Lecture :..... 5

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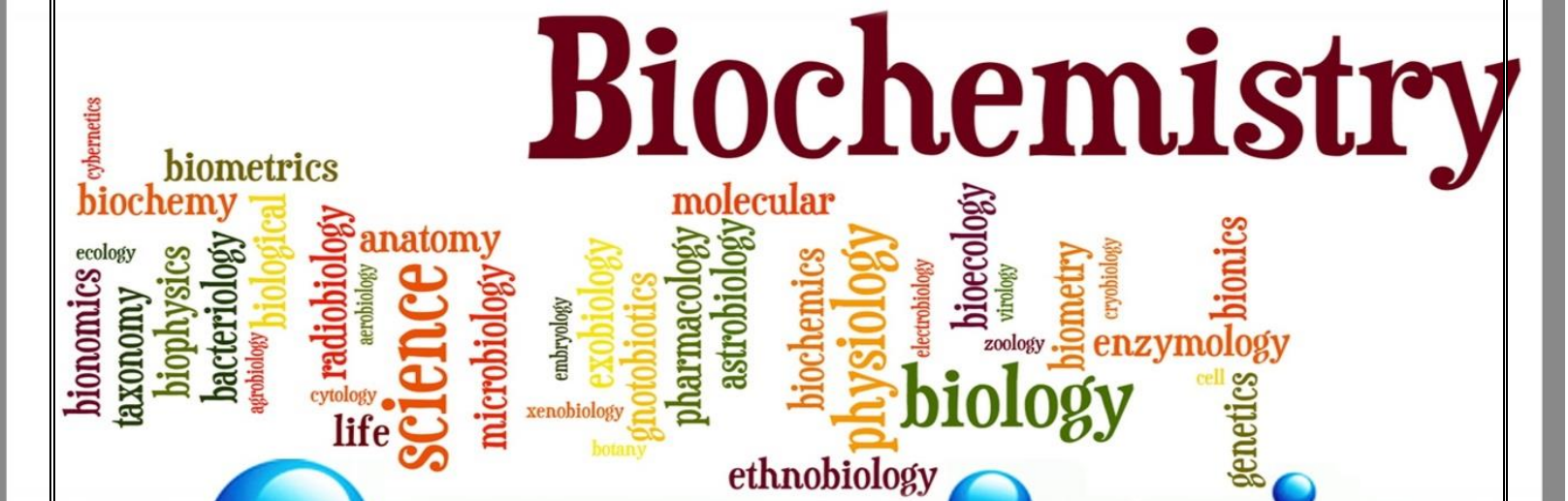
☐ Slide ■ Sheet

Medical Committee
The University of Jordan

Biochemistry



Mousa Subeh

Protein Structure

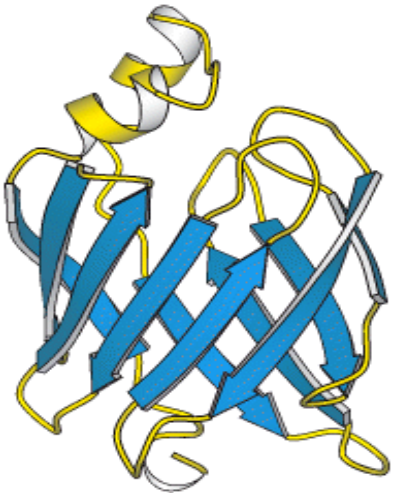
Quick revision ☺

- Levels of protein structure: primary, secondary, tertiary & quaternary.
- Primary structure is the sequence of amino acids residues. It determines the other levels of structure.
- Secondary structure is the local arrangement of the backbone of a polypeptide chain. Most common: α-helix, β-pleated sheet, turns & loops.
- Super secondary structures are regions in proteins that contain an ordered organization of secondary structures. They are of 2 types: motifs & domains.

Today's lecture:

A domain

It is a super secondary structure presented by a specific folding of a protein that results in accumulation (clustering) of multiple secondary structures, that can be of the same type or different types.



- Domains can be present in multiple proteins with similar structure or function. Examples: enzymes that catalyze the same reaction would have the same domains / DNA binding regions have particular domains.

*Question: what are the main differences between domains and motifs?

