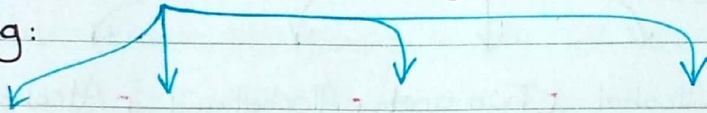


" Proteins 2 "

Tertiary structure of proteins

- its three-dimensional structure, as defined by the atomic coordinates.
 - Le¹ 2² 3³ Proteins Biological active - 3-D structure.
- is the final specific geometric shape that a protein assumes.
- This final shape is determined by a variety of bonding interactions between the "side chains" on the amino acids.
- These bonding interactions may be stronger than the hydrogen bonds between amide groups holding the helical structure.
- As a result, bonding interactions between "side chains" may cause a number of folds, bends, and loops in the protein chain.
- There are four types of bonding interactions between "side chains" including:

- 
- hydrogen bonding.
 - Between side chain -
 - Salt bridges
 ↳ Ionic
 - disulfide bonds
 S-S (covalent bond).
 - non-polar hydrophobic interactions.
 Van-der-Waals

- Disulfide Bonds - covalent bond.
- are formed by oxidation of the sulfhydryl groups on cysteine.
- different protein loops within a single chain are held together by the strong covalent disulfide bonds.
 والتالي

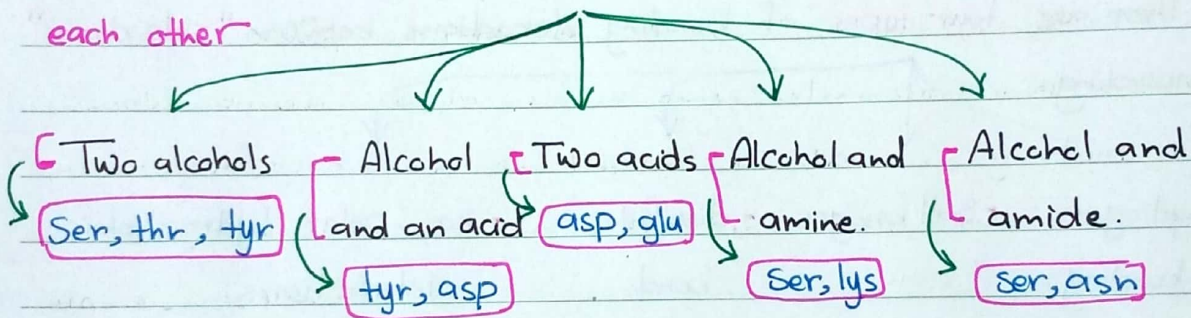
3-D structure في الشكل

- Hydrogen Bonding - [non-covalent bond]

- Between "side chains" occurs in a variety of circumstances.
- The most usual cases are between two alcohols, an alcohol and an acid, two acids, or an alcohol and an amide.

Example: In the prion protein, tyr 128 is hydrogen bonded to asp 178, which cause one part of the chain to be bonding with a part some distance away.

- Examples of amino acid side chains that may hydrogen bond to each other



- Salt Bridges -

result from the neutralization of an acid with an amine on side chains.

The final interaction is **ionic** between the **Positive ammonium group** and the **negative acid group**.

Ex: from the Prion protein with the salt bridge of [glutamic acid 200] and [lysine 204].

لغترسيوا كترسيوا

~ In this case a very small loop is made because there are only three other amino acids between them.

- Non-Polar Hydrophobic Interactions -

- the hydrophobic interactions of non-polar side chains are believed to contribute significantly to the stabilizing of the tertiary structures in proteins. This interaction is really just an application of the solubility rule that "likes dissolve likes".

الشبيه يذيب الشبيه

- The non-polar groups mutually repel water and other polar groups and results in a net attraction of the non-polar groups for each other. Hydrocarbon alkyl groups on [ala, val, leu, and ile] interact in this way. In addition, benzene [aromatic] rings on phe and tyr can "stack" together.

الصورة في الامتحان

- non-polar side chains of amino acids being on the inside of a globular protein.

عادةً - يرتبطان جنباً إلى جنب

- polar groups. outside of the proteins.

DOMAINS

* the tertiary structure of many proteins is built from several domains.

* A domain is a section of protein structure sufficient to perform a particular chemical or physical task such as binding of a substrate or other ligand.

Small proteins may consist of → a single domain.

✓ Each domain is structurally independent of the other domains in the polypeptide chain.
 Secondary structure (البنية الثانوية) - domain structure

✓ Protein domains may be considered as elementary units of protein structure.

Enzyme ^{إنزيم} Pyruvate kinase, it is possible to distinguish three domains.

- α -helix → ^{حلزوني} coil (البنية الثانوية) → three different secondary structure.
- β -sheet → ^{نموذج} zig-zag (البنية الثانوية) ⇒ loops.

