An Introduction to Practical Biochemistry

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Preface

Biochemistry is the chemistry of life. Biochemists study the elements, compounds and chemical reactions that are controlled by enzymes and take place in all living organisms. The findings of biochemistry are used in many areas, from genetics to molecular biology and from agriculture to medicine.

Biochemistry processes concern not only chemists and biochemists, but also physicists and physicians. Over the past 20 years there have been considerable changes in the way we consider biochemistry. In view of the results obtained in chemistry and biochemistry, biochemistry appears to be much more than a simple tuning of the properties of the molecule itself, and important remote effects have been highlighted. It is now obvious that the inversion about either a double bond or a restrained single bond generates extensive changes.

It is an important component of all disciplines of biology. In the era of multidisciplinary approach, the basic techniques in biochemistry and molecular biology are much needed by the students of botany, zoology, microbiology, biotechnology, fisheries, veterinary, pharmacology, physiology, medicine, genetics, agriculture and allied subjects.

Author

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Chapter 1

Protein as Coenzymes

Proteins are present in every cell of humans, animals, plant tissues, tissue fluids and in micro organisms. They account for about 50% of the dry weight of a cell. The term protein is derived from the Greek word *proteios* meaning holding first place or rank in living matter.

MEDICAL AND BIOLOGICAL IMPORTANCE

Proteins perform wide range of essential functions in mammalians.

- Proteins are involved in the transport of substances in the body.
  Example: Haemoglobin transports oxygen.
- Enzymes which catalyze chemical reactions in the body are proteins.
- Proteins are involved in defence function. They act against bacterial or viral infection.
  Example: Immunoglobulins.
- Hormones are proteins. They control many biochemical events.
  Example: Insulin.
- Some proteins have role in contraction of muscles.
  Example: Muscle proteins.
- Proteins are involved in the gene expression. They control gene expression and translation.
  Example: Histones.
- Proteins serve as nutrients. Proteins are also involved in storage function.
  Examples: Casein of milk, Ferritin that stores iron.

- Proteins act as buffers.
  Example: Plasma proteins.
- Proteins function as anti-vitamins.
  Example: Avidin of egg.
- Proteins are infective agents.
  Example: Prions which ca9. use mad cow disease are proteins.
- Some toxins are proteins.
  Example: Enterotoxin of cholera microorganism.
- Some proteins provide structural strength and elasticity to the organs and vascular system.
  Example: Collagen and elastin of bone matrix and ligaments.
- Some proteins are components of structures of tissues.
  Example: α-keratin is present in hair and epidermis.

In order to understand how these substances though they are all proteins play such diverse functions their structures, and composition must be explored.

CHEMICAL NATURE OF PROTEINS

All proteins are polymers of aminoacids. The aminoacids in proteins are united through "Peptide" linkage. Sometimes proteins are also called as polypeptides because they contain many peptide bonds.

PROPERTIES OF PROTEINS

- Proteins have high molecular weight, *e.g.*, the lactalbumin of milk molecular weight is 17000 and pyruvate dehydrogenase molecular weight is 7×10^6.
- Proteins are colloidal in nature.
- Proteins have large particle size.
- Different kinds of proteins are soluble in different solvents.
- Proteins differ in their shape.
- Some proteins yield amino acids only on hydrolysis where as others produce amino acids plus other types of molecules.

- *Charge properties*: Charge of a protein depends on the surroundings like amino acids. So, by changing the pH of surroundings the charge of protein can be altered. This property is used for separation of proteins.
  Isoelectric point: Proteins have characteristic isoelectric points. At the isoelectric point its net charge is zero because the number of positive charges are equal to number of negative charges.
  So proteins are insoluble or have minimum solubility at isoelectric point. This property is used for the isolation of casein from milk. The isoelectric point for casein is 4.6. If the pH of the surrounding is raised above the isoelectric point, the protein is negatively charged *i.e.,* it exists as anion. Likewise, if the pH of the surrounding is lowered, the protein is positively charged *i.e.,* it exist as cation. Further, proteins do not move in an electrical field at isoelectric point like amino acids. However, if the pH of the medium is raised or lowered protein moves towards anode or cathode respectively. This property is exploited for the separation of proteins.
- *Proteins act as buffers*: Since proteins are amphoteric substances, they act as buffers. Hemoglobin (Hb) of erythrocytes and plasma proteins are important buffers. Hb accounts for 60% of buffering action with in erythrocytes and plasma proteins contributes to 20% of buffering action of blood.

