1

INTRODUCTION

Biochemistry is the science which deals with the chemistry of living tissues and the substances that take part in their metabolism. Optimal level of biochemicals in a living organism keep the organism in healthy condition. Either excess or low concentration of biochemicals will affect the function of a living organism which leads to a disease state. Therefore, understanding of biochemistry is very useful in the diagnosis and treatment of various diseases.

Biochemistry in preventive medicine

Knowledge of biochemistry can be utilized to prevent malnutrition. Similarly estimations of biochemicals like blood cholesterol, or enzymes can help the clinicians to find atherosclerosis or cancer at an early stage. Thus, doctors can advise the patients to take preventive steps according to the nature of the disease.

Many genetic disorders like phenylketonuria, glucose-6-phosphate dehydrogenase deficiency can be identified by the use of clinical biochemistry.

Knowledge of biochemistry is very essential for designing many drugs useful in variety of pathological conditions. A sound knowledge of biochemistry is required to understand the metabolism of drugs by different enzymes. Toxic substances act on biochemical reactions or processes in the living cells, thus study of toxicology also requires a better understanding of biochemistry. Advances in nuclear biochemistry is useful in the field of recombiant DNA technology to synthesize many bioactive polypeptide like human insulin.

2

PROTEINS

Proteins are complex nitrogenous substance of high molecular weight which on decomposition by acids, alkalis or enzymes yield aminoacids.

Proteins play a vital role in our body. Following are some of the chief function of the proteins in our body.

(i) Protein is a part of the cell membrane structure.

(ii) Structural proteins are present in different organs of our body.

(iii) All enzymes are proteins

(iv) Contractile proteins, like actin myosin and others are essential for muscular contraction.

(v) Many clotting factors are proteins.

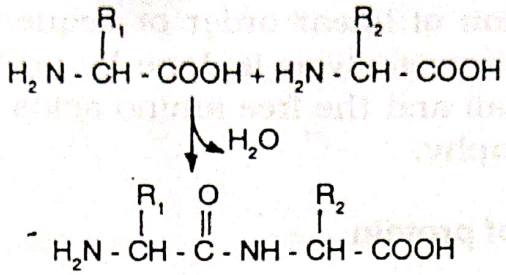
(vi) Immunoglobulins are proteins in nature.

(vii) Conjugate protein-like hemoglobin is very essential for oxygentransport from blood into the cells.

(viii) Many hormones are polypeptides in nature (e.g. Insulin, Parathormone).

Peptide bonds

Peptide bonds, Unite the amino end (N-terminal) of one amino acid to the carboxyl end (C-terminal) of another.



Peptide bond

Each aminoacid in a polypeptide chain is termed a residue.

Oligopeptide : An amino acid chain with less than 25 amino acid is called an oligopeptide.

e.g. TRH (Thyrotrophin releasing hormone)

Enkephalins	Motilin
Angiotensin II	Gastrin
Vasopressin	Substance P
Oxytocin	Somatostatin
Bradykinin	Bombesin
Glucagon	Endothelin

Polypeptide : An amino acid chain with more than 25 amino acids is called a polypeptide.

e.g. Insulin
VIP (Vasoactive intestinal peptide)
ACTH (Adreno corticotrophic hormone)
Neuropeptide Y

Protein : A protein may consist of a long polypeptide chain or several polypeptide subunits.

e.g. Streptokinase
Tissue plasminogen activator
Interferons
Clotting factors
Antibodies

Determination of primary structure of protein

It involves two steps :

(a) Qualitative identification and quantitative estimation of aminoacid residue.

(b) Determination of linear order or sequence: peptide bond hydrolysis or proteolysis is done by proteolytic enzymes, acids or alkali and the free amino acids are separated by chromatography.

Acid hydrolysis of protein

$$\text{Protein} \xrightarrow[\text{at } 110^{\circ}C \text{ for } 24 \text{ hrs.}]{6\ NHCl} \text{Aminoacids}$$

Set back of acid hydrolysis

Amino acids like tryptophan and cysteine are destroyed where as serine, threonine and tyrosine are partially destroyed.

