Proteins

Proteins are the most abundant macromolecules in living cells. Proteins are linear chains of amino acids that are linked together by covalent, peptide bonds. During human digestion, proteins are broken down in the stomach to smaller polypeptide chains via hydrochloric acid and protease actions. This is crucial for the synthesis of the essential amino acids that cannot be biosynthesized by the body. A linear chain of amino acid residues is called a polypeptide. A protein contains at least one long polypeptide. Short polypeptides, containing less than 20–30 residues, are rarely considered to be proteins and are commonly called peptides, or sometimes oligopeptides. The sequence of amino acid residues in a protein is defined by the sequence of a gene, which is encoded in the genetic code. In general, the genetic code specifies 20 standard amino acids. There was many function of protein for example:

1-Transporter like Hemoglobin transports oxygen, Transferrin transports iron, Albumin carries fatty acids and bilirubin.
2-proteins act as enzymes like Glucokinase, Dehydrogenases, Transaminases, Hydrolytic enzymes, pepsin, trypsin, etc .
3-Many proteins serve as storage form like Apoferritin stores iron in the form of ferritin, Myoglobin stores oxygen in muscles.
4-Replication and transcription genes, process mRNAs, etc.
5-Many proteins serve as biological structure, strength or protection like Collagen in bone, Cartilage, elastin of ligaments, Keratin of hair, nail.
6-Many proteins involved in defense mechanism against invasion of foreign substances such as viruses, bacteria and cells. Examples of defence proteins are: Immunoglobulins or antibodies, Fibrinogen and thrombin are blood clotting proteins that prevent loss of blood when the vascular system is injured.
7- Proteins act as hormone like insulin, regulate sugar metabolism; growth hormone of pituitary gland regulates growth of the cells.
8-Receptors enable cells to sense and respond to hormones.

Classification of Protein:

a) Simple Proteins: Classified according to solubility

- Albumins
- Globulins
- Protamine

b) Conjugated Proteins: Contain amino acid + other compounds:

-Glycoproteins
- Lipoproteins
- Nucleoproteins
-Phosphoprotein

c) Derived Proteins: derived from simple or conjugated proteins like peptide.

Denaturation of Proteins : Denaturation of proteins involves the disruption and possible destruction of both the secondary and tertiary structures.

Denaturing Agents

• Denaturation is brought about by certain:
– Physical agents
– Chemical agents
– Mechanical means.

• **Physical agents :**

-Heat
-Ultraviolet rays
-ionizing radiations can denature proteins.

• **Chemical agents:**

-Acids
-alkalies
- certain acid solutions of heavy metals, e.g. mercury, lead.
-detergents; organic solvents like alcohol, acetone, etc.

•**Mechanical means :** Vigorous shaking or grinding leads to denaturation of the protein.