CLASSIFICATION OF PROTEINS

There is no single universally satisfactory system of protein classification so far.

- One system classifies proteins according to their composition or structure.
- One system classifies them according to solubility.
- One system classifies them according to their shape.
- Classification of proteins based on their function also found in literature.

Classification of Proteins Based on their Composition

Proteins are divided into three major classes according to their structure.

- *Simple proteins*: Simple proteins are made up of amino acids only. On hydrolysis, they yield only amino acids.
  Examples: Human plasma albumin, Trypsin, Chymotrypsin, pepsin, insulin, soyabean trypsin inhibitor and ribonuclease.
- *Conjugated proteins*: They are proteins containing non-protein part attached to the protein part. The non-protein part is linked to protein through covalent bond, non-covalent bond and hydrophobic interaction. The non-protein part is loosely called as prosthetic group. On hydrolysis, these proteins yield non-protein compounds and amino acids.
  Conjugated protein - Protein + Prosthetic group
  The conjugated proteins are further classified into subclasses based on prosthetic groups.

Table. Different classes of conjugated proteins

	Subclass	Prosthetic group	Examples	Type of linkage
1.	Lipoproteins	Lipids	Various classes of Lipoproteins. Lipovitellin of egg	Hydrophobhic Interaction
2.	Glycoproteins	Carbohydrates	Immunoglobulins of blood, Egg albumin	Covalent
3.	Phosphoproteins	Phosphorus	Casein of milk, vitellin of egg yolk	Covalent
4.	Nucleoproteins	Nucleic acids	Chromatin, Ribosomes	Non-covalent
5.	Hemoproteins/ Chromoproteins	Heme	Hemoglobin, Myoglobin, Cytochromes	Non-covalent
6.	Flavoproteins	Flavin nucleotides FMN, FAD	Succinate Dehydrogenase	Covalent
7.	Metalloproteins	Iron	Ferritin, Cytochromes	Non-covalent
8.	Visual pigments	Retinal	Rhodopsin	Covalent

- *Derived proteins*: As the name implies this class of proteins are formed from simple and conjugated proteins. There are two classes of derived proteins.
  - *Primary derived proteins:* They are formed from natural proteins by the action of heat or alcohol etc. The peptide bonds are not hydrolysed. They are synonymous with denatured proteins.
    Example: Coagulated proteins like cooked-egg

albumin.

- *Secondary derived proteins:* They are formed from partial hydrolysis of proteins.
  Examples: Proteoses, peptone, gelatin, and peptides.

Proteins Classification According to their Solubility

- *Albumins*: Soluble in water and salt solutions.
  Examples: Albumin of plasma, egg albumin and lactalbumin of milk.
- *Globulins:* Sparingly soluble in water but soluble in salt solutions.
  Examples: Globulins of plasma, ovoglobulins of egg, lactoglobulin of milk.
- *Glutelins:* Soluble in dilute acids and alkalies.
  Examples: Glutenin of wheat, oryzenin of rice, zein of maize.
- *Protamins:* Soluble in ammonia and water.
  Examples: Salmine from salmon fish, sturine of sturgeon.
- *Histones:* Soluble in water and dilute acids.
  Example: Histones present in chromatin.
- *Prolamines:* Soluble in dilute alcohol and insoluble in water and alcohol.
  Examples: Gliadin of wheat, zein of corn.
- *Clero proteins*: Insoluble in water and dilute acids and alkalies.
  Examples: Collagen, elastin and keratin.

Classification of Proteins Based on Shape

Proteins are divided into two classes based on their shape.

- *Globular proteins:* Polypeptide chain(s) of these proteins are folded into compact globular (Spherical) shape.
  Examples: Haemoglobin, myoglobin, albumin, lysozyme, chymotrypsin.
- *Fibrous proteins:* Poly peptide chains are extended along one axis.
  Examples: α-keratin, β-keratin, collagen and elastin.

