

**AL- MUSTAQBAL UNIVERSITY**  
**College Of Health And Medical Techniques**  
**Prosthetic Dental Techniques Department**  
**Second Grade**  
**Second Semester**



**Advanced chemistry**

**Lecture 18 (The theoretical part)**

# **(Proteins)**

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## **Lecture 18**

Primary Structure of Proteins-Secondary Structure of Proteins-Tertiary Structure of Proteins-Forces Controlling Structure-Quaternary Structure-Complex Protein Structures-Clinical Significances-Analysis of Protein Structure -Myoglobin and Hemoglobin.

# Proteins

## Protein Functions:

1. Enzyme catalysis: e.g. All enzymes that catalyze cell reactions are proteins.
2. Transport and storage: Hemoglobin transport oxygen, Ferritin store iron.
3. Coordinated motions: e.g. muscles contain two motion proteins (myosin and actin)
4. Mechanical support: e.g. collagen (a fibrous protein)
5. Immune and Protection: e.g. Antibodies (immunoglobulin-A,-G,-M, and-E)
6. Generation and transmission of nerve pulses: e.g. Rhodopsin (a light-sensitive protein in retinal rod cells).
7. Control of growth and differentiation: e.g. (Hormones: insulin, growth hormone...)

## Protein Structure

Protein is a polymer of more than 100 amino acids. Each of them is called residue. There are 4 basic levels of structure in protein architecture:-

### **1-Protein Primary Structure**

The primary structure of peptides and proteins refers to the linear number and order (sequence) of the amino acids present.

### **2-Protein Secondary Structure**

The ordered array of amino acids in a protein confers regular conformational forms upon that protein. These conformations constitute the secondary structures of a protein.

Note: In general proteins fold into two broad classes of structure termed:

- globular proteins** are compactly folded and coiled,
- fibrous proteins** are more filamentous or elongated.

@Two elements of secondary protein structure:

#### **A. The Alpha-Helix:**

- The  $\alpha$ -helix is a common secondary structure encountered in proteins of the globular class. Example; is lysozyme.
- The formation of the  $\alpha$ -helix is spontaneous and is stabilized by H-bonding between amide nitrogens and carbonyl carbons of peptide bonds spaced four residues apart.
- This orientation of H-bonding produces a helical coiling of the peptide backbone such that the R-groups lie on the exterior of the helix and perpendicular to its axis.
- The disruption of the helix is important as it introduces additional folding of the polypeptide backbone to allow the formation of globular proteins.

#### **B. $\beta$ -Pleated Sheets:**

- $\beta$ -sheets are composed of 2 or more different regions of stretches of at least 5-10 amino acids. The folding and alignment of stretches of the polypeptide backbone aside one another to form  $\beta$ -sheets is stabilized by H-bonding between amide nitrogens and carbonyl carbons. Example; silk fibers protein
- The H-bonding residues are present in adjacently opposed stretches of the polypeptide backbone as opposed to a linearly contiguous region of the backbone in the  $\alpha$ -helix.

Q/ Why  $\beta$ -Sheets are said to be pleated?

A/ This is due to positioning of the  $\alpha$ -carbons of the peptide bond which alternates above and below the plane of the sheet.

\* $\beta$ -Sheets are either parallel or antiparallel.

### **3-Tertiary Structure**

- Tertiary structure refers to the complete three-dimensional structure of the polypeptide units of a given protein. It is the spatial relationship of different secondary structures to one another within a polypeptide chain and how these secondary structures themselves fold into the three-dimensional form of the protein.
  - The interactions of different domains is governed by several forces: These include **hydrogen bonding, hydrophobic interactions, electrostatic interactions** and **van der Waals forces**.
- #### **Forces Controlling Protein Structure**

1. **Hydrogen Bonding:** Polypeptides contain numerous proton donors and acceptors both in their backbone and in the R-groups of the amino acids. H-bonding, therefore, occurs not only within and between polypeptide chains but with the surrounding aqueous medium.
2. **Hydrophobic Forces:** The hydrophobicity of certain amino acid R-groups tends to drive them away from the exterior of proteins and into the interior. This driving force restricts the available conformations into which a protein may fold.
3. **Electrostatic Forces:** Electrostatic forces are mainly of three types; **charge-charge**, **charge-dipole** and **dipole-dipole**.
  - Charge-charge interactions are between oppositely charged R-groups such as K or R and D or E.
  - Charge-dipole interactions refers to the interaction of ionized R-groups of amino acids with the dipole of the water molecule.