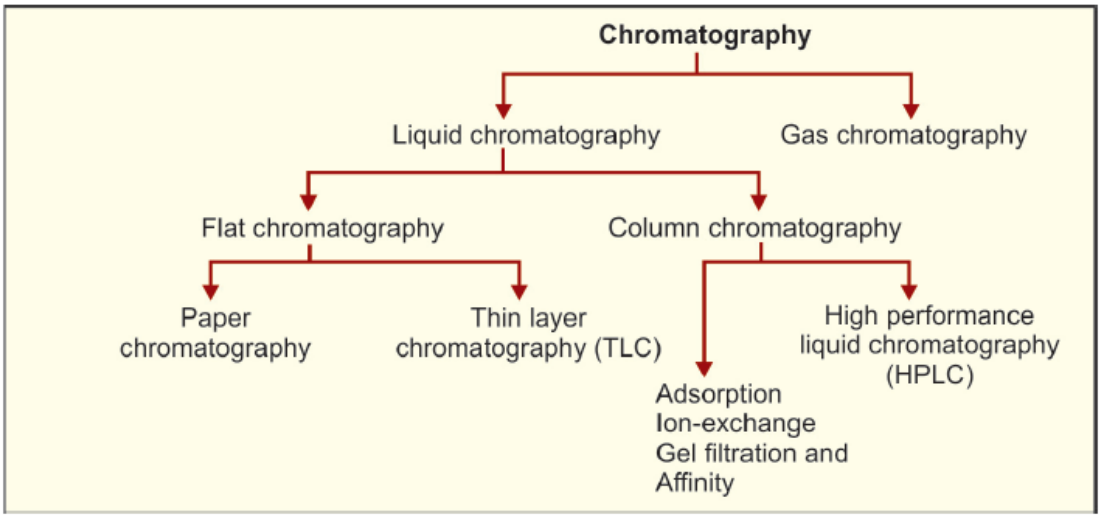
Purification of protein

Highly purified protein is essential for determination of its amino acid sequence, there was many technique for protein purification:

Chromatography

Chromatography is laboratory techniques for the separation of mixtures. The mixture is dissolved in a fluid called the mobile phase, which carries another material called the stationary phase. The various constituents of the mixture travel at different speeds, causing them to separate. The separation is based on differential partitioning between the mobile and stationary phases.

There was many type of chromatography like: Paper chromatography**,** Thin layer chromatography (TLC)**,** Affinity chromatography**,** Ion exchange chromatography also there is techniques used physical state of mobile phase like: Liquid chromatography(HPLC) , Gas chromatography.



-Column Chromatography

Column chromatography of proteins employs as the stationary phase a column containing small spherical beads of modified cellulose, acrylamide, or silica and the liquid mobile phase is percolated through it.

These stationary phase matrices interact with proteins based on their charge, hydrophobicity, and ligand-binding properties. A protein mixture is applied to the column.

-Cation exchange chromatography

The method mostly commonly used for separation is cation exchange chromatography. During ion exchange column chromatography the amino acids are separated by sulphonated polystyrene cation exchange resin, mixed with deriveatization agent (mainly ninhydrin), passed through column and detector . In pH (pH=2.2) hence all of them bear a positive charge. In These conditions all of the amino acids will link to the resin. Therefore, the slower elution the more basic in the solution and the faster elution is the acidic amino acids during the cation exchange. The Process of ion exchange is the following:

$$\mathbf{Matrix{-}SO_3^-{-}H_3N^+{-}CH(R)COOH + Na^+}$$

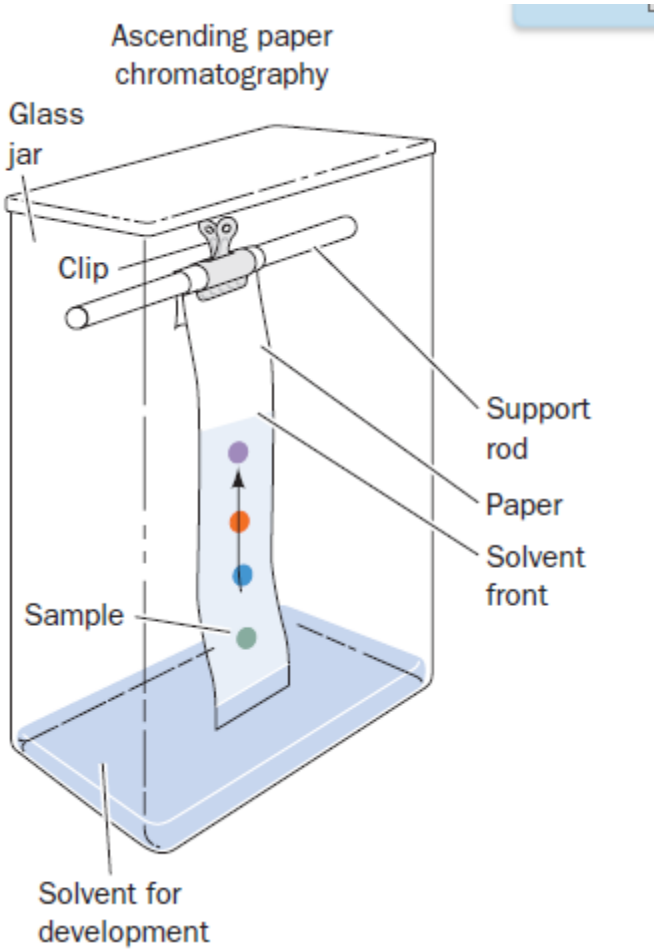
-Anion Resins

Anion resins may be either strongly or weakly basic for anion resins, regeneration typically involves treatment of the resin with a strongly basic solution, e.g. aqueous sodium hydroxide. For example of anion resins is polyethylene amine**.**