1) Motifs can tell us about folding or structure of a protein, but not necessarily the function. On the other hand, domains can indicate a certain function for a certain protein.

2) Domains are larger than motifs. A domain may consist of 100-200 residues in various combinations of α helices, β sheets, turns & random coils.

-domains can fold independently of the rest of the protein.

** Properties of proteins:

- **Denaturation**

-It is the disruption of native conformation of a protein (disruption of the tertiary structure of a protein). If the protein has disulfide bonds, the complete disruption is accomplished by reducing (or breaking) them.

-Denaturation involves breaking the non-covalent bonds between the R-groups & the non-covalent bonds between the backbones (we are disrupting almost everything).

*Remember:

Non-covalent bonds –not the covalent bonds and not the disulfide bonds- are the ones that determine the protein's structure.

- **Denatured proteins** lose their properties & activities. **Why?**

Because the activity of a protein is determined by a specific domain or a specific structure, so disrupting this domain results in losing the activity & proteins will become insoluble.

***Question:** is denaturation a special property for the tertiary structure?

No, it's for secondary and quaternary as well.

***Question:** are super secondary structures examples on tertiary structures?

Yes, of course!

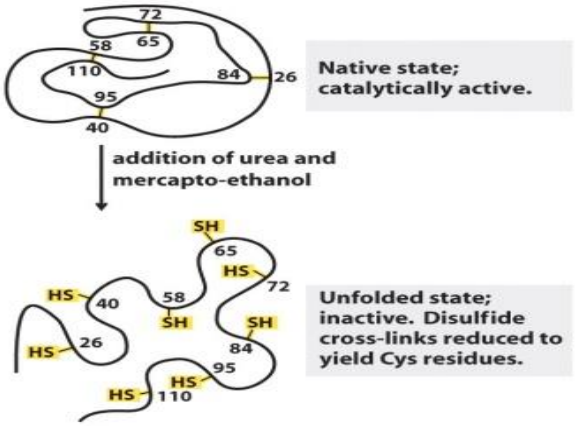
Denaturing agents

1) Heat disrupts (breaks) van der waals forces in proteins. Why? These weak forces are dependent on electrons movement & raising the temperature will increase electrons kinetic energy, so they'll keep moving very fast breaking van der waals forces.
2) Extremes of PH. If we have the protein at acidic or alkaline PH, that will disrupt the protein's structure. Why? The structure is dependent on the non-covalent interactions & one of these interactions is the electrostatic interaction (salt bridges) which can be disrupted by changing the protonation states (protonation or deprotonation) of the amine or carboxyl groups. Hydrogen bonding can be disrupted as well.
3) Detergents (Triton X-100 / SDS). When a protein folds the hydrophobic region clusters inside hiding from water & the hydrophilic region becomes to the outside (on the surface). Adding a detergent whether it is ionic or non-ionic, will form a more hydrophobic environment, so the protein's hydrophobic region will turn from the inside to the outside.
4) Urea & guanidine hydrochloride disrupt hydrogen bonding & hydrophobic interactions.

Reducing agents

Such as: β-mercaptoethanol (βME) & dithiothreitol (DTT)

- Both reduce disulfide bonds to accomplish complete denaturation. Check the example→→



- ## Renaturation

If you **remove** urea (denaturing agent) & βME (reducing agent) from the previous solution, does the protein come back to its original folded structure? Yes, in some cases especially if the protein is small.

- **Renaturation** occurs quickly & spontaneously. The disulfide bonds are formed correctly.

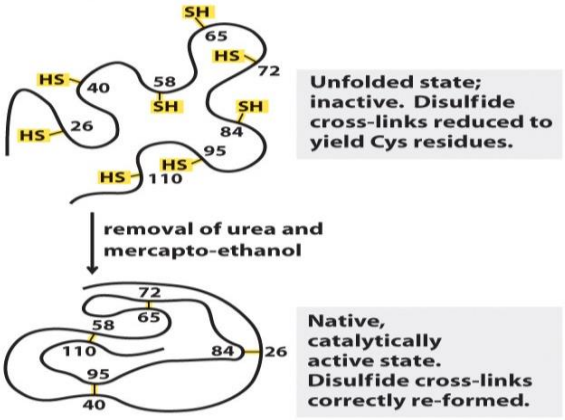
***Question:** why does renaturation occur spontaneously (in some cases, if the protein is small)?