Domain functions.

Each domain has a separate function to perform for the protein,

1) Can bind a small ligand.	2) Spanning the plasma membrane (transmembrane proteins).	3) It might contain the catalytic site (enzymes).	4) DNA-binding (in transcription factors).	5) providing a surface to bind specifically to another protein.
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• Protein Quaternary structure

• Is the assembly of ^① several polypeptides to make a unique functional protein, stabilized through several ^② non-covalent interactions between the R-side chain of amino acids from different peptide chains.

① more ^{than} one polypeptides \longleftrightarrow ② non covalent bonds.

- Monomeric proteins consist of a single polypeptide chain.

- Quaternary proteins consist of two or more polypeptide chains that may be structurally identical or totally unrelated.

• dimeric [two subunits]. • trimeric [three subunits].

• multimeric [Several subunits].

✓ The non covalent interactions that maintain the quaternary structure: Hydrogen bonds, Ionic interactions, hydrophobic interactions.....

✓ Not all proteins show a quaternary level of organization.

- For having a quaternary structure -

↳ [a] The protein should be formed by more than one peptide chain.

↳ [B] These chains can not be attached by covalent bonds among them.

..... Some examples to clarify the concept

MYOGLOBIN

Is formed by a single peptide chain and a heme group, since Myoglobin is formed by just one peptide chain, it does not show quaternary structure.

INSULIN

for ex, is formed by ¹two peptide chains, but since these two chains are linked by disulfide linkage [Covalent bonding], insulin does not qualify as a protein with quaternary structure.

HEMOGLOBIN

is formed by ²four peptide chains [and four Heme groups] that are forming a unique functional protein. These peptide chains are associated through ²non covalent bonds between their lateral chains: Hemoglobin is the typical example of a protein with quaternary structure.

كل وحدة تستقل كالم أو يمكن مع بعضها لتعاونوا :

- subunits may either function independently of each other, or may work cooperatively, as in hemoglobin, in which the binding of oxygen to one subunit of the tetramer increases the affinity of the other subunits for oxygen.

اربع وحدات [H₂O] ، الارتباط ما بعد كل وحدة وحدة ، الريبوسيد انو الاكسجين الاولى والحزبي الاول مع يتبد مع chain وحدة ، ارتباط الاولى يستعمل ارتباط الثانية ، ارتباط الاولى والثانية مع يستعمل ارتباط الثانية ، ارتباط الاولى والثانية ، ارتباط الاولى والثانية [اصفة سمي] .

Protein folding It is the physical process by which a polypeptide folds into its characteristic and functional 3-D structure from an random coil.

↳ unfolded

✓ Most proteins fold spontaneously into their native conformation, even in the test tube.

← 3-D structure

✓ Amino acids sequence of a polypeptide chain contains all the information required to fold the chain into its native, 3-D structure.

✓ Each protein exists as an unfolded polypeptide or random coil when translated from a sequence of mRNA to a linear chain of amino acids.

✓ As a peptide folds, its amino acid side chains are attracted and repulsed according to their chemical properties.

⇒ The process of folding often begins co-translationally, so that the N-terminus of the protein begins to fold while the C-terminal portion of the protein is still being synthesized by the ribosome.

✓ **Chaperones** :- specialized proteins, assist in the folding of other proteins.

✓ The folding process depends on the solvent, salt concentration, the temperature, and the presence of molecular chaperones.

✓ Failure to fold into native structure produces inactive proteins that are usually toxic. مثال: البروتينات

✓ Several neurodegenerative and other diseases are believed to result from the accumulation of amyloid fibrils formed by misfolded proteins.

structure ← 3-D مثال: البروتينات

• Role of chaperones in protein folding

✓ One major function of chaperones is to prevent both newly synthesized polypeptide chains and assembled subunits from aggregating into non-functional structures.

✓ also used to prevent ^{من} misfolding and aggregation that may occur as a consequence of exposure to heat or other changes in the cellular environment.

✓ In the cellular environment, newly synthesized proteins are at great risk of aberrant folding and aggregation, potentially forming toxic species.

~ To avoid these dangers, cells invest in a complex network of molecular chaperones, which use ingenious mechanisms to prevent aggregation and promote efficient folding.

~ For many proteins, completion of folding requires the subsequent interaction with one of the large oligomeric ring-shaped proteins of the chaperon family. پروتینان با حجم بزرگ

~ These chaperone proteins bind partially folded polypeptide in their central cavity and promote folding by [ATP-dependent cycles] of release and rebinding.

~ In these reactions, molecular chaperones interact predominantly with the hydrophobic surfaces exposed by non-native polypeptides, thereby preventing incorrect folding and aggregation.