Paper Chromatography

Paper chromatography is a partition type of chromatography. Partition chromatography is a process whereby the solutes of a sample are separated by differences in their relative solubilities between two liquid phases. In paper chromatography, filter paper serves as a solid support to hold the stationary phase. Solvent system provides mobile phase consisting of aqueous and organic components; for example, water/ butanol /acetic acid. Keep the paper in the chamber dipping the end of the paper close to spots inside the solvent 2 cm above one end of a strip of filter paper. After drying, that end of the paper is dipped into a solvent mixture. The paper should also be in contact with the equilibrium vapors of the solvent. Solvent mixture is then allowed to run almost to the upper edge of paper. The paper is then removed, dried and sprayed with appropriate staining solution **.**The colored spots seen are identified on the basis of Rf values, which are specific for different substances. The ratio of fronts termed Rf, which is given by:

$$\text{Rf (ratio of fronts)} = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solven}}$$

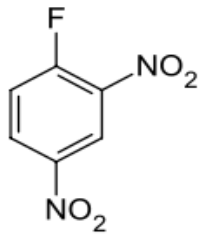


Thin Layer Chromatography (TLC)

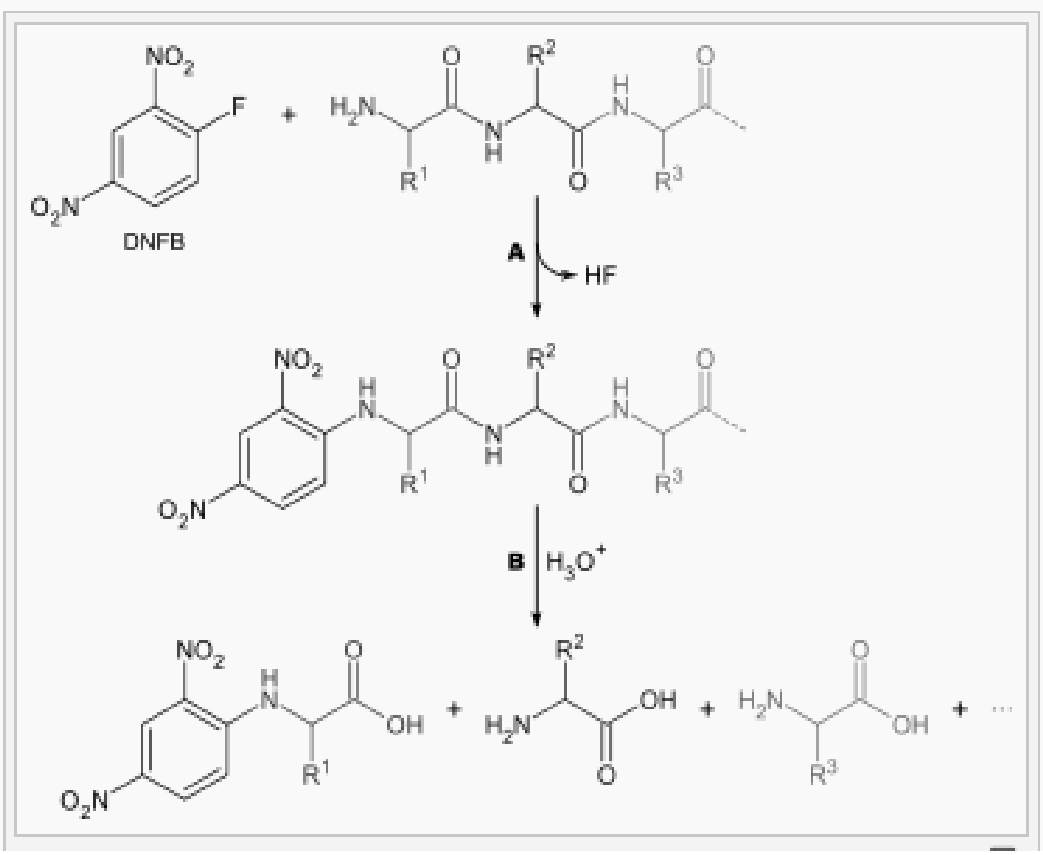
In thin layer chromatography (TLC), a thin (0.25 mm) coating of a solid material spread on a glass or plastic plate is utilized in a manner similar to that of the paper in paper chromatography.