PROTEIN STRUCTURE

Since proteins are built from amino acids by linking them in linear fashion, it may be viewed as proteins having long chain like structures. However, such arrangement is unstable and polypeptide or protein folds to specific shape known as *conformation,* which is more stable.

Various stages involved in the formation of final conformation from linear chain are divided into four levels or orders of protein structure.

Primary Structure

The linear sequence of amino acid residues in a polypeptide chain is called as primary structure. Generally disulfide bonds if any are also included in the primary structure. *Bonds responsible for the maintenance of primary structure* are mainly peptide bonds and *disulfide* bonds. Both of them are covalent bonds.

Primary Structure of Insulin

This protein consist of two polypeptide chains A and B. The two chains are covalently linked by disulfide bonds. The A chain has N-terminal glycine and C-terminal aspargine. The B chain has phenylalanine and alanine as N-and C-terminal residues, respectively. Insulin is a hormone and its molecular weight is 5,700.

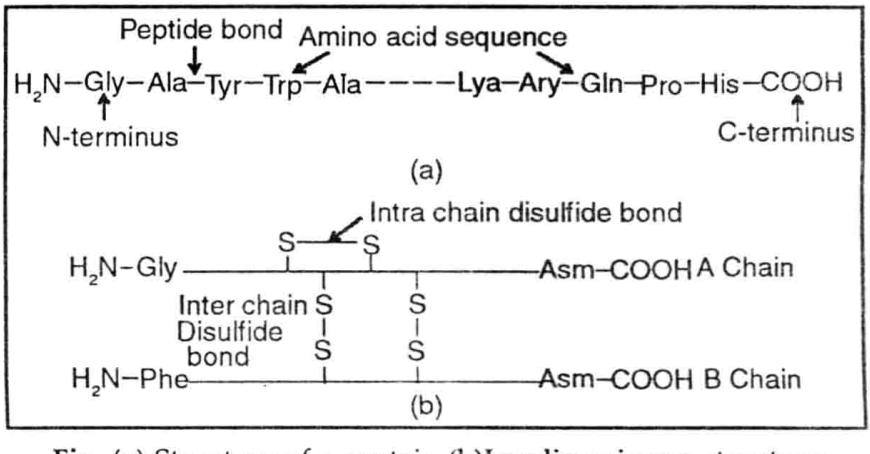

Fig. (a) Structure of a protein (b)**Insulin primary structure**

Secondary Structure

Folding of polypeptide chain along its long axis is called as secondary structure of protein. Folding of polypeptide chain can be ordered, disordered or random. Secondary structure is often referred as conformation. So, proteins has ordered secondary structure or conformation and random or disordered secondary structure or conformation.

Ordered Conformation of Polypeptides

The polypeptide chain of some proteins may exist in highly ordered conformation. The conformation is maintained by *hydrogen bonds* formed between peptide residues.

Hydrogen Bond

It is a weak ionic interaction between positively charged hydrogen atom and negatively charged atoms like oxygen, nitrogen, sulfur etc. It is indicated with broken lines (—-).

There are two types of ordered secondary structure observed in proteins.

- The polypeptide chain of keratin, which is present in hair, nails, epidermis of the skin is arranged as α-*Helix*: α-letter is given to this type of structure because it was first ordered structure noticed in proteins.
- Polypeptide chain of keratin, which is present in silk fibroin and spider web is arranged in β-*pleated* sheet. The β-letter is given because it was observed later.

Main Features of α-Helix

- In a-helix polypeptide, backbone is tightly wound round (coiled) long axis of the molecule.
- The distance between two amino acid residues is 1.5 Å.
- α-helix contain 3.6 amino acid residues per turn. The R-group of amino acids project outwards of the helix.
- The pitch of the α-helix is 5.4 Å long and width is 5.0 Å.

- The α-helix is stabilized by intra chain hydrogen bonds formed between –N–H groups and –C=O groups that are four residues back, *i.e.*, –N–H group of a 6th peptide bond is hydrogen bonded to –C=O group of 2nd peptide bond.