Q/Why the majority of the amino acids, contain charged or polar R-groups, found on the exterior surfaces of globular proteins.

A/ The slight dipole moment that exist in the polar R-groups of amino acid influences their interaction with water.

4. **van der Waals Forces:** There are both attractive and repulsive van der Waals forces that control protein folding. The repulsion is the result of the electron-electron repulsion that occurs as two clouds of electrons begin to overlap.

\*Note: Although van der Waals forces are extremely weak, relative to other forces governing conformation, it is the huge number of such interactions that occur in large protein molecules that make them significant to the folding of proteins.

5-**Disulfide bonds (Bridge):** The thiol of cysteine is able to form a disulfide bond with other cysteines in the same P.P.Ch. in the Tertiary structure. For example; Ribonuclease A enzyme has 4 disulfide binds.

#### 4-Quaternary Structure:

Proteins with multiple polypeptide chains are termed **oligomeric** proteins. The structure formed by monomer-monomer interaction in an oligomeric protein is known as **quaternary structure**.

e.g. **Hemoglobin**, the oxygen carrying protein of the blood, contains two  $\alpha$  and two  $\beta$  subunits arranged with a quaternary structure in the form,  $\alpha_2\beta_2$ . Hemoglobin is, therefore, a hetero-oligomeric protein.

#### \*Complex Protein Structures

**I-glycoproteins:** Proteins covalently associated with carbohydrates.

Proteins covalently conjugated with carbohydrates following the synthesis (**translation**) of proteins and are, therefore, termed (**post-translational modifications**).

-Glycoproteins are of two classes: (1) N-linked sugars are attached to the amide nitrogen of the R-group of asparagines (2) O-linked sugars are attached to the hydroxyl groups of either serine or threonine.

-There are extremely important glycoproteins found on the surface of erythrocytes. There are at least 100 blood group determinants, most of which are due to carbohydrate differences. The most common blood groups, A, B, and O, are specified by the activity of specific gene products whose activities are to incorporate distinct sugar groups onto RBC membrane glycosphingolipids as well as secreted glycoproteins.

**II-lipoproteins:** protein associated with lipid via noncovalent interactions are termed lipoproteins. Their major function in the body is to aid in the storage transport of lipid and cholesterol.

#### Clinical Significances

**I-**The substitution of a hydrophobic amino acid (V) for an acidic amino acid (E) in the  $\beta$ -chain of hemoglobin results in **sickle cell anemia** (HbS).

\*the deoxygenated proteins polymerize and precipitate within the erythrocyte, leading to their characteristic sickle shape.

**II-**Collagens are the most abundant proteins in the body.

\*Alterations in collagen structure arising from abnormal genes or abnormal processing of collagen proteins results in numerous diseases, including (**Larsen syndrome**, **scurvy**, **osteogenesis imperfecta** and **Ehlers-Danlos syndrome**).

**a- Ehlers-Danlos syndrome** defective collagen structure.

**b-Osteogenesis imperfecta** multiple fractures and resultant bone deformities.

c-**Marfan's syndrome** results from mutations in the extracellular protein, **fibrillin**, which is an integral constituent of the non-collagenous microfibrils of the extracellular matrix.