Sequence of polypeptide

1-Sanger reaction: (1-fluoro-2,4-dinitrobenzene):



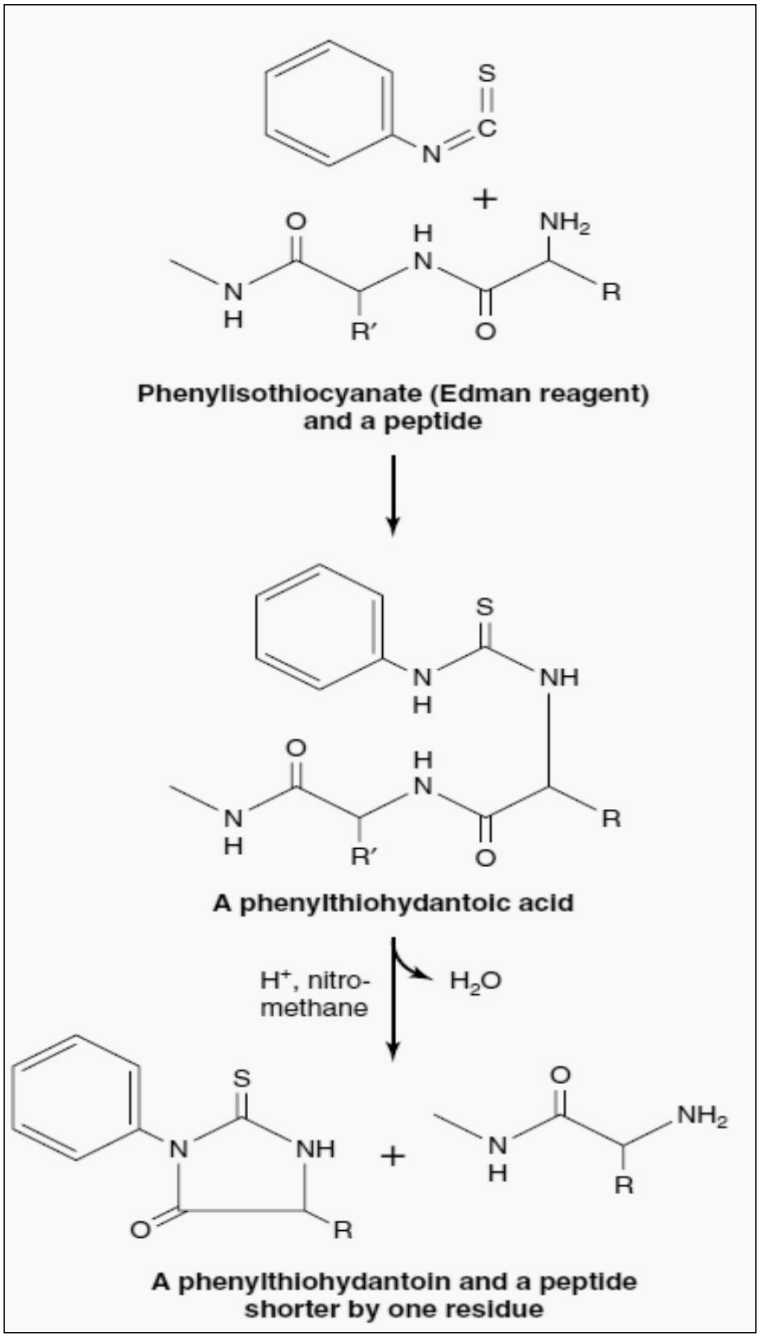
Frederick Sanger described its use for determining the N-terminal amino acid in polypeptide chains. Dinitrofluorobenzene reacts with the amine group in amino acids to produce dinitrophenyl-amino acids.