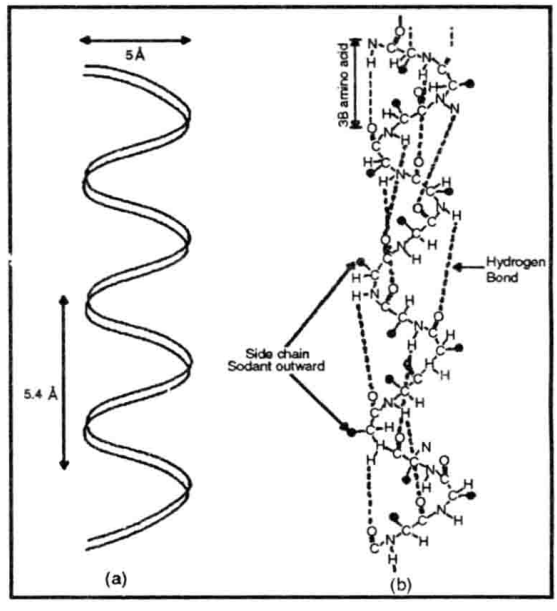

Fig. (a) Right handed a-helix

- Each peptide bond participates in the hydrogen bonding. This gives maximum stability to α-helix.
- α-helix present in most fibrous proteins is right handed. The right handed α-helix is more stable than the left handed helix.
- α-helix is hydrophobic in nature because of intra chain hydrogen bonds.
- An α-helix forms spontaneously since it is the most stable conformation of polypeptide chain.
- Some amino acids act as terminators for α-helix. *Example:* Proline.
- Aromatic amino acids stabilizes α-helix.
- Charged and hydrophobic amino acids destabilize α-helix.
- Content of α-helix varies from protein to protein.

β-Pleated Sheet Features

- In β-pleated sheet, the polypeptide chain is fully extended.4
- In β-pleated sheet, polypeptide chains line up side by side to form sheet. The side chains are above or below the plane of the sheet.
- From 2 to 5, adjacent strands of polypeptides may combine and form these structure.
- When the adjacent polypeptide chains run in same direction (N to C terminus) the structure is termed as parallel β-pleated sheet.
- When the adjacent polypeptide chains run in opposite direction the structure is termed as anti-parallel β-pleated sheet.
- The β-pleated sheet is stabilized by inter chain hydrogen bonds.
- β-keratin contains anti parallel β-pleated sheet.
- Both parallel and anti-parallel β-pleated sheet occur in ot function of proteins as those of helices and β-pleated sheet.It accumulates in the CNS.

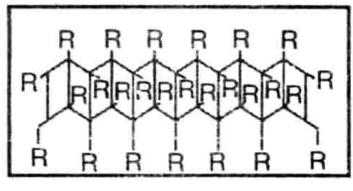

Fig. β Pleated Sheet Showing Arrangement of R groups all the R-groups Project Above (solid line) or Below (broken- line)the Plane.

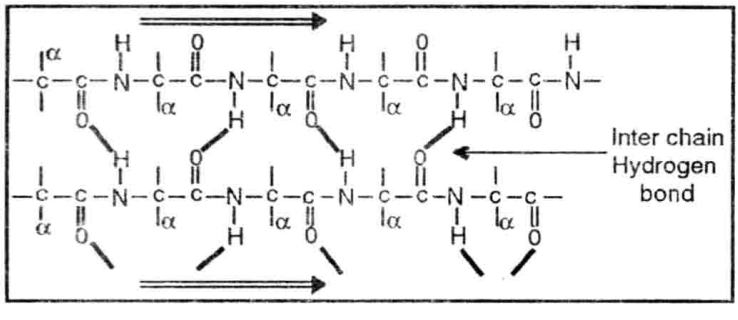

Fig. Parallel β-structure Showing Interchain Hydrogen Bonds

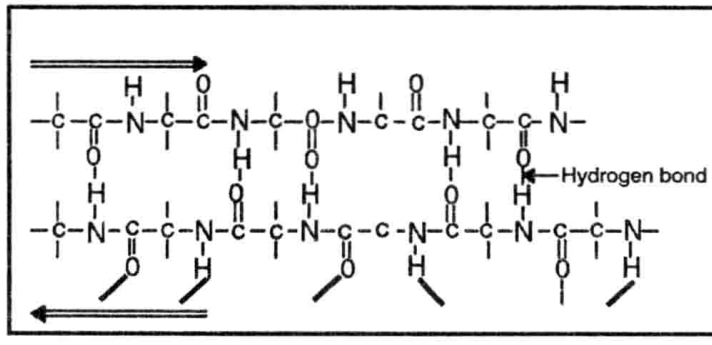

Fig. Anti-Parallel β-Structure

Random Coil (Disordered) Conformation

Regions of proteins that are not organized as helices and pleated sheet are said to be present in andom coil conformation. These are also equally important for biological function of proteins as those of helices and pleated sheet turn or bends (Reverse Turn).

