Lecture : 7 section 1 , 2 , 3

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☐ Slide ■ Sheet

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Biochemistry

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The Three-Dimensional Structure of Proteins

Tertiary Structure of Proteins :

Forces involved in tertiary structure:-

Higher-order level of structure depends on noncovalent interactions. Noncovalent stabilizing forces contribute to the most stable structure for a given protein, the one with the lowest energy

1-Noncovalent interactions
2-Hydrogen bonding
3-electrostatic attraction
4-Hydrophobic interactions

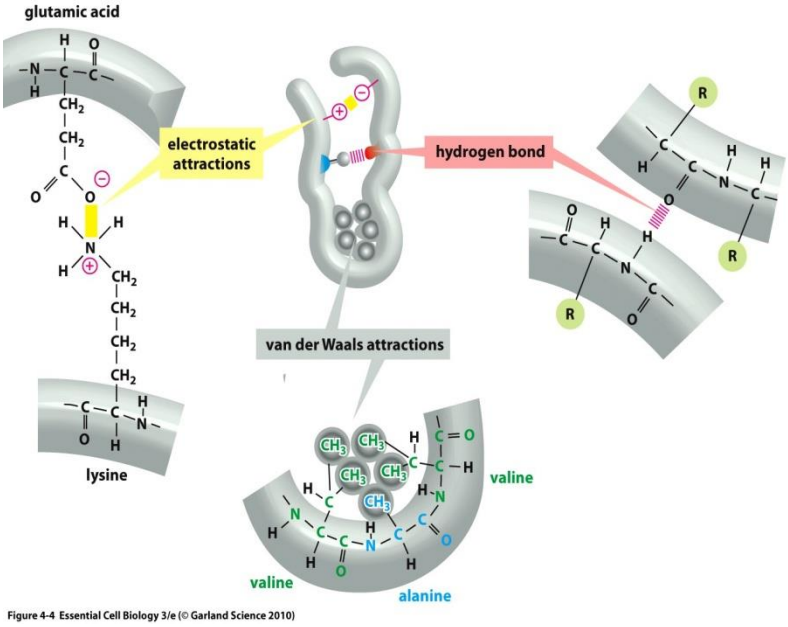
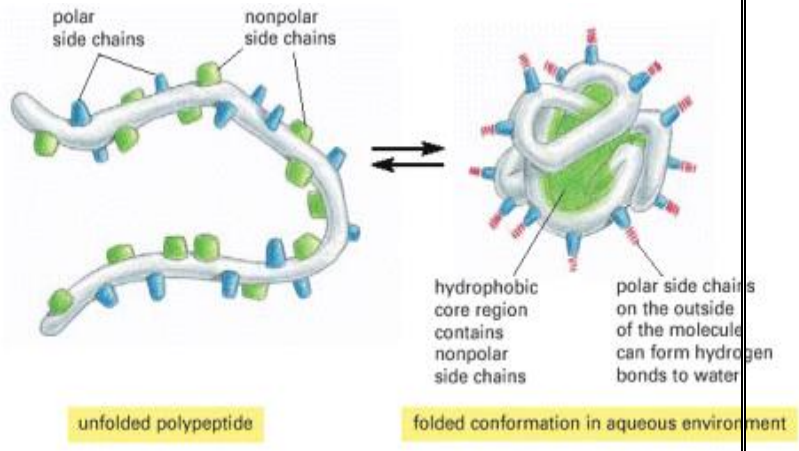


Figure 4-4 Essential Cell Biology 3/e (© Garland Science 2010)