Sanger reaction

2-The Edman reaction

Edman reaction (Phenylisothiocyanate) is reacted with an uncharged N-terminal amino group, under mildly alkaline conditions, to form a cyclical phenylthiohydantoic acid. Then, under acidic conditions, form thiazolinone derivative.



Edman reaction.

Protein cleavage

There are different methods of cleavage peptide. This reaction is used to reduce the size of polypeptide segments for identification and sequencing.in Table Methods for cleaving polypeptides:

Reagent	Cleavage site
Chemical cleavage	
Cyanogen bromide	Carboxyl side of methionine residues
O-Iodosobenzoate	Carboxyl side of tryptophan residues
Hydroxylamine	Asparagine-glycine bonds
2-Nitro-5-thiocyanobenzoate	Amino side of cysteine residues
Enzymatic cleavage	
Trypsin	Carboxyl side of lysine and arginine residues
Clostripain	Carboxyl side of arginine residues
Staphylococcal protease	Carboxyl side of aspartate and glutamate residues (glutamate only under certain conditions)
Thrombin	Carboxyl side of arginine
Chymotrypsin	Carboxyl side of tyrosine, tryptophan, phenylalanine, leucine, and methionine
Carboxypeptidase A	Amino side of C-terminal amino acid (not arginine, lysine, or proline)

Protein structure

To understand the functions of proteins at a molecular level, it is often necessary to determine their three dimensional structure. This is the topic of the scientific field of structural biology, which employs techniques such as X-ray crystallography, NMR spectroscopy, and dual polarization interferometer to determine the structure of proteins. The four order of protein structure was**;**

1-Primary Structure: the sequence of the amino acids in a polypeptide chain.

2-Secondary Structure: the folding of short (3- to 30-residue).

3-Tertiary Structure: the three-dimensional assembly of secondary structural units to form larger functional units such as the mature polypeptide and its component domains.

4-Quaternary Structure: polypeptide units of oligomeric proteins.

Primary structure

The primary structure of a protein refers to the linear sequence of amino acids in the **polypeptide chain**. The primary structure is held together by covalent bonds such as peptide bonds. The two ends of the polypeptide chain are referred to as the carboxyl terminus (C-terminus) and the amino terminus (N-terminus) based on the nature of the free group on each extremity.