Because of energy. This is the best structure! The factor that determines proteins renaturation is the least amount of energy needed to stabilize the protein.

- The least amount of energy needed to stabilize the protein is determined by:
  1) The amino acids sequence (primary structure).
  2) The proper angles between the R-groups of amino acids.
     And this proper angle is determined (or stabilized) by:
     A) The different sets of weak non-covalent bonds between the R-groups (electrostatic/hydrophobic/hydrogen bonding/van der waals).
     B) Non-protein molecules.

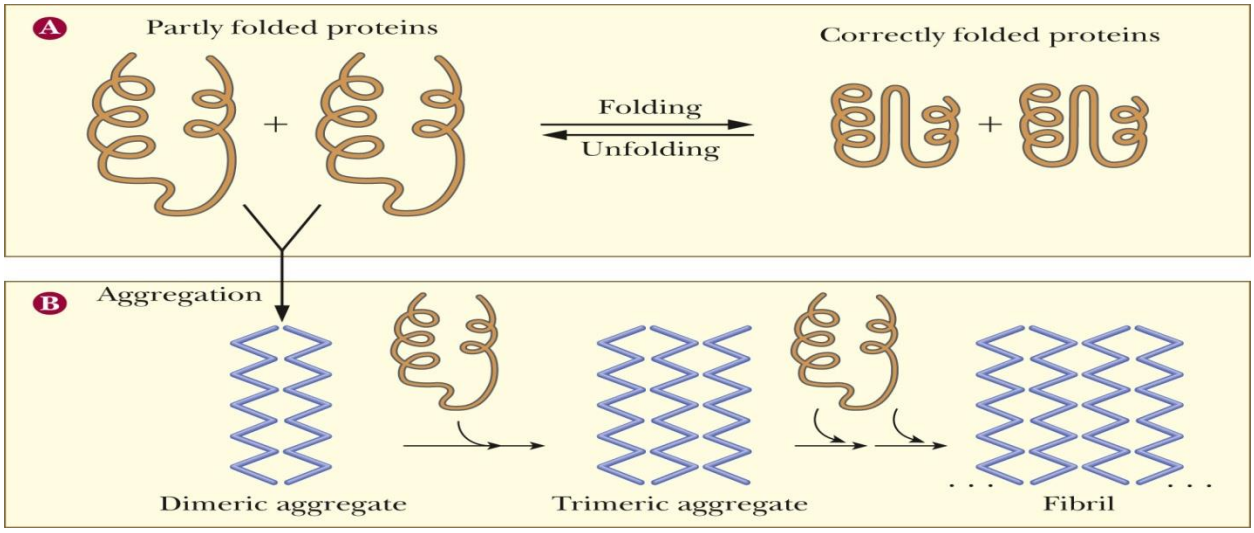
*Question: how does re-folding occur?

Basically, it's stepwise. Each step presents the best energy consumed. After a protein is folded, we have the re-formation of the disulfide bonds between cysteines that are close to each other.



The problem of misfolding

In this case, the internal hydrophobic regions become exposed & meet other hydrophobic regions from other proteins forming aggregates.



Picture explanation: (A) here we have a partly folded protein with exposed hydrophobic regions, but they correctly fold. (B) But sometimes, the two partly folded proteins will aggregate which results in changing the structure of a third polypeptide forming an aggregate.

Problem solvers: chaperones

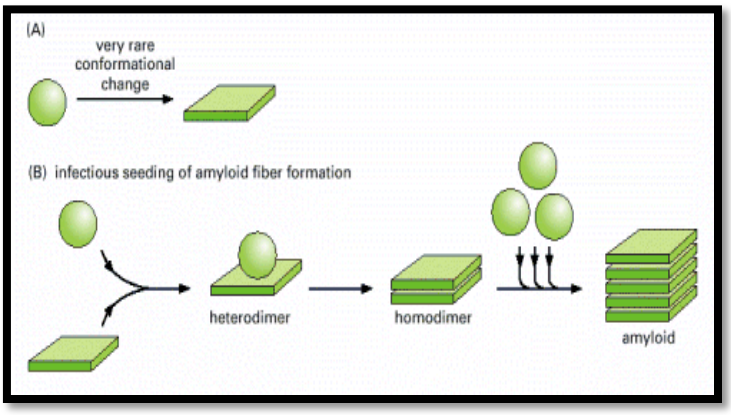
- During protein synthesis inside the cell, hydrophobic regions may fold improperly forming aggregates. Here the **chaperones** are needed as **problem solvers.**
- **Chaperone** (an English word means **المرافق**): a large protein that takes unfolded protein and helps the protein to get folded inside it, releasing the correctly folded protein and the chaperon is then re-used. So, chaperones prevent protein aggregation via hydrophobic interactions.