Hydrolysis of protein by alkali

Alkaline hydrolysis does not damage tryptophan, hence, alkaline hydrolysis can be used to separate tryptophan from peptides. However, alkaline hydrolysis destroys cysteine, serine, and threonine.

Hydrolysis of protein by enzymes

Enzymes catalysed hydrolysis depends upon the nature of the enzymes

e.g. Peptidase hydrolyses all proteins but very slowly.

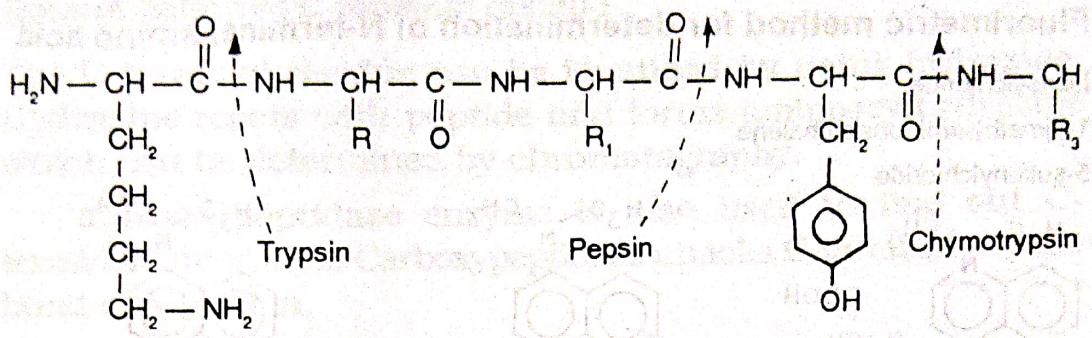
Chymotrypsin : Attacks peptide bond at the carboxyl side of tyrosine and phenylalanine.

Pepsin : Attacks peptide bond at the amino side of tyrosine and phenylalanine.

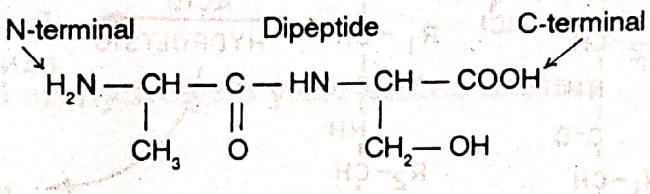
Trypsin : Hydrolyses the peptide bond at the carboxyl side of lysine and arginine

Carboxypeptidase

It splits peptide bonds adjacent to a free carboxyl group. As soon as the first amino acid is split from the chain the enzyme attacks the second peptide bond of the polypeptide. The residue bearing the free aminogroup of peptide chain is called as N-terminal residue and the one bearing the α-carboxyl group is called as C-terminal residue.



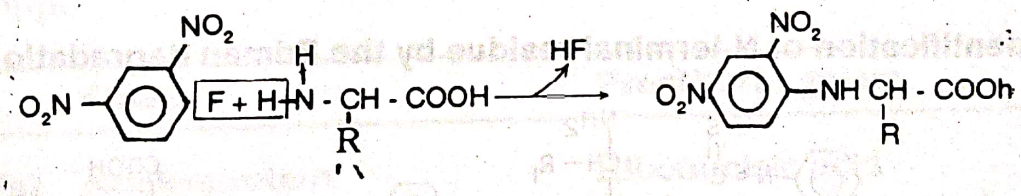
Lysine Tyrosine



Ala (Alanine) Ser (Serine)

Determination of N-terminal aminoacid residue

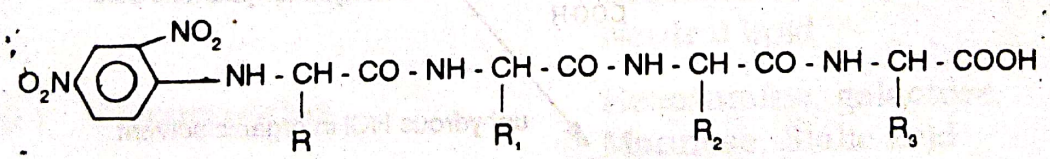
In 1945 Sanger introduced the use of 1-fluoro-2, 4 dinitrobenzene to determine the N-terminal aminoacid residue.



