15

BIOCHEMISTRY

Faculty of medicine – JU2018

Sheet Slides

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Revision

In this lecture the doctor mentioned the concept of ionization of amino acids and isoelectric point :

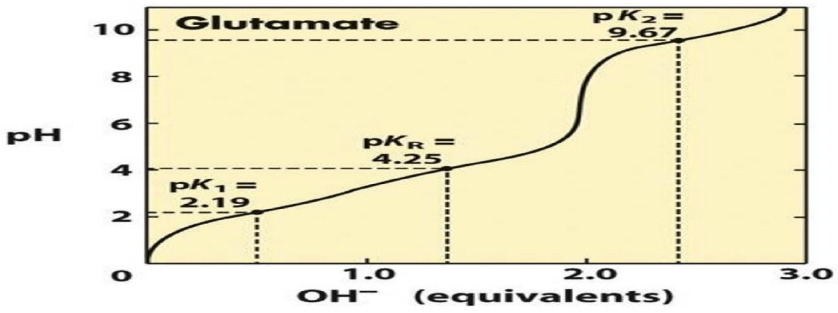
*The figure below shows the titration curve of Glutamate (which is a negatively charged amino acid, meaning that it has carboxylic acid in its R-group which can be ionized) .

Remember:

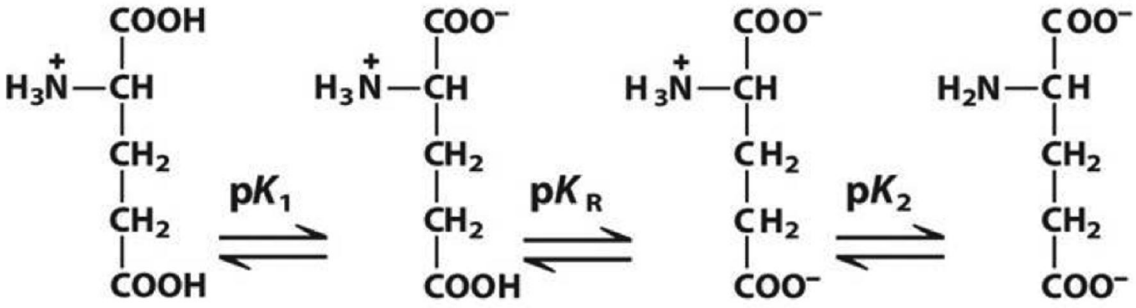
Rule 1: if the carboxylic group is protonated it won't have a charge , that happens when the PH of the solution where Glutamate exists is lower than the (***Pka***) of its carboxylic group.

Rule 2 : if the PH is lower than the ***Pka*** of any group, it will be protonated. On the other hand, if the PH is higher than the ***Pka*** of any group, it will be unprotonated.

-While the amino group when it is protonated it carries positive charge, and when it gets unprotonated it carries no charge.



Look at this figure below:



*From the left ,the first form of Glutamate is in very low PH ,so the carboxylic groups (of the R-group and the backbone) are protonated ,the amino group is also protonated. So the total net charge on Glutamate in the very low PH is **+1**.

*The second form of Glutamate is a **Zwitterion form** ,when the PH=2 it reaches the ***Pka*** of the carboxylic group of the backbone ,so it's unprotonated and has a negative charge. Now the total net charge on Glutamate in Zwitterion form is **zero**.

*The third form of Glutamate is when PH reaches the ***Pka*** of the carboxylic group of the R-group ,so it gets unprotonated and becomes negatively charged. The total net charge on Glutamate is **-1**.

*By increasing the PH further, it will reach the ***Pka*** of the amino group (which it is about 9) ,this will make the amino group also gets unprotonated. The net charge on the Glutamate will be **-2**.

So, we have **3 buffering ranges in Glutamate**, the first one is around PH=2 ,the second one is around 4 and the third one is around 9.

What about isoelectric form? In other words what is the PH when we have **Zwitterion form** as predominant? We take the average of the two PH's that surrounded the Zwitterion form (from the figure above). The answer is around 3.