Diseases resulting from protein misfolding

When a protein folds improperly, aggregates are formed. These aggregates vary in size; they can be soluble dimers or insoluble fibrillar structures (large structures, also called amyloids). Both soluble & insoluble can be toxic to cells causing cell damage.

Prion disease

- At the beginning, scientists thought that all infectious diseases are caused by living organisms that contain DNA or RNA. Such as: viruses & bacteria.
- In the 1960s, it was discovered that prion disease is caused by a protein & this protein can be transmitted from one person to another.
- Prion disease is caused by a misfolded protein called prion protein (PrP), the normal prion protein is full of helical structures. But when it is misfolded, it contains β-pleated



sheets (a completely different structure). Misfolded prion protein can interact with a correctly folded protein forming a dimer & changing it into a misfolded protein that is full of β-pleated sheets instead of alpha helices. And these two dimers can interact with other proteins forming aggregates.

- This is common in the CNS, specifically in the brain causing brain damage.

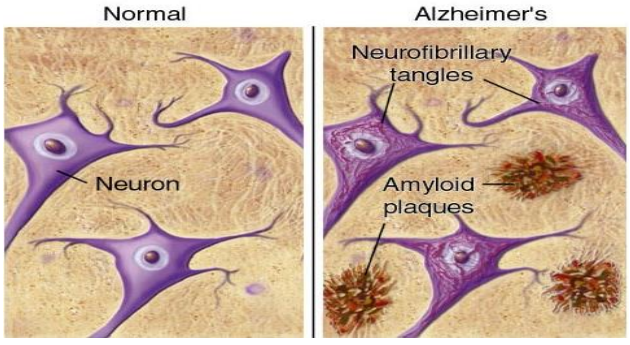
- Prion disease is found in different organisms with different names: Creutzfeldt-Jacob disease (in humans), mad cow disease (in cows) & scarpie (in sheep).
  * The major problem is that prion disease can be transmitted from a cow with mad cow disease (especially if the cow's nutrition is meat) to humans causing Creutzfeldt-Jacob disease by eating its meat.

- Prion disease can be:

1) Infectious by eating a cow or sheep with prion disease.
2) Inherited, if the person himself has a mutation in the DNA that causes the formation of a misfolded prion protein which causes prion disease.
3) Spontaneous.

Alzheimer's disease

- It is caused by a misfolded protein that forms aggregates. It is not transmissible between individuals like prion disease.
- In the normal brain section, you can see the neurons and tissues with the ECM outside. In someone with Alzheimer's disease, you can recognize the formation of plaques which result from aggregation of proteins.



- There are two proteins, one is known as tau another known as amyloid peptide (Aβ). Amyloid peptide is a membrane protein that can be cut (cleaved) by a certain enzyme known as α-secretase. But if it's abnormally cut by 2 enzymes: β-secretase & γ-secretase, this will result with Aβ peptide which can form aggregates with other Aβ peptides forming a plaque.
  - Formation of a plaque or protein aggregates can be toxic to the tissues.

Quaternary structure

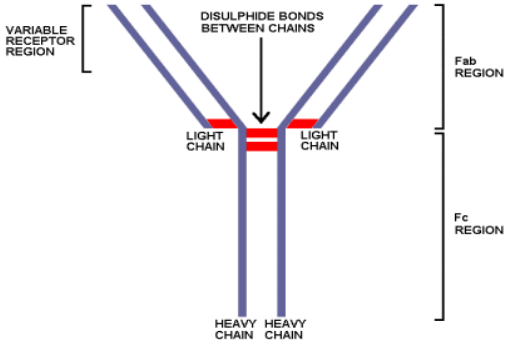
It is composed of more than one polypeptide chain. Called oligomeric proteins (oligo= short / mer= part or unit). Basically, it's the spatial arrangement of subunits & the nature of their interactions.