1-fluoro-2, 4 dinitrobenzene DNFB

Nitrophenyl peptide

OR

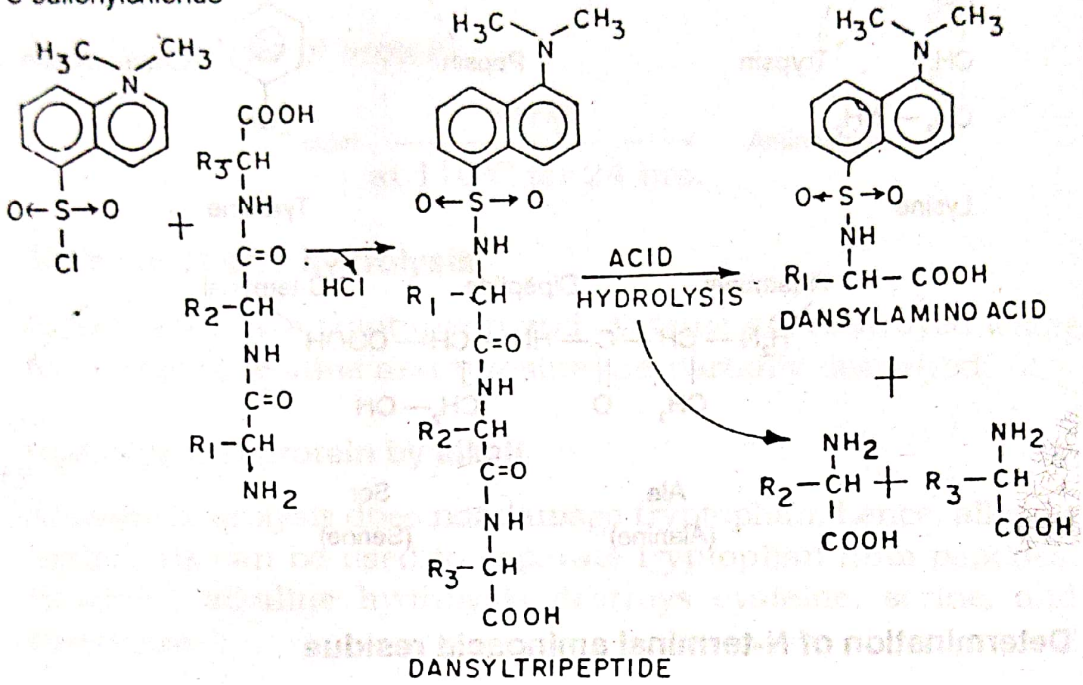


α-aminogroup of peptide reacts with DNFB to form a yellow 2, 4 dinitrophenyl derivative when this derivative is subjected to acid hydrolysis by 6NHCl all the peptide bonds are hydrolysed except the bond between DNFB and N-terminal aminoacid which is more stable to acid hydrolysis.

This nitrophenyl derivative is separated from free aminoacid, identified and compared with standard dinitrophenyl derivative.