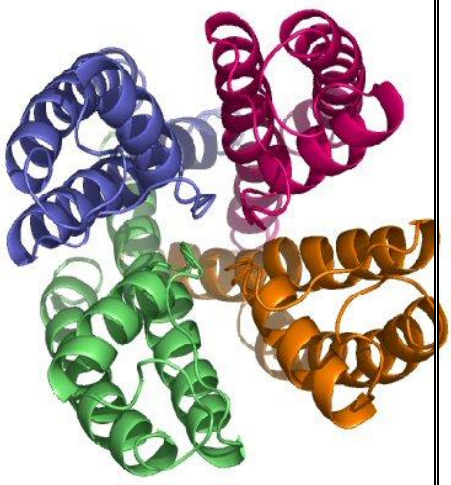
.**Van der Waals forces .**

Hydrophobic interactions are the ones responsible for making hydrophobic (nonpolar) residues cluster in the interior of the protein molecule and the hydrophilic (polar) residues remain exteriorly.
Nevertheless, we can find hydrophilic amino acids in the core interiorly and they are stabilized by hydrogen bonding to the backbone of the amino acids .



These hydrophilic amino acids that are found in the interior of the protein have functional reasons. For example: In Myoglobin, the interior of the protein contains almost exclusively nonpolar amino acid residues. But we can also find two polar histidine residues in the interior; they are involved in interactions with the heme group and bound oxygen, and thus play an important role in the function of the molecule.

Quaternary Structure of Proteins :-



It is the final level of protein structure and it's the structure in which proteins consist of more than one polypeptide chain , Each chain is called a subunit The number can range from two or more than a dozen , and the subunits can be identical or different

If the subunits are identical they are called
*Homooligomers
if the subunits are different they are called
*Heterooligomers

Oligomer is the generic term for a molecule made up of a small number of amino acids: dimers, trimers, and tetramers

Each polypeptide chain folds by itself creating the final shape. These chains interact with one another noncovalently via electrostatic attractions, hydrogen bonds, and hydrophobic interactions

What is the importance of the quaternary structure?

The quaternary structure regulates the protein function whether it is a dimer, trimer, or tetramer , It isn't a rigid structure, instead the subunits move with respect to one another,Consider Hemoglobin as an example Structural changes during binding and unbinding of oxygen from one subunit to another occur through the rotation of the subunit for about 15 degree , during this movement non-covalent bonds are breaking and reforming continuously and this wouldn't occur if we had covalent bonds .

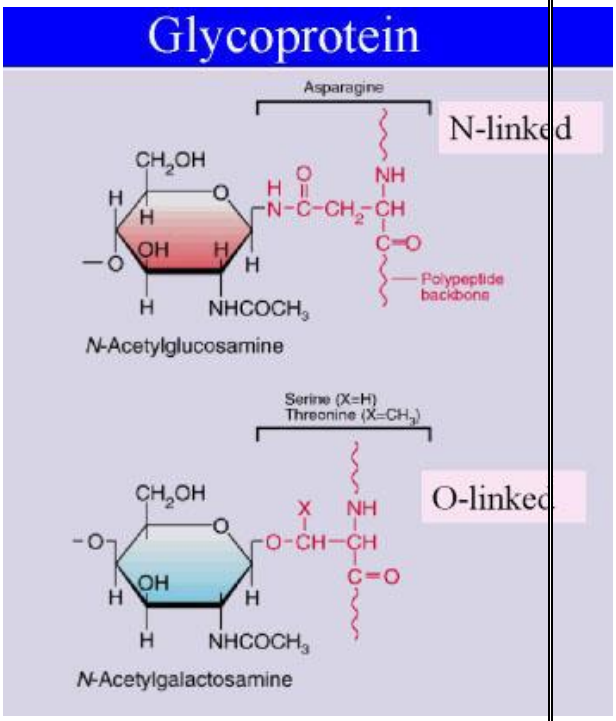
-Disulfide bonds also occur in the quaternary structure, which hold the subunits together and further stabilize the protein molecule

For example, Insulin and the antibody, they are dimers, two subunits held together by a disulfide bond .