### Analysis of Protein Structure

#### **&&-Determination of Amino acid composition:**

Protein  $\xrightarrow{\text{Hot } 6\text{N HCl} + 110\text{C}^+ / 24\text{Hours}}$  Free amino acids  $\longrightarrow$  Separation  $\longrightarrow$  Identification by Chromatography

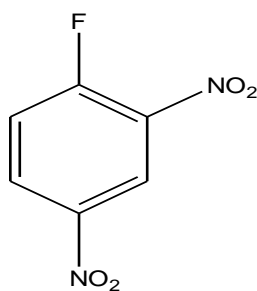
\* In this method all tryptophane and some serine and methionine are destroyed.

#### **I-Amino-Terminal Sequence Determination:**

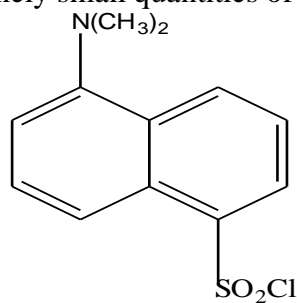
**2-mercaptoethanol** or **dithiothreitol**: used in order to permit separation of peptide strands and prevent protein conformations that are dependent upon disulfide bonds. Both of these chemicals reduce disulfide bonds. To prevent reformation of the disulfide bonds the peptides are treated with **iodoacetic acid** in order to alkylate the free sulfhydryls.

There are three major chemical techniques for sequencing peptides and proteins from the N-terminus. These are the **Sanger, Dansyl chloride** and **Edman techniques**.

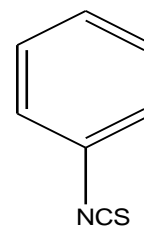
1. **Sanger's Reagent:** This sequencing technique utilizes the compound, 2,4-dinitrofluorobenzene (DNF) which reacts with the N-terminal residue under alkaline conditions. The derivatized amino acid can be hydrolyzed and will be labeled with a dinitrobenzene group that imparts a yellow color to the amino acid. Separation of the modified amino acids (DNP-derivative) by electrophoresis and comparison with the migration of DNP-derivative standards allows for the identification of the N-terminal amino acid.
2. **Dansyl chloride:** Like DNF, dansyl chloride reacts with the N-terminal residue under alkaline conditions. Analysis of the modified amino acids is carried out similarly to the Sanger method except that the dansylated amino acids are detected by fluorescence. This imparts a higher sensitivity into this technique over that of the Sanger method.
3. **Edman degradation:** This method utilizes phenylisothiocyanate to react with the N-terminal residue under alkaline conditions. The resultant phenylthiocarbonyl derivatized amino acid is hydrolyzed in anhydrous acid. The added advantage of the Edman process is that the remainder of the peptide is intact. The entire sequence of reactions can be repeated over and over to obtain the sequences of the peptide. This process has subsequently been automated to allow rapid and efficient sequencing of even extremely small quantities of peptide.



2,4-dinitrofluorobenzene



Dansyl chloride



phenylisothiocyanate

#### **II-Protease Digestion**

Enzymes, **endopeptidases**, cleave at specific sites within the primary sequence of proteins. The resultant smaller peptides can be chromatographically separated and subjected to Edman degradation sequencing reactions. Peptides longer than around 50 residues can not be sequenced completely by Edman degradation technique.

\*For example **Trypsin** can cleave peptide bond C-terminal to R, K, but not if next to P. Other example is **Pepsin** can cleave peptide bond N-terminal to L, F, W, Y, but when next to P

#### **III-Carboxy-Terminal Sequence Determination**

**exopeptidases** enzymes have been identified that cleave peptides at the C-terminal residue which can then be analyzed chromatographically and compared to standard amino acids. This class of exopeptidases is called, **carboxypeptidases**.

\* For example *Carboxypeptidase B* cleaves when C-terminal residue = R or K; not when P resides next to terminal residue, and *Carboxypeptidase C* that cleaves all free C-terminal residues, pH optimum = 3.5

#### IV-Chemical Digestion of Proteins

1. **cyanogen bromide (CNBr)** cleaves peptide bonds at the C-terminal side of M residues.
2. **Hydrazine (NH<sub>2</sub>-NH<sub>2</sub>)** at (90°C) for (20-100hr) cleaves all of the peptide bonds yielding amino-acyl hydrazides of all the amino acids excluding the C-terminal residue which can be identified chromatographically compared to amino acid standards. only used on carboxypeptidase resistant peptides.