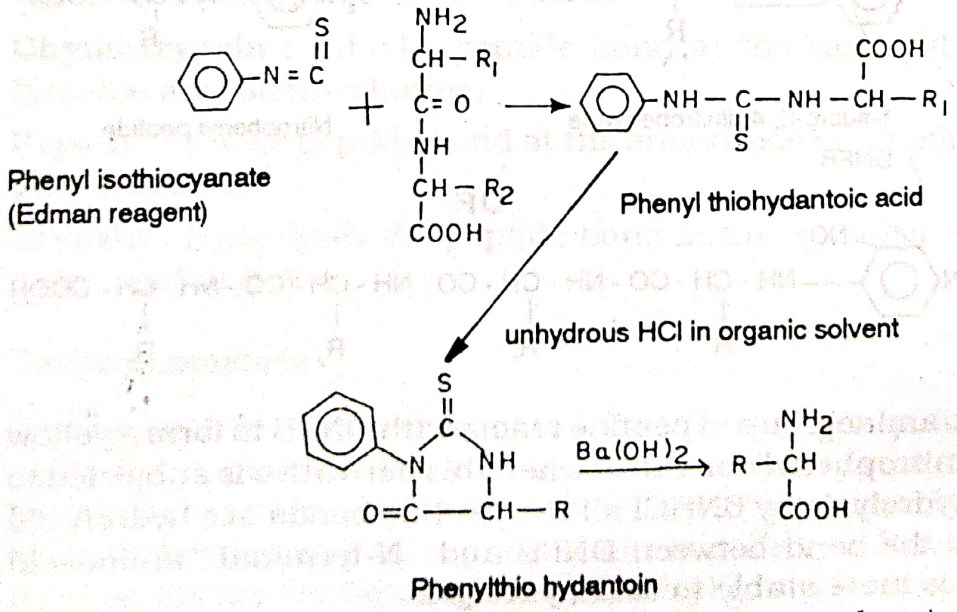
Fluorimetric method for determination of N-terminal amino acid

Dansylchloride
1-dimethylaminonaphthalene
5-sulfonylchloride



Dansylaminoacid is measured by fluorimetric method (This method is 100 times more sensitive than the DNFB method).

Identification of N-terminal residue by the Edman degradation.



Phenylthiohydantoin is soluble in organic solvent and is determined by gas liquid chromatography.

Determination of C-terminal residue

The C-terminal residue can be identified by using hydrazine. Hydrazine reacts with peptide and forms aminoacylhydrazine which can be determined by chromatography.

Carboxypeptidase enzyme is also used to find out C-terminal amino acid. Carboxypeptidase attacks C-terminal peptide bond of a protein.

CLASSIFICATION OF PROTEINS

I. Simple Protein

These proteins on hydrolysis yield amino acids.

e.g. Albumin
Globulin
Prolamine
Glutelins
Scleroprotein

II. Conjugated protein

These proteins on hydrolysis yield amino acid and a prosthetic group.

	Class	Prosthetic group
(a)	Nucleoprotein	Ribonucleic acid Deoxy ribonucleic acid
(b)	Lipoprotein	Phospholipid Cholesterol Neutral lipid
(c)	Glycoproteins	Hexosamine, galactose Mannose, Sialic acid
(d)	Phosphoprotein Casein	Phosphate esterified with serine
(e)	Hemoproteins Hemoglobin Cytochrome Catalase	Iron protoporphyrin

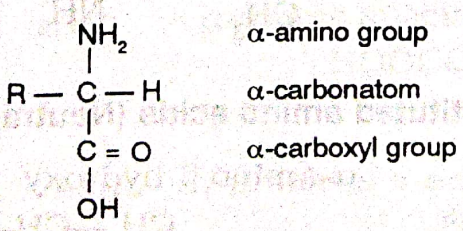
	Class	Prosthetic group
(f)	Flavoproteins	
	Succinic acid dehydrogenase	FAD
	D-amino acid oxidase	
(g)	Metalloproteins	
	Ferritin	Fe
	Cytochrome oxidase	Fe and Cu
	Alcoholdehydrogenase	Zn
	Xanthine oxidase	Mo

Classification of protein based upon biological function

Types and Examples	Function
I. Enzymes	
Hexokinase	Phosphorylation
Cytochrome C	Transfer of electron
DNA polymerase	Replication and repair of DNA
II. Storage proteins	
Ova albumin	Egg white protein
Casein	Milk protein
Ferritin	Iron storage protein
III. Transport protein	
Hemoglobin	Transport of oxygen in blood of vertebrates
Hemocyanin	Transport of oxygen in blood of in-vertebrate
Myoglobin	Transport of oxygen in muscle cells
Lipoproteins	Transport of lipid in blood
Iron binding globulin	Transport of iron in blood
Ceruloplasmin	Transport of Cu ++ in blood