Complex Protein Structures :-

Proteins are associated with other macromolecules such as: carbohydrates, lipids and phosphates and they perform different functions

Glycoproteins :-

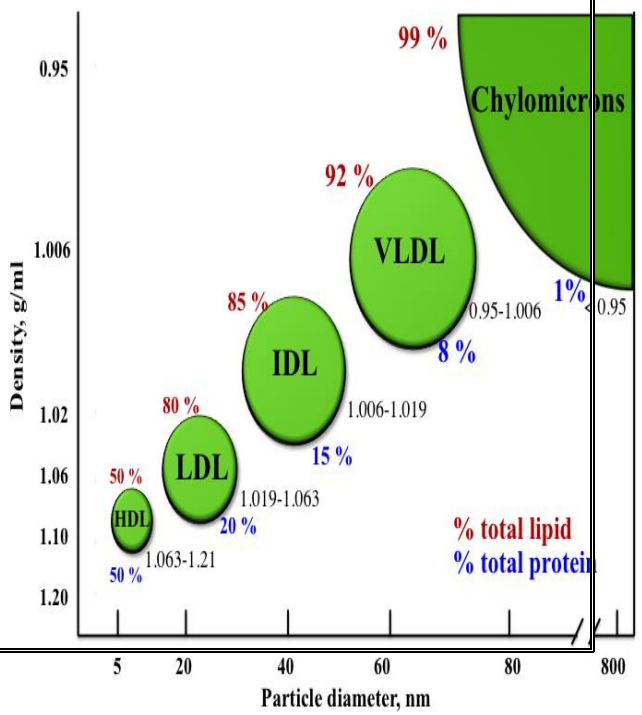


Binding of proteins to carbohydrates occur at specific sites unlike binding to lipids which is random , They can bind either to oxygen or nitrogen, We have several amino acids with nitrogen in their side chains like Lysine, Arginine, and Glutamine, But the only amino acid which can bind to carbohydrates to form a complex protein structure is **Asparagine** .

On the other hand, carbohydrates can bind to three amino acids with hydroxyl groups in their side chains that are: Serine, Threonine and Tyrosine .

Occasionally if there is a modification to Lysine to become hydroxylysine, it will link through its nitrogen with the carbohydrates

Lipoproteins :-



There are five major groups of lipoproteins: cholymicrons, very-low density lipoprotein (VDL), intermediate density lipoprotein (IDL), low density lipoprotein (LDL) and high-density lipoprotein (HDL). (fats) such as cholesterol, phospholipids and triglycerides within the bloodstream .

Phosphoproteins :-

They are proteins which are posttranslationally modified by the covalent attachment of a phosphate group through phosphorylation ,Phosphate group binds to oxygen and forms phosphodiester bond with serine, threonine and tyrosine (Amino acids with hydroxyl group in their side chain)

What is the purpose of phosphorylation ?

Phosphorylation regulates the function of many proteins and enzymes through kinases and phosphatases , kinases phosphorylate proteins while Phosphatases dephosphorylate proteins

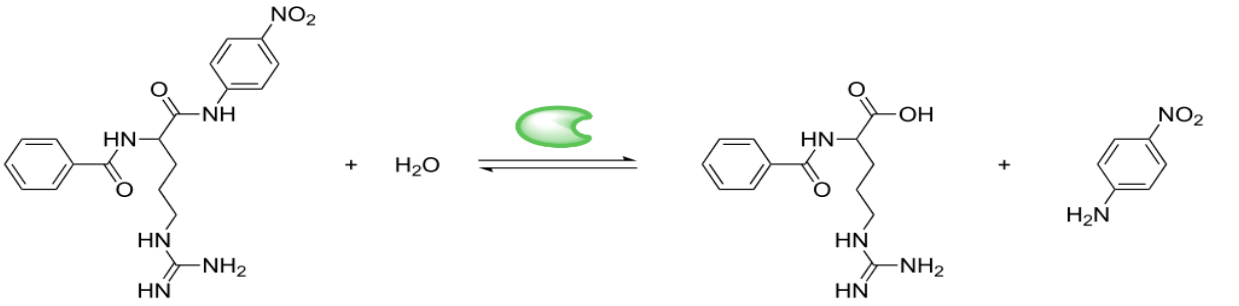
-many enzymes and receptors are switched on and off through phosphorylation and dephosphorylation