~ Protein Denaturation ~

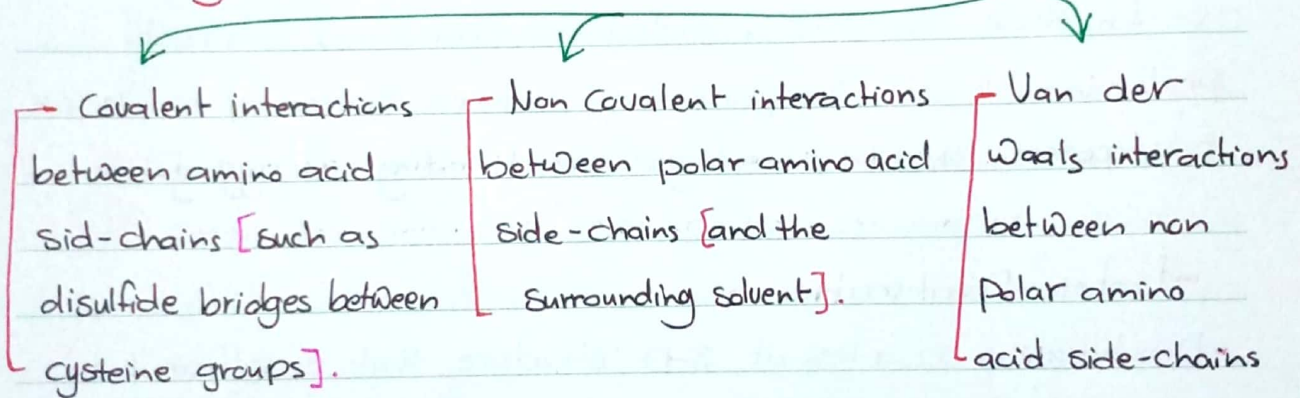
- Denaturation is a loss of 3-D structure that is sufficient to cause loss of function. renaturation ← عودت
- Denaturation involves the breaking of the non-covalent bonds which determine the structure of a protein.
- If proteins in a living cell are denatured, this results in disruption of cell activity and possibly cell death.
- Denatured proteins can exhibit a wide range of characteristics, from loss of solubility to communal aggregation.

- Communal aggregation is the phenomenon of aggregation of the hydrophobic proteins to come closer and form the bonding between them, so as to reduce the total area exposed to water.

How denaturation occurs at levels of protein structure

- In quaternary structure denaturation, protein sub-units are dissociated and/or the spatial arrangement of protein subunits is disrupted.

- Tertiary structure denaturation involves the disruption of



- In secondary structure denaturation, proteins lose all regular repeating patterns such as alpha-helices and beta-pleated sheets, and adopt a random coil configuration.

- Primary structure, such as the sequence of amino acids held together by covalent peptide bonds, is not disrupted by denaturation.

- A protein can be denatured, by denaturing agents :-

- Acids

Acetic acid, Trichloroacetic acid 12% in water.

- Solvents

Ethanol, Methanol - - -

- Cross-linking reagents

formaldehyde, glutaraldehyde.

- Chaotropic agents

urea 6-8M, Guanidinium chloride 6M.

- Disulfide bond reducers

2-Mercaptoethanol, Dithiothreitol (DTT).

~ Protein Renaturation ~

~ the process of returning a denatured protein structure to its original structure and normal level of biological activity.

~ In a renatured protein, the primary structure of the biopolymer remains the same but the protein which had been denatured gets restored back to its former native structure and is able to function as effectively as before, because a renatured protein merely undergoes the process of reversal of a denatured protein.

~ Experimental results prove that the amino acids sequence of a polypeptide chain contains all the information required to fold the chain into its native, 3-D structure.

✓ Thus dismiss any remaining doubt that enzymes fold spontaneously ~

[Refolding of the solubilised proteins]

✓ is initiated by the removal of the denaturant.

✓ To slow down the aggregation process, refolding is usually carried out at low protein concentrations, in the range of 10-100 mg/ml.

- Important variables are:-

- buffer composition (pH, ionic strength).
- Temperature.

- Different methods for the refolding of proteins -

1) Dialysis the most used method is the removal of the solubilising agent by dialysis.

During dialysis the concentration of the solubilizing agent decreases slowly which allows the protein to refold optimally.

2) Slow dilution the concentration of the solubilizing agent is decreased by dilution allowing the protein to refold. Usually the dilution is carried out slowly by step-wise addition of buffer or by continuous addition using a pump.

3) Chromatography

← فصل المواد عن الوسط

- the solubilising agent is removed using a chromatographic step.
- Size exclusion chromatography [eg. gel filtration].
[فصل المواد بناءً على حجمها] (المواد الكبيرة لا تدخل المسام الصغيرة)
- ion exchange chromatography.
[فصل المواد بناءً على شحنتها]
- affinity chromatography.
[الصورة في الـ 1 لا يد]