Types and Examples	Function
IV. Contractile proteins	
Actin	Contraction of muscles.
Myosin	
V. Protective proteins	
Antibodies	Form complex with foreign protein
Complement	Form complex with antigen antibody
Fibrinogen	Precursor of fibrin
Thrombin	Component of clotting mechanism
VI. Toxins	
Clostridium botulinumtoxin	Protein causing food poisoning
VII. Hormones	
Insulin	Regulates blood sugar
VIII. Structural protein	
α-keratin	Skin
Collagen	Fibrous connective tissue.

All living organisms contain α-amino acids and all aminoacids share the same structural backbone.



All α-aminoacids have three main features

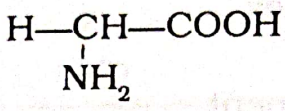
1. They have an α-amino group

2. They possess a side chain, or R group, that is bound to that α-carbonatom.
3. They contain an α-carboxyl group.

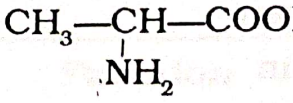
Classification of amino acid : About twenty amino acids are generally found in most proteins

I. Aminoacids with aliphatic side chain (Neutral amino acids)

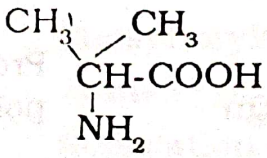
1. Glycine Gly — Aminoacetic acid



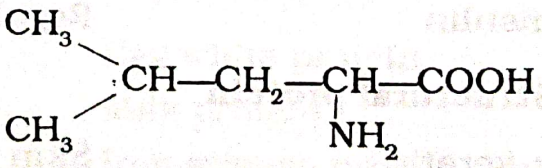
2. Alanine Ala — α-aminopropionic acid



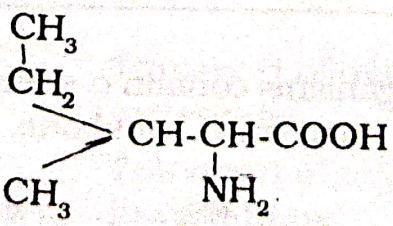
3. Valine Val — α-aminoisovaleric acid



4. Leucine Leu — α-aminoisocaproic acid

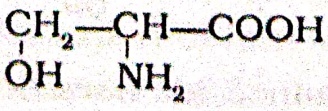


5. Isoleucine Ileu — α-amino β-methyl valeric acid

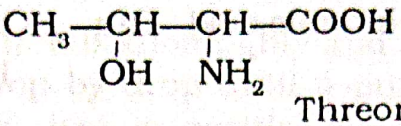


II. Hydroxylsubstituted amino acids (Neutral amino acids)

1. Serine Ser — α-amino β-hydroxy propionic acid



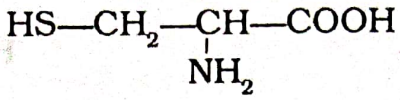
2. Threonine Thr. — α-amino β-hydroxy n-butyric acid



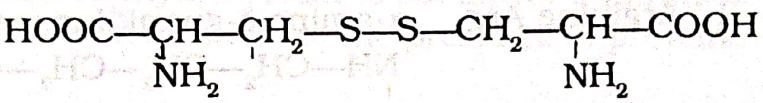
Threonine

III. Sulphur containing amino acids

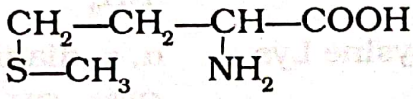
1. Cysteine cys α-amino β-mercapto propionic acid



2. Cystine cys-s-s-cys β, β, dithio α-amino propionic acid

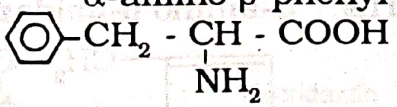


3. Methionine Met α-amino-γ-methylthio n-butyric acid

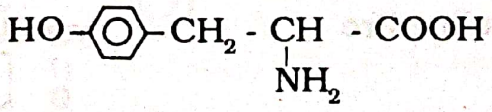


IV. Aromatic amino acids derived from alanine

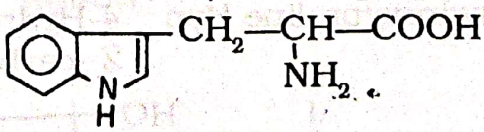
1. Phenylalanine Phe α-amino β-phenyl propionic acid