-Phosphorylation doesn't mean activation sometimes dephosphorylation causes activation

Chemical properties of proteins :-

All different kinds of proteins share two main chemical properties :-

1- Protein hydrolysis :-

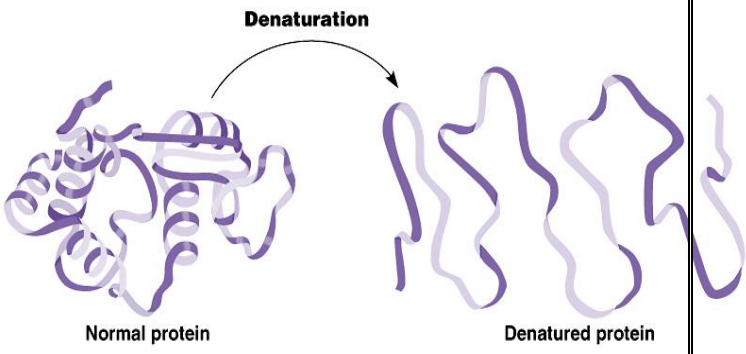


benzoyl-Arg *p*-nitroanilide (BAPNA) | benzoyl-Arg | *p*-nitroaniline (yellow, A: 410 nm)

-proteins can be hydrolyzed or broken down into their components of amino acid by the addition of water and this won't happen unless we have proteolytic enzymes called proteases ,proteins are formed through condensation reaction by the elimination of water molecule and forming the peptide bond and broken down through hydrolysis
-examples of proteases : Pepsin ,Trypsin ,Chymotrypsin and they are all digestive enzymes .

Protein denaturation :-

The noncovalent interactions that maintain the three-dimensional structure of a protein are weak and can be easily disrupted, The unfolding of a protein (disruption of the tertiary structure) is called denaturation .



Due to denaturation, the protein becomes inactive and loses its function by affecting its binding ability. The unfolding of the protein results in the primary structure where the protein is linear and enzymes can act on it.

What is the purpose of denaturation ?

Although denaturation is linked to loss of biological functions and destroying protein, it has an important

function. Denaturation of proteins doesn't affect the primary structure so it remains linear and enzymes can hydrolyze it like in stomach and intestine .

Why do boiled and fried eggs become hard ?

Denatured proteins can exhibit a wide range of characteristics, from loss of solubility to the aggregation of hydrophobic proteins. When food is cooked, proteins become denatured like in eggs. Globular proteins in eggs (albumin) have hydrophobic amino acids interiorly and hydrophilic ones on the exterior surface. When denaturation occurs due to heat, and the protein unfolds to its primary structure, hydrophobic amino acids become in contact with the water medium which is unstable. That's why they accumulate together from several proteins decreasing the solubility and forming the solid state.

❖ Causes of denaturation :-

Heat: Most of the proteins denature on a temperature above 50°

.

Mechanical agitation: Repetitive movement causes the noncovalent interactions to break leading to denaturation. This can be observed when preparing cream due to the continuous stirring of the egg whites .

Peptide bonds are not broken through the denaturation process

Detergents: These cleaning solutions tend to disrupt hydrophobic interactions Examples :-

Triton X-100 (nonionic, uncharged)

▪ Sodium dodecyl sulfate (SDS, anionic, charged) – because it's negatively charged it also affects electrostatic interactions.

.Organic compounds: like acetone and alcohol -

Why alcohol causes denaturation ?