- Proteins can be: monomer (1 subunit "polypeptide") / dimer (2 subunits) / trimer (3 subunits).....etc
- A protein can be composed of up to 60 different polypeptide chains.
- The simplest protein with quaternary structure is: homodimer (its polypeptide chains are identical)
- If the polypeptide chains are different, it's heterodimer.

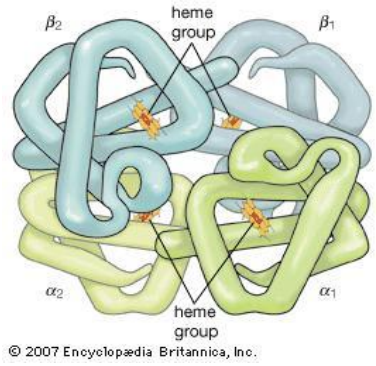
***Question:** how are these polypeptide chains connected?

Depends on the protein. Sometimes by covalent bonds, other times by non-covalent. Here are some examples:

1) Antibodies (are tetramers "composed of 4 polypeptide chains", and still called a heterotetramer. These chains are connected to each other by disulfide bonds).



2) Hemoglobin (a heterotetramer protein in which polypeptide chains are connected to each other via hydrophobic interactions as well as salt-bridges).



Complex protein structures :

Proteins composed of (polypeptide + non-protein component)

Proteins linked to non-protein molecules such as: sugars, lipids & metal ions.

- Holo- & apo- proteins
  Holo- tells you that this complex is composed of a protein component & a non-protein component. If we remove the non-protein component from this protein then the holoprotein is converted into apoprotein.

- Apo- is a protein that is normally composed of a protein and non-protein component except that in this case it doesn't have the non-protein component.

- If I say this is an apolipoprotein… it's a complex of a protein & lipids, but the lipids are extracted (they aren't a part of the protein anymore).

Glycoproteins

Proteins that have sugars covalently bonded (conjugated) to them.

An example is the antibody which can have sugars attached to it.

- **glycoproteins** are of two classes depending on how the sugars are attached to the protein:
    1) N-linked sugars (attached to the amide nitrogen of the R-group of asparagine)
    2) O-linked sugars (attached to the hydroxyl groups of serine or threonine & occasionally to hydroxylysine)

- We can also have **lipoprotein** (protein attached to lipids) which functions in transporting lipids within the system such as: HDL and LDL & **phosphoprotein** (protein attached to a phosphate group>> phosphorylated protein)

Metal ions

They can form a holoprotein. Sometimes they're linked to the protein via non-covalent interactions (electrostatic interactions for example) or they can be linked covalently. Examples:

1) Carbonic anhydrase which has zinc as part of it non-covalently bonded to 3 histidines.

2) heme (part of hemoglobin) which has iron covalently bonded to the protein.

***Question:** is apoprotein functional?

No, it's only functional when bonded to the non-protein component.

Structure-function relationship

Biological functions of proteins DO NOT MEMORIZE ☺

- They can be enzymes, transport molecules (lipoproteins/hemoglobin), storage proteins (myoglobin), involved in contraction & motion of the muscles, structural (collagen), involved in defense (antibodies "immunoglobulin"), signaling (hormones/receptors) & toxins (certain bacterial proteins).

* Structurally, we can divide proteins into 2 types:
1) Fibrous (straight chains) such as collagen, elastins and keratins
2) Globular (rounded) such as myoglobin, immunoglobulin, hemoglobin.

* Cells are surrounded by an extracellular matrix environment; its components are bound to the inner cell proteins in order to work together.

Collagens

Collagen is a protein that exists in the ECM.

It's not just one protein; it's a family of fibrous proteins. There are up to 25 different types. They are the most abundant proteins in mammals, constituting 25% of the total body mass. We called them type 1 collagen; type 2 collagen, type 25 collagen & so on. Their main function is to provide structural support to tissues. In order to provide support, collagen must be stiff!

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RAZAN JARAS