2. Tyrosine Tyr α-amino β (p-hydroxyphenyl) propionic acid

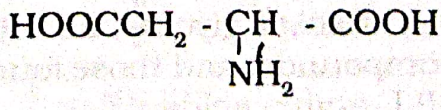


3. Tryoptophan Trp. α-amino β-indole propionic acid

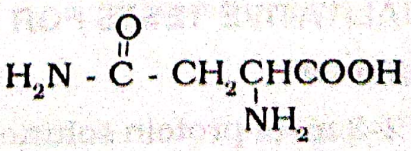


V. Acidic aminoacids

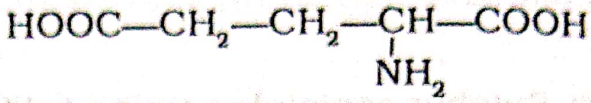
1. Aspartic acid Asp α-amino succinic acid



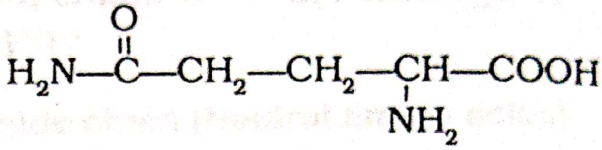
2. Asparagine Asn γ-amide of α-amino succinic acid



3. Glutamic acid Glu α-aminoglutaric acid

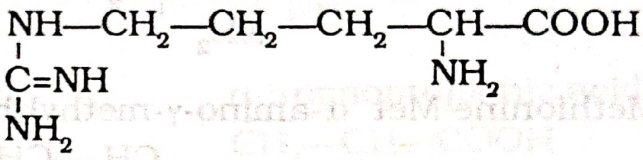


4. Glutamine Gln δ-amide of α-amino glutaric acid

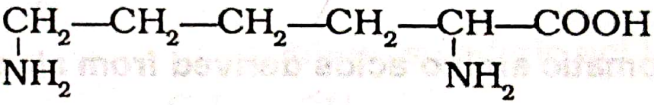


VI. Basic amino acid

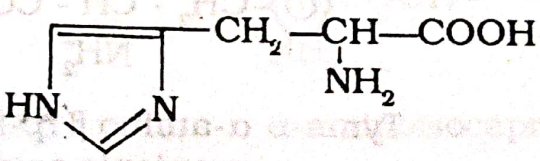
1. Arginine Arg. α-amino δ-guanidino n-valeric acid



2. Lysine Lys α, ε- diamino caproic acid

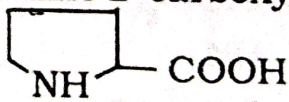


3. Histidine His α-amino β-imidazole Propionic acid

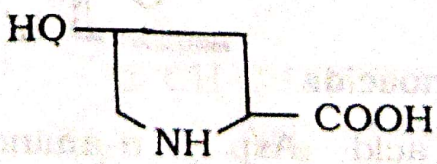


VII. Imino acids

1. Proline Pro Pyrrolidine 2-carboxylic acid



2. 4-hydroxyproline Hyp 4-Hydroxypyrrolidine 2 -carboxylic acid



Except glycine, all other amino acids are optically active compounds, and those found in naturally occuring proteins are all L-amino acids.