Alcohols bind polar amino acids and causes denaturation of the bacterial proteins (found in the cell wall) and this leads to the

death of the bacterial cells that are found on the hand. That's why it's widely used in hospitals

- pH change: This causes denaturation of proteins in stomach beside digestive enzymes. pH in the stomach (around=2) alters due to food entering the stomach. This leads to the change of the charged amino acids that are found in the protein structure and so electrostatic interactions that stabilize the shape of the protein are .drastically reduced
- Other reagents that are used: Urea and guanidine hydrochloride and they are used if the protein doesn't contain cysteine which means it doesn't contain a disulfide .bond
- Other reducing agents :β-mercaptoethanol (βME) and) dithiothreitol (DTT) that are frequently used to reduce .disulfide bridges to two thiol groups

Most denaturations are irreversible. Sometimes certain proteins .can be renaturated

Factors That Determine Protein Structure :-

The least amount of energy needed to stabilize the protein. This is determined by

- The amino acid sequence (the primary structure), .mainly the internal residues- hydrophobic
- Proper angles between amino acids which depend on

The different sets of weak noncovalent bonds that form between the atoms in the polypeptide backbone and in the amino acid side chains .

Non – protein molecules like heme. The presence of heme in myglobin and hemoglobin preserves the tertiary structure of these .proteins

Chaperones: these special proteins preserve the structure of the .molecule and the word chaperone means protector or companion

The Problem of Misfolding:

Sometimes proteins fail to fold into their normal configuration and they become misfolded. The Misfolding of the tertiary white structure of globular proteins will result in

During misfolding, some of hydrophobic amino acids will be exposed to the surface and this will lead to the attraction of other hydrophobic materials to reach stability and decrease energy. And the most favorable way to do this is by attaching to similar proteins and aggregates will form as a result.

Protein misfolding propagates in the body because the formed aggregates attach to the newly formed proteins during folding causing errors in the folding process and leading to more aggregates.

Problem Solvers: Chaperones

To help avoid the protein misfolding problem, chaperones aid in the correct and timely folding of many proteins. The first chaperones discovered were a family called hsp70 (MW heat-shock proteins for 70,000). Chaperones exist in organisms from prokaryotes through humans and they are of great importance. They are barrel- shaped and are attached to ribosomes. When the polypeptide exits the ribosome during translation, it enters the chaperone for assistance in the folding process. When it is finally folded, it leaves the chaperone with its final structure.

To sum up: If you have three proteins that have 5th ,cysteine in the 2nd and 8th positions: The first protein will have a disulfide bond between the 2nd and the 5th cysteine, the second protein will a disulfide bond between the 5th and the 8th cysteine and the last one will have a disulfide bond between the 2nd and the 8th cysteine. Who is behind this difference?

The presence of chaperones that allow the proteins to fold in a certain way.

Protein Folding Diseases:

- Prion Diseases:
  - Creutzfeldt-Jacob disease (in humans).
  - Mad cow disease (in cows)(also known as bovine spongiform encephalopathy or BSE) and it is transmissible.
  - Scrapie (in sheep) and causes death.

The causative agent of these diseases is the misfolding of the Prion protein; a small protein found in the nervous system (28-kDa). Regardless of the type of the mutation that will occur, regardless the type of the disease, the methionine 129 in the prion protein (the 129^{th} amino acid is methionine) will change regardless to which amino acid it has changed and this leads to misfolding.

The normal prions have a large percentage of α-helix, but the abnormal forms have moreβ-pleated sheets. This will result in decreased solubility and forming aggregates within the neurons. These aggregates will attach to normal folded proteins making them abnormal and this will badly affect the function of neural cells and diseases will occur.

-Alzheimer's disease:

A disease results from protein aggregates within the neural cells. Cells in Alzheimer's patient have two types of aggregates: Intracellular aggregates called tau tangles and extracellular ones called amyloid plaques .The protein causing these aggregates is the amyloid precursor protein that is crossing the neuron cell membrane. The part of it that lies within the membrane is hydrophobic and the parts that are extended outside are hydrophilic so the solubility is not affected. When a mutation occur, the cleavage will be affected, the hydrophobic part will be exposed to the water medium, the solubility will decrease forming aggregates and attaching to other hydrophobic molecules on top of it. This effects the function of neural cells especially in hippocampus region resulting in the loss of memory and causing Alzheimer disease.

Done by :Hiba Mihyar

-Extracts from this sheet are also found in the study notes #4 .