#### V- Chromatographic methods for separation:

##### 1. Size Exclusion Chromatography

A porous insoluble gel in the form of beads placed into a column. As a solution of proteins is passed through the column, small proteins can penetrate into the pores of the beads and, therefore, are retarded in their rate of travel through the column. The larger proteins a protein is the less likely it will enter the pores.

##### 2. Ion Exchange Chromatography

Fine cellulose resins are used that are either negatively (**cation exchanger**) or positively (**anion exchanger**) charged. Proteins of opposite charge to the resin are retained as a solution of proteins is passed through the column. The bound proteins are then eluted by passing a solution of ions bearing a charge opposite to that of the column.

##### 3. Affinity Chromatography

Proteins have high affinities for their substrates or co-factors or prosthetic groups or receptors or antibodies raised against them. This affinity can be exploited in the purification of proteins. A column of beads bearing the high affinity compound can be prepared and a solution of protein passed through the column. The bound proteins are then eluted by passing a solution of unbound soluble high affinity compound through the column.

##### 4. Thin layer chromatography:

Same technique but thin layer of adsorbate like silica gel or cellulose on glass or aluminum plate were used instead of column.

##### 5. High Performance Liquid Chromatography (HPLC)

HPLC utilizes tightly packed fine diameter resins to impart increased resolution and overcomes the flow limitations by pumping the solution of proteins through the column under high pressure. An additional separation technique commonly used with HPLC is to utilize hydrophobic resins to retard the movement of nonpolar proteins. The proteins are then eluted from the column with a gradient of increasing concentration of an organic solvent. This latter form of HPLC is termed **reversed-phase HPLC**.

#### VI-Electrophoresis of Proteins

Proteins also can be characterized according to size and charge by separation in an electric current (electrophoresis) within solid sieving gels made from polymerized and cross-linked acrylamide. The most commonly used technique is termed **SDS polyacrylamide gel electrophoresis (SDS-PAGE)**. The gel is a thin slab of acrylamide polymerized between two glass plates. This technique utilizes a negatively charged detergent (sodium dodecyl sulfate) to denature and solubilize proteins. SDS denatured proteins have a uniform negative charge such that all proteins will migrate through the gel in the electric field based solely upon size.

The larger the protein the more slowly it will move through the matrix of the polyacrylamide. Following electrophoresis the migration distance of unknown proteins relative to known standard proteins is assessed by various staining or radiographic detection techniques.

The use of polyacrylamide gel electrophoresis also can be used to determine the isoelectric charge of proteins (**pI**). This technique is termed **isoelectric focusing**.

@ *isoelectric point (pI)*: the PH at which the the compound bears no net charge and does not move when an electrical field applied

Isoelectric focusing utilizes a thin tube of polyacrylamide made in the presence of a mixture of small positively and negatively charged molecules termed ampholytes. Proteins cease migration in the gel when they reach the point where the ampholytes have established a pH equal to the proteins pI.

### **VII-Centrifugation of Proteins**

Proteins will sediment through a solution in a centrifugal field dependent upon their mass. The most common solution utilized is a linear gradient of sucrose (generally from 5-20%). Proteins are layered atop the gradient in an ultracentrifuge tube then subjected to centrifugal fields in excess of 100,000 x g. The sizes of unknown proteins can then be determined by comparing their migration distance in the gradient with those of known standard proteins.

### **VIII-(X-Ray Diffraction):**

A single crystal of protein or layers of protein or protein fibers will deflect x-rays, and the resultant image on the photographic plate can be analyzed to yield information on the crystal or on the structure of the protein. It used for secondary and tertiary structures.