QUALITATIVE TESTS FOR PROTEINS

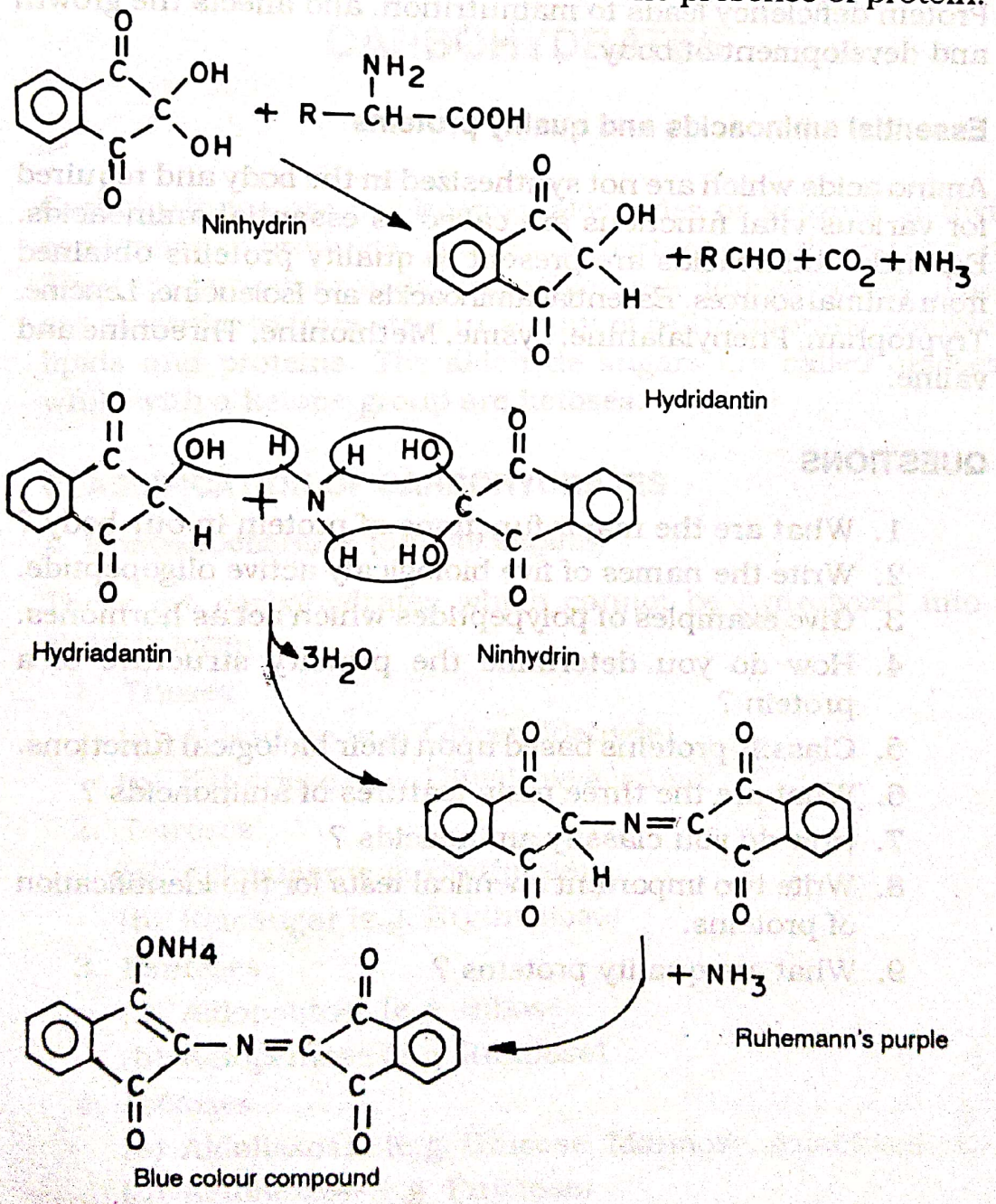
Biuret test

To 2-3 ml of protein solution in a test tube add an equal volume

of 10% sodium hydroxide solution. Mix thoroughly and add a 0.5% coppersulphate solution drop by drop until a purplish violet colour is produced. Biuret test is positive for those molecules which contain two carbamyl group (Co-NH_2) joined either directly together or through a single atom of nitrogen or carbon.

