen with a light microscope and appear as biconvex discs about 3–10 µm in diameter.
- There may be only a few chloroplasts in a cell or many as 100 in some palisade mesophyll cells.
- Each chloroplast is surrounded by an envelope of two phospholipid membranes.
- ~~Each~~ A system of membranes also runs through the ground substance, or stroma.
- The membrane system is the site of the light dependent reactions of photosynthesis.
- It consists of a series of flattened fluid-filled sacs or thylakoids, which in places form stacks called grana that

are joined to one another by membranes.
- The membranes of the grana provide a large surface area, which hold the pigments, enzymes and electron carriers needed for the light dependent reactions.
- The membranes make it possible for a large number of pigment molecules to be arranged so that they can absorb as much light as necessary.
- The pigment molecules are also arranged in particular light-harvesting clusters for efficient light absorption.

- In each photosystem, the different pigments are arranged in the thylakoid in funnel-like structures.

- Each pigment passes energy to the next member of the cluster, finally 'feeding' it to the chlorophyll a reaction centre (primary pigment).
- The membranes of the grana hold ATP synthase and are the site of ATP synthesis by chemiosmosis.
- The stroma is the site of the light independent reactions.
- It contains the enzymes of the Calvin cycle, sugars and organic acids.
- It bathes the membranes of the grana and so receive the products of the light dependent reactions.

- Also, within the stroma are small (70s) ribosomes, a loop of DNA, lipid droplets and starch grains.
- The loop of DNA codes for some of chloroplast proteins, which are made by the chloroplast ribosomes.
- However, other chloroplast proteins are coded for the DNA in the plant cell nucleus.



Trapping Light Energy

- Chloroplasts contain several different pigments, and these different pigments absorb different wavelengths of light.
- The photosynthetic pigments of higher plants form two groups; the chlorophylls (primary pigments) and the carotenoids (accessory pigment).

Group	Pigment	Colour
Chlorophylls	Chlorophyll a	Yellow-green
	Chlorophyll b	blue-green
Carotenoids	B carotene	orange
	Xanthophyll	~~orange~~ yellow