Secondary structure

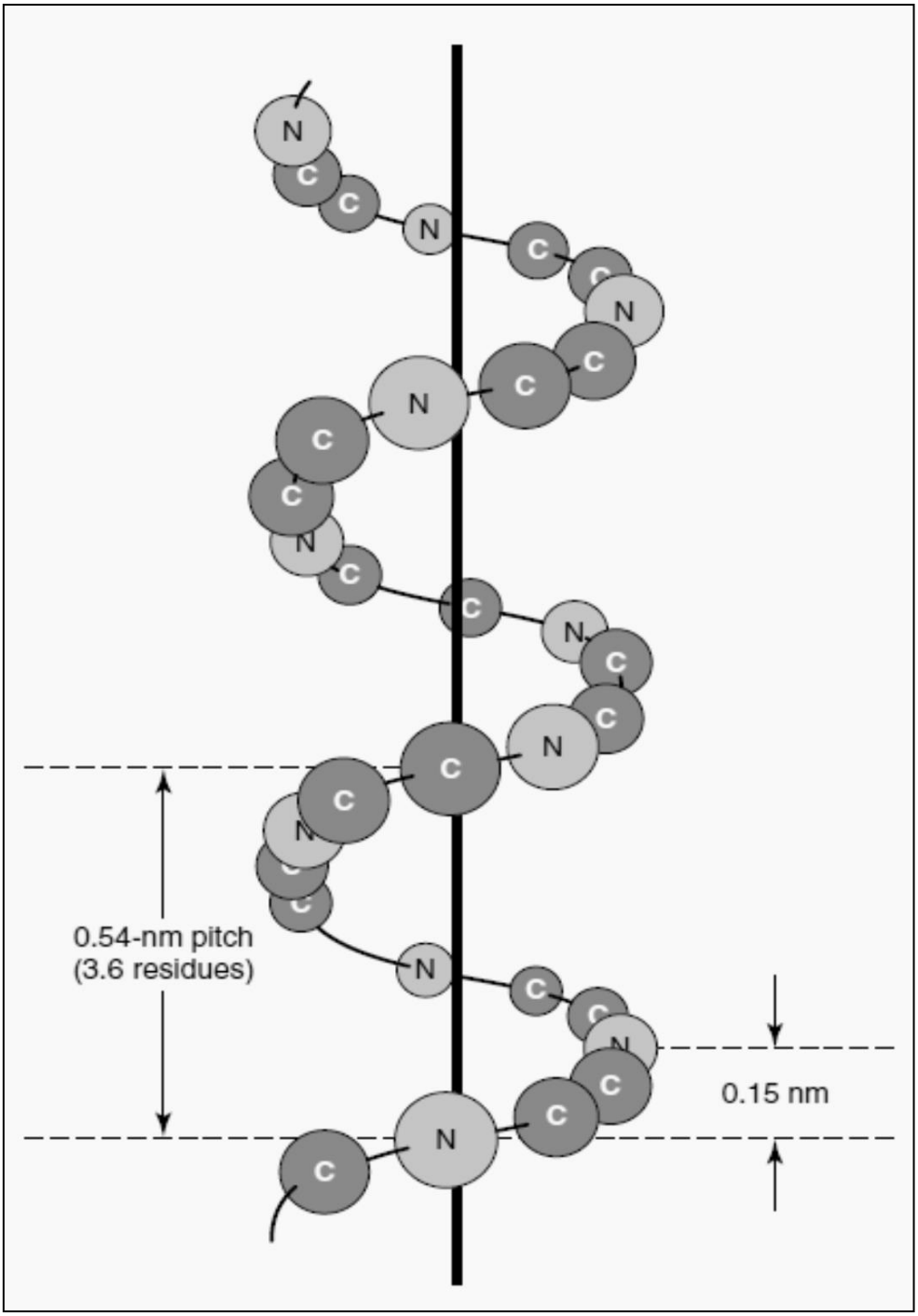
For stability of primary structure, hydrogen bonding between the hydrogen of NH and oxygen of C=O groups .Two main types of secondary structure, **the alpha helix and the beta sheets**.

a-The Alpha Helix

A complete turn of the helix contains an average of 3.6 aminoacyl residues, and the distance it rises per turn (pitch) is 0.54 nm. Proteins contain only L-amino acids, for which a right-handed &-helix is by far the more stable.

The stability of an &-helix arises primarily from hydrogen bonds formed between the oxygen of the peptide bond carbonyl and the hydrogen atom of the peptide bond nitrogen. The ability to form the maximum number of hydrogen bonds, supplemented by vander Waals interactions in the core of this tightly packed structure, provides the thermodynamic driving force for the formation of an &-helix.

Since the peptide bond nitrogen of proline lacks a hydrogen atom can only be stably accommodated within the first turn of an &-helix. When present elsewhere, proline disrupts the conformation of the helix, producing a bend. Because of its small size, glycine also often induces bends in &-helices.