Ninhydrin reaction

To 5 ml of dilute protein solution (pH between 5 and 7) add 0.5 ml of a 0.1% solution of ninhydrin. Heat to boiling for 2 minutes and allow to cool. Blue colour indicate the presence of protein.



The ninhydrin reaction can be used to estimate the quantity of amino acid present in the sample. Individual amino acids can be separated by chromatoraphic techniques for estimation.

Biological value

Amino acids are very essential for the biosynthesis of enzymes, storage proteins, hemoglobin, actin myosin, antibodies, clotting factors and hormones.

Deficiency diseases

Protein deficiency leads to malnutrition, and affects the growth and development of body.

Essential aminoacids and quality proteins

Amino acids which are not synthesized in the body and required for various vital functions are called as essential aminoacids. Essential aminoacids are present in quality proteins obtained from animal sources. Essential aminoacids are Isoleucine, Leucine, Tryptophan, Phenylalanine, Lysine, Methionine, Threonine and valine.

QUESTIONS

1. What are the major functions of protein in our body ?
2. Write the names of five biologicaly active oligopeptide.
3. Give examples of polypeptides which act as hormones.
4. How do you determine the primary structure of a protein ?
5. Classify proteins based upon their biological functions.
6. What are the three main features of aminoacids ?
7. How do you classify aminoacids ?
8. Write two important chemical tests for the identification of proteins.
9. What are quality proteins ?

3

CARBOHYDRATES

Carbohydrates are polyhydroxy aldehydes or ketones or their condensation products. They are important constituent of diet. They are important fuels from which cell derives energy. They are essential to from structural unit of cell membrane complex lipids and proteins. The aldehyde sugars are called aldoses, while with a ketone group are ketoses.

CLASSIFICATION OF CARBOHYDRATES

A. Monosaccharides (Simple sugars)

These are carbohydrates which cannot be hydrolyzed into a simpler form.

1. Trioses
   (a) Aldotriose (e.g. Glyceraldehyde)
   (b) Ketotriose (e.g. Dihydroxyacetone)
2. Tetroses
   (a) Aldosugar (e.g. Erythrose)
   (b) Ketosugar (e.g. Erythrulose)
3. Pentoses
   (a) Aldopentose (e.g. Ribose)
   (b) Ketopentose (e.g. Ribulose)
4. Hexoses
   (a) Aldohexoses (e.g. Glucose, Mannose, galactose)
   (b) Ketohexose (e.g. Fructose)

B. Disaccharides

These are carbohydrates which yield two molecules of same or different monosaccharides on hydrolysis.

(a) Reducing disaccharide e.g. lactose, Maltose

(b) Non-reducing disaccharide e.g. Sucrose

C. Oligosaccharides

On hydrolysis oligosaccharides yield 3-6 monosaccharide units.

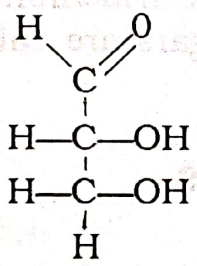
e.g. Maltotriose

D. Polysaccharides

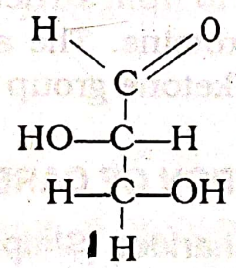
These carbohydrates on hydrolysis yield more than 6 monosaccharide units.

1. Homopolysaccharides e.g. Starch, glycogen
2. Heteropolysaccharides e.g. Heparin, Hyaluronic acid.

Monosaccharides exhibit isomerism,



D-Glyceraldehyde



L-Glyceraldehyde

D and L indicate spatial relationship and not optical activity. Optical activity is denoted by + (d-form) and - (l-form).

When two mono saccharide units condense together with elimination of water a disaccharide is formed.

Mutarotation

When glucose is dissolved in water, the solution has specific rotation of a D + 111°, when the solution is allowed to stand rotation falls slowly ot 52.5 Similarly β-glucose gives rotation from 19° to 52°.

It indicates that glucose can exist in two forms. The change in rotation to a common value which occurs when either of this