Hair pin turn of a polypeptide chain is called as turn. The change in the direction of a polypeptide chain is achieved by turn. turn connects anti parallel sheets. Usually four aminoacids make up turn. Gly, Ser, Asp, proline are involved in turns.

Super Secondary Structure

In some globular proteins regions of helix and pleated sheet join to form super secondary structure or motifs. They are very important for biological function.

Super Helix

keratin consist of right handed helix as basic unit. Three such helices get cross linked by disulfide bonds and form super secondary structure.

Triple Helix

Collagen present in skin, cartilage, bone and tendons consists of left handed helix as basic unit. Three left handed helices are wrapped around each other to right handed super secondary structure triple helix.

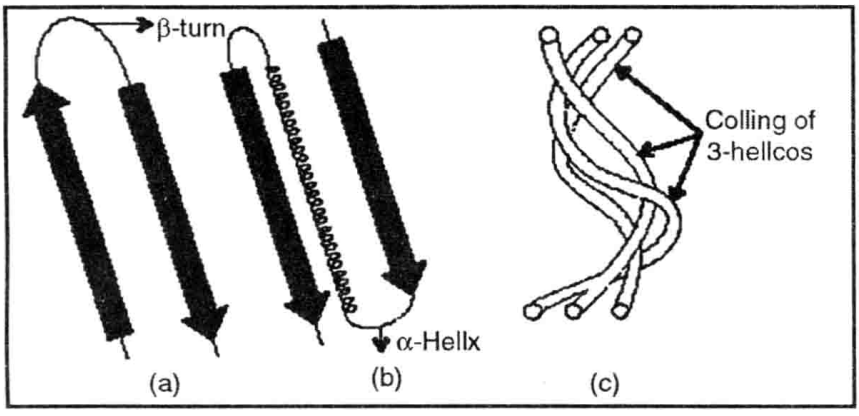

Fig. (a) Two Anti-parallel Chains are Joined by β-turn
(b) Motif (c) Super Secondry Structure

Tertiary Structure

Three-dimensional folding of polypeptide chain is called as tertiary structure. It consists of regions of helices, pleated sheet, turns, motifs and random coil conformations. Interrelationships between these structures are also a part of tertiary structure. Tertiary structure of a protein is mainly stabilized by non-covalent bonds.

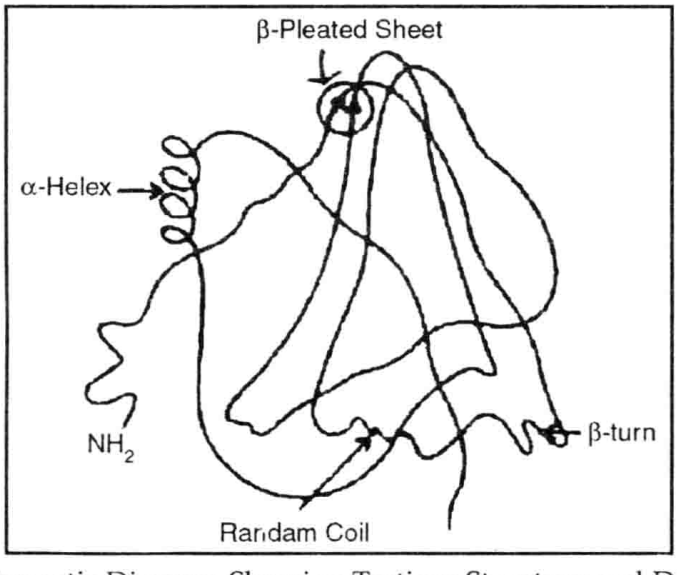

Fig. Schematic Diagram Showing Tertiary Structure and Different Types of Secondary Structures of a Protein Molecule