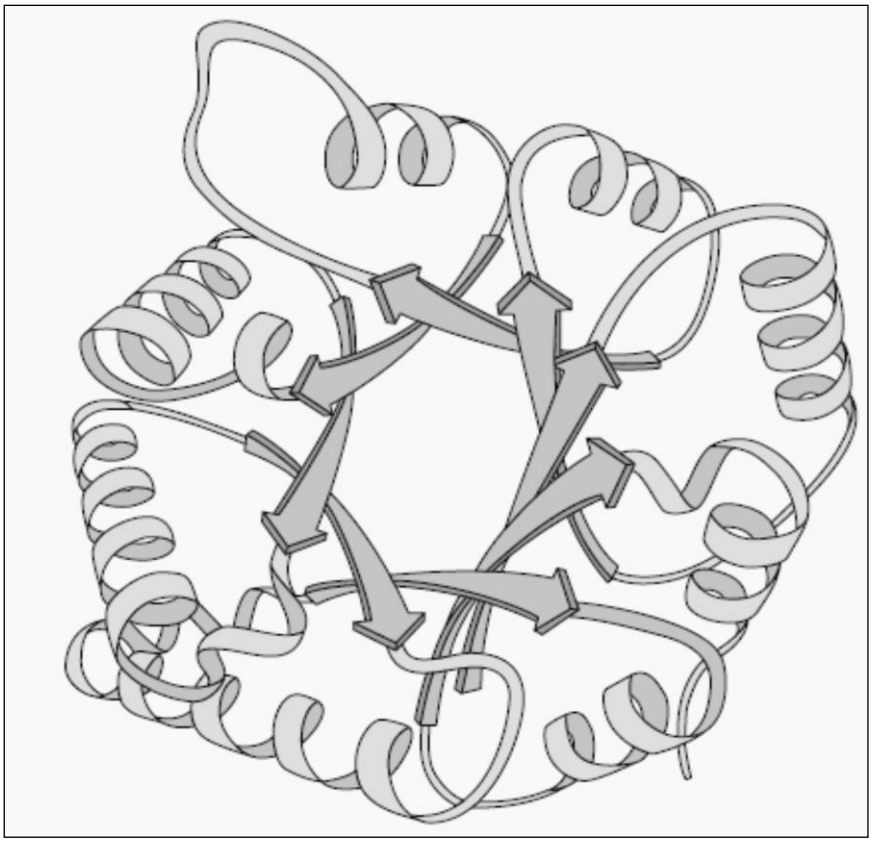
&- helix of secondary structure.

b-The Beta Sheet

Unlike the compact backbone of the &-helix, the peptide backbone of the β-sheet is highly extended. But like the &-helix, β-sheets derive much of their stability from hydrogen bonds between the carbonyl oxygens and amide hydrogens of peptide bonds.

Tertiary Structure

The term "Tertiary Structure" refers to the entire three dimensional conformation of a polypeptide. It indicates, in three-dimensional space, how secondary structural features helices, sheets, bends, turns, and loops assemble to form **domains** and how these domains relate spatially to one another. A domain is a section of protein structure sufficient to perform a particular chemical or physical task. The three-dimensional tertiary structure of a protein is stabilized by: Hydrogen bonds, Van der Waals forces, Hydrophobic interactions, Disulfide bond, Ionic (electrostatic) bonds or salt bridges:



Examples of Tertiary structure of proteins.

Quaternary structure

The polypeptide composition of a protein and the spatial relationships between its **subunits** include **monomeric proteins** consist of a single polypeptide chain. **Dimeric proteins** contain two polypeptide chains. **Homodimers** contain two copies of the same polypeptide chain, while in **a heterodimer** the polypeptides differ. Quaternary Structure Stabilizing Forces. The subunits of polymeric protein are held together by noncovalent interactions or forces such as: Hydrophobic interactions, Hydrogen bond, Ionic bonds.

Globular and fiber proteins

Globular proteins are spherical in shape and soluble in water, acids and bases, while fibrous proteins are not soluble, except in strong concentrations of acid and alkali. Fibrous proteins found in scleroprotein that make up connective tissue, bone matrices, tendons and muscle and are more rod-like in structure like collagen.

Collagen

Collagen is the most abundant of the fibrous proteins that constitute more than 25% of the protein mass in the human body. Other prominent fibrous proteins include keratin and myosin. These proteins represent a primary source of structural strength for cells (ie, the cytoskeleton) and tissues. Skin derives its strength and flexibility from a crisscrossed mesh of collagen and keratin fibers, while bones and teeth are buttressed by an underlying network of collagen. Collagen also is present in connective tissues such as ligaments and tendons.

The high degree of tensile strength required to fulfill these structural roles requires elongated proteins characterized by repetitive amino acid sequences and a regular secondary structure.

Salting out method

When neutral salts such as ammonium sulfate or sodium sulfate are added to a protein solution, the addition may precipitate a protein from its solution. Mineral ions attract water molecules and consequently remove the shell of hydration from around protein molecules. Since water layer around protein particles is removed, the protein is precipitated. This is called salting out.