Continuing protein structure:

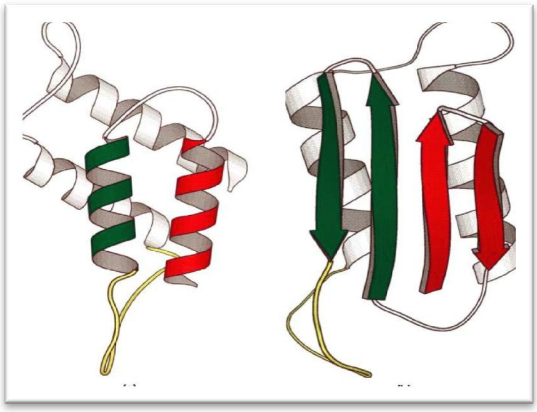
-The tertiary structure of protein is important, and it is determined by the primary structure which is folded in a certain way to hide the hydrophobic amino acids inside the protein and exposing the hydrophilic and charged amino acids to surface.

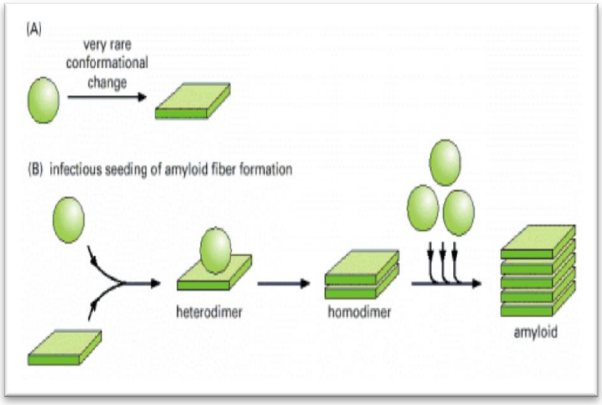
-Sometimes the protein is misfolded, which means that the protein didn't fold in the *native conformation,* which is the most stable 3-dimentional structure of the protein.

-In this case What cells do is they either degrade the protein or refold it again or they can utilize proteins like Chaperones to help the protein refold itself again.

- The misfolding of a protein may be associated with pathological conditions ,an example is Prion disease.

Prion disease:





Prion disease can affect cows (mad cow disease) ,sheep (scrapie) and humans (Creutzfeldt-Jacob disease).

it is related to brain protein known as prion protein (PrP). The misfolded form is called (PrP^{sc}).

Because of mutations or external factors, the prion protein reforms having a lot of beta strands instead of alpha helices. The abnormal form can then interact with other normal prion changing its structure to abnormal form. The abnormal prions tend to aggregate together and become toxic to the cells and damage tissues. **The disease is caused by transmissible agent.**

**Abnormal proteins can be acquired by:

1-Infection 2-Inheritance 3-Spontaneouse mutation

Alzheimer's Disease:

A disease where 2 proteins known as **tau and amyloid peptides (Aβ)** are misfolded.

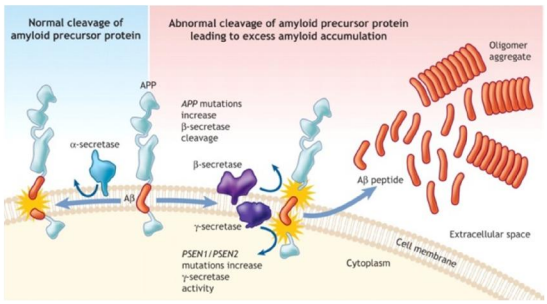
Then they aggregate in brain tissues leading to damage of the brain cells and that what causes dementia. (Not transmissible between individuals)

****The mechanism**: we have amyloid protein on the surface of cells and normally it gets cleaved by a protease releasing the protein ,this is the normal process that when cells do something known as shedding (like when an animal changes its skin) ,in other words cells remove the cell surface proteins to renew them, or to control the number of cell surface receptors, or to produce new better proteins.

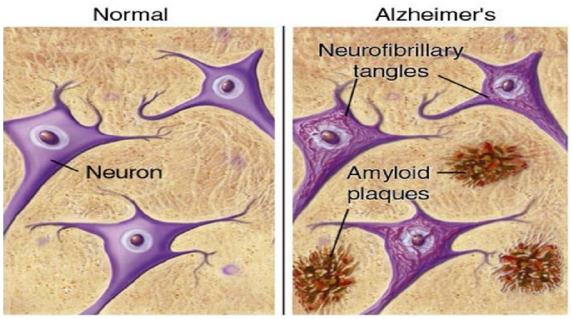
The problem happens when abnormal cleavage of the peptide takes place.

(look at pic 1): the peptide is released from the membrane(millions of peptides get released), so when they get out of the cell they aggregate as you see in pic2 (media outside is hydrophilic, and the peptides are hydrophobic they will cluster to hide from the water), so it cause the damage in the brain .

EXRTA INFO: There is some evidence that misfolded amyloid Aβ can induce tau to misfold too.



Pic 1



Pic 2

Quaternary structure

1-Primary structure =Amino acids sequence (its important cause it determines the structure of the protein at the end).

2-Secondary structure =localized structures within the protein (like alpha or beta sheet)

3-Tertiary structure = formation of a single polypeptide .

Some proteins can be made of multiple polypeptides , **if it is composed of :-

A. two subunits: dimer **B**. three subunits: trimer **C**. four subunits: tetramer

These subunits can be :

-identical → homooligomers (homo = same).

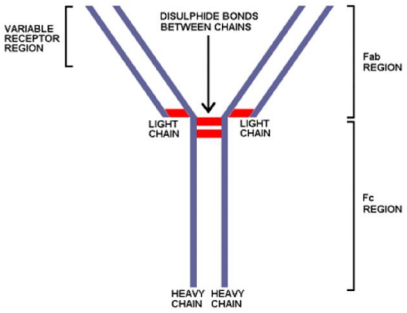
-different → heterooligomers (hetero = different).

The simplest one is homodimer.

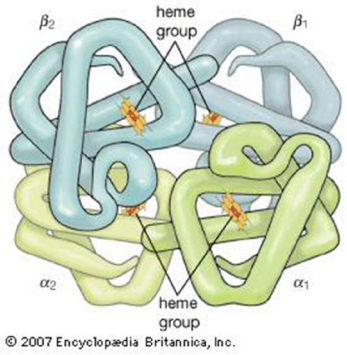
The question now how are the subunits connected ?

Proteins like hemoglobin which is heterotetramer, are connected via noncovalent interactions .

While antibodies (immunoglobulin, are also heterotetramer), have two identical light chains and two identical heavy chains, are connected via covalent interaction (disulfide-bonds).



Antibod



Hemoglobin

-P.S: all proteins have tertiary structure , but not all proteins have quaternary structure .

Complex protein structures

Some protein can be linked to non-protein parts, so we call it holoprotein. When we remove the non-protein part it is called apoprotein.

Like lipoprotein it composed of lipid part + protein part :

*Holoprotein = non-protein part (lipid, metals...etc.)+ protein part.

*Apoprotein = protein part only.

e.g. Enzymes can have metal part so:

*enzyme + metal = holoenzyme *enzyme alone = apoenzyme

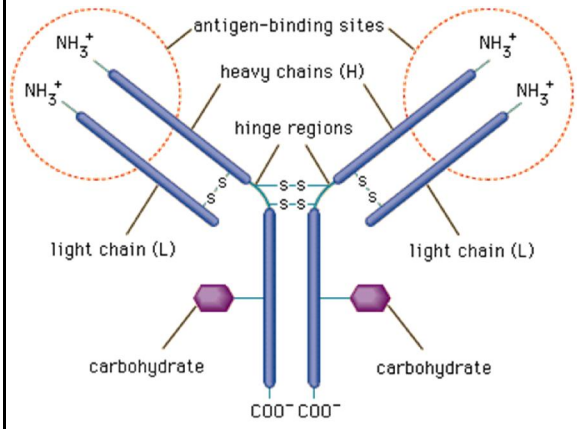
Now let's talk about Glycoproteins:

1-Glycoproteins (sugar + protein).

2-The sugar part can be linked via two linkages : O-linkage or N-linkage.it depends on which amino acid the sugar molecule is attached to.

For example : if it is linked to **Asparagine** it will be N-linkage because its R-group contains amino group. But if it is an O-linkage that means there are 3 amino acids that can be modified (**serine** ,**threonine** ,**hydroxylysine**).

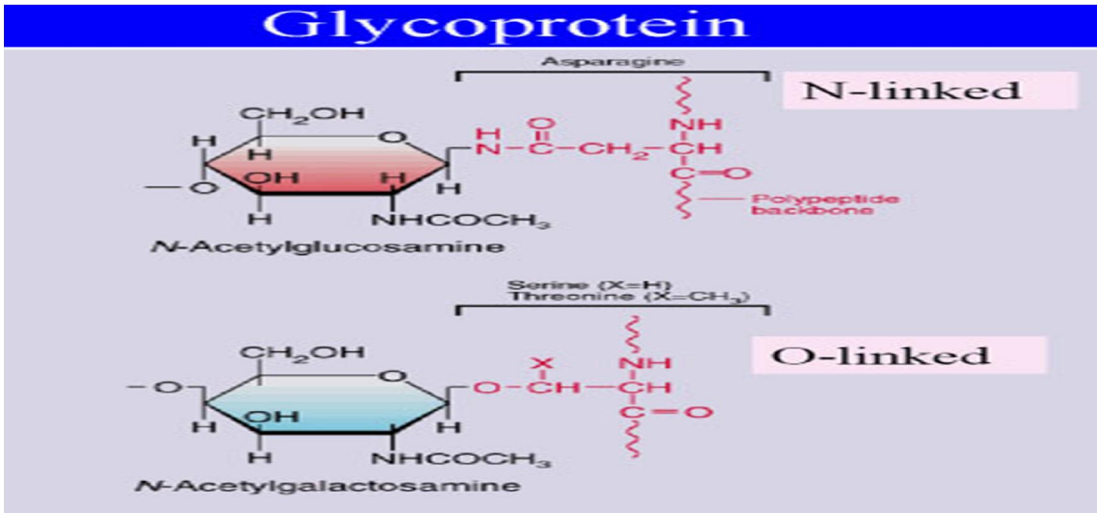
***An example on Proteins which are found to be covalently conjugated with carbohydrates are Immunoglobulins.*



1-Sugars are bound on a certain region on immunoglobulins.

2- N-linked sugars: linked to the amide nitrogen of the Asparagine R-group

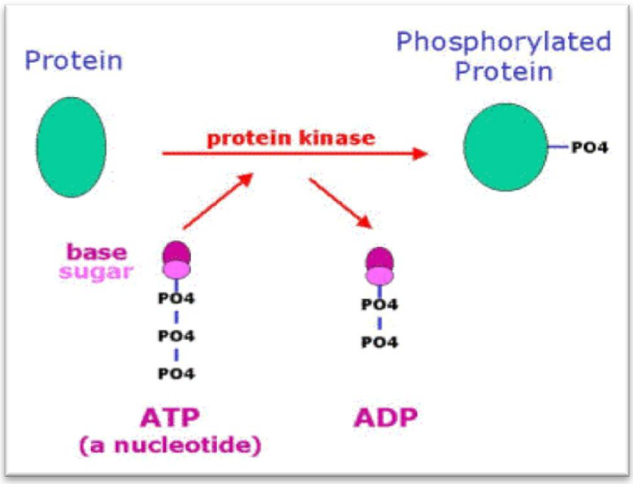
3- O-linked sugars: liked to the hydroxyl group of serine, threonine and occasionally hydroxylysine

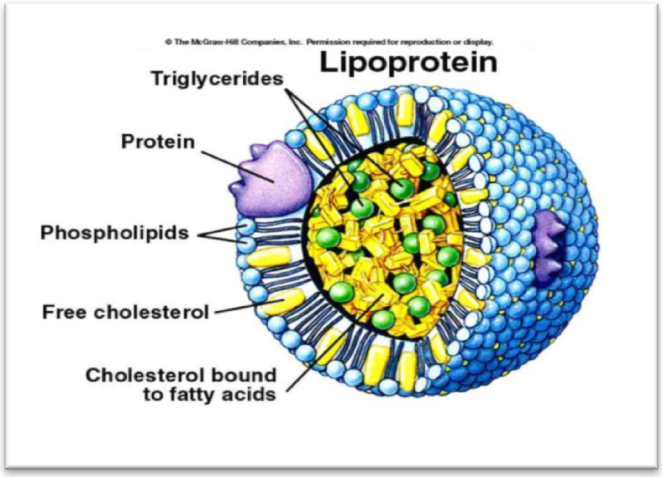


Lipoproteins: proteins that have lipids associated with them.

Other proteins are **phosphorylated,** and these are known as **phosphoproteins**.

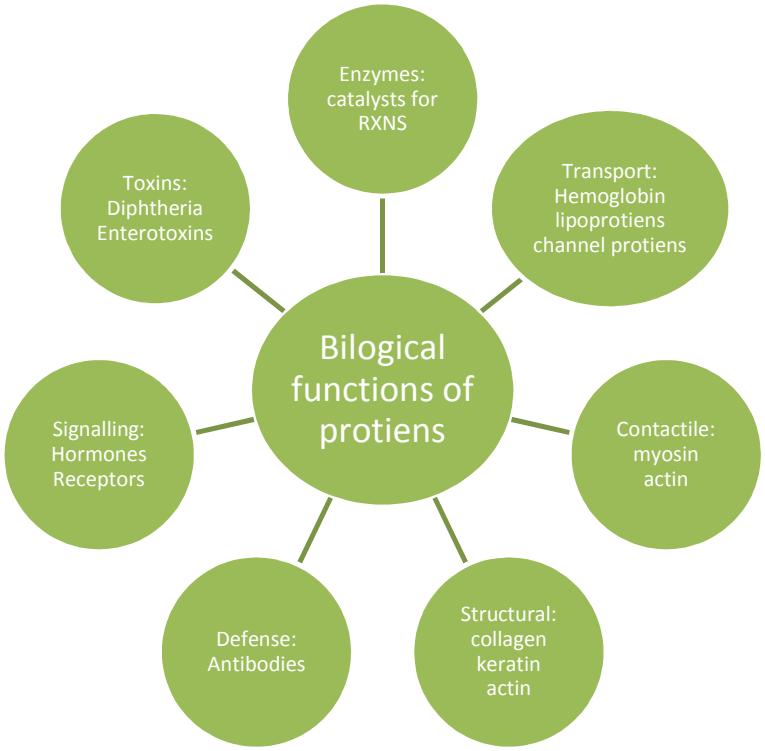
Phosphorylation: adding phosphate group to the protein (this is important in proteins regulation) .





Fibrous proteins

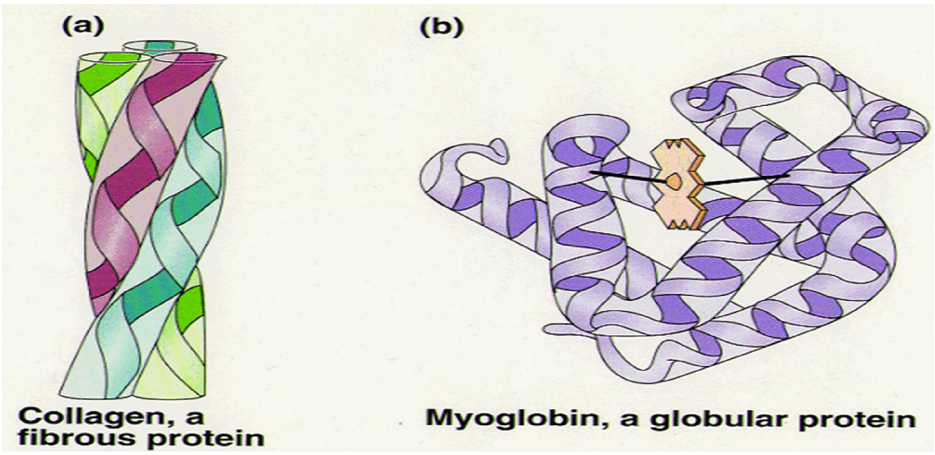
Proteins can have different structures ,and they can have different function as well:



In general proteins can be divided into two classes according to structure:

1-fibrous (fiber-like with a uniform secondary-structure only)

2-globular (globe-like with three-dimensional compact structures)



Examples:

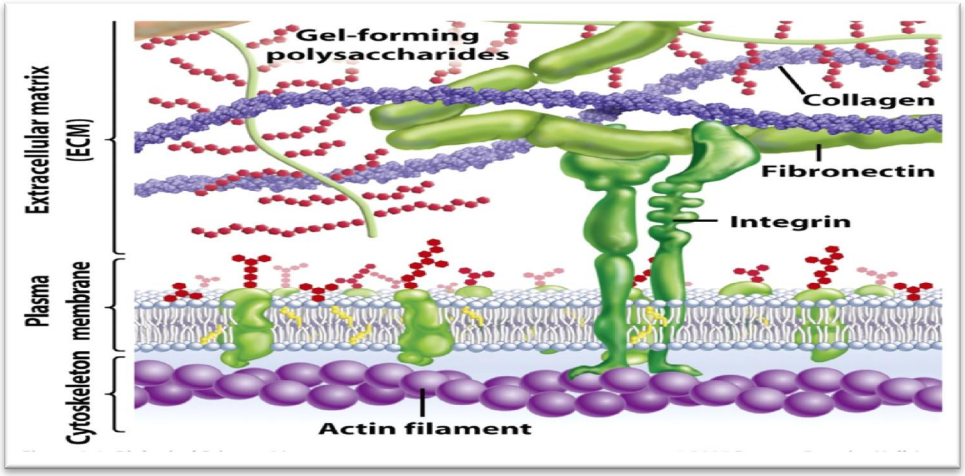
*Fibrous proteins: collagens, elastins , and keratins.

*Globular proteins: myoglobin, hemoglobin, and immunoglobulin.

In globular proteins ,there are hydrophobic amino acids are inside the protein, and the hydrophilic amino acids are outside.

Fibrous proteins in general have one basic function: structural function(They support the structure).

**They exist outside the cells (they create a meshwork in the ECM). By this meshwork they preserve the nutrients and signal molecules in the matrix.



Collagens

Collagens are the predominant protein in our body ,constituting **25%** of the total protein mass ,Collagen molecules are named as type I collagen, type II collagen, type III collagen, and so on.

-Collagens are fibrous proteins with 25 different types found in all multinuclear animals.

-The main function of collagen molecules is to ***provide structural support to tissues***.

-Since the main function of collagen is to provide structural support, then it should be **rigid** and **stiff**.

**Why collagens are stiff molecules? Because they have certain amino acids in their structure.

The structure of collagen molecule:

*It is a left-handed, triple-stranded (**each strand is made of different gene**), helical protein, in which three collagen polypeptide chains, called α chains, are wound around one another in a ropelike superhelix.

P.S. Compared to the α -helix, the collagen helix is much more extended with 3.3 residues per turn.

*This basic unit of collagen is called tropocollagen.

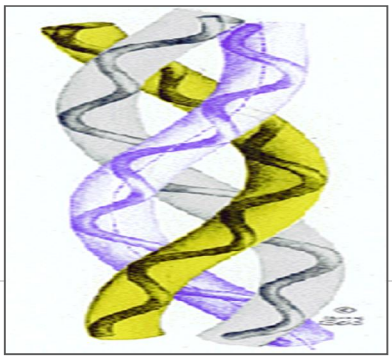
Primary structure of collagens:

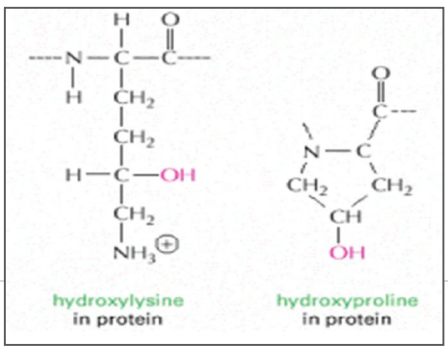
1-Collagens are rich in glycine (33%) and proline (13%).

2-It also contains hydroxyproline (9%) and hydroxylysine.

3-Every third residue is glycine, which, with the preceding residue being proline or hydroxyproline in a repetitive fashion as follows:

1. Gly-pro-Y(any amino acid). 2. Gly-X(any amino acid)-hydroxyproline





-In collagen ,if you look at the top of the triple-stranded structure, you will see no spaces between them ,**why?** Because of *glycine*. Glycine is a small amino acid ,it doesn't cause repulsion (its R-group doesn't need a distance from other amino acids' R-groups) ,also it is found ***inside*** the collagen not outside it.

-*Proline* creates the kinks and stabilizes the helical conformation in each alpha chain.

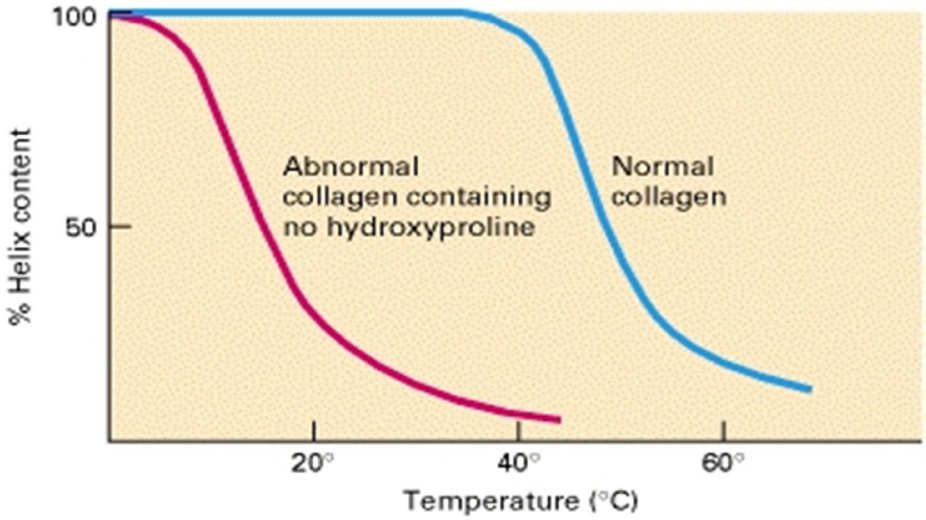
What makes the molecule **tightly packed?**

1-The glycine amino acids.

2- hydroxyproline ,why? Because it can form hydrogen bonds.

The importance of hydroxyproline is illustrated in the figure below:

Normal collagen is stable even at 40 °C ,without hydrogen bonds between hydroxyproline residues, the collagen helix is unstable and loses most of its helical content at temperatures above 20 °C.



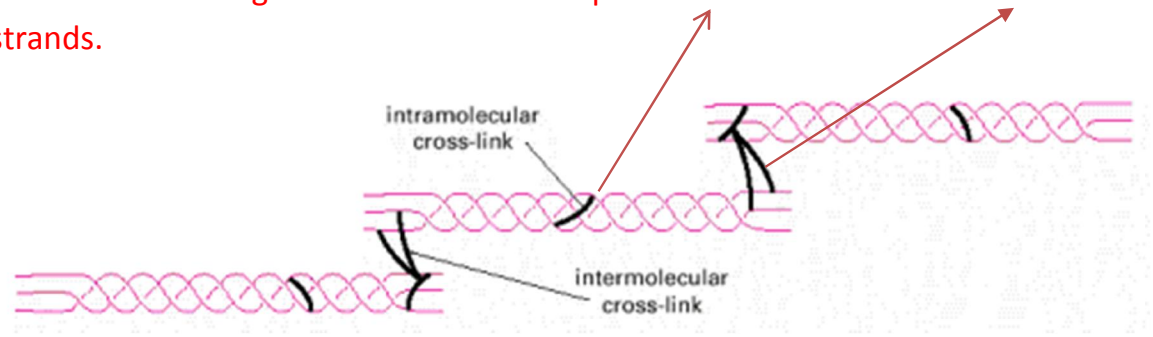
To make the 3 strands stronger linked to each other ,there is also covalent linkage between them, done by lysine .

The lysine gets modified (oxidized) to aldehyde derivatives known as allysine (instead of the amino group in the R-group ,it is converted to aldehyde group).

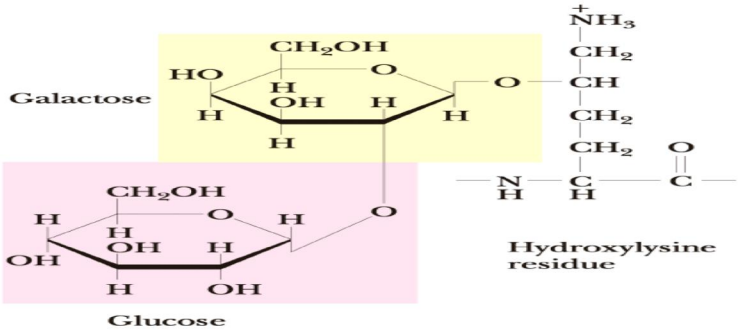
When two allysines are next to each other or allysine with lysine ,it will form covalent linkage between them.

Allysine----Allysine Allysine----Lysine

This covalent linkage can curve within the protein itself or between the different strands.



We also have Hydroxylysine which serves as attachment sites of polysaccharides, making collagen a glycoprotein.



Formation of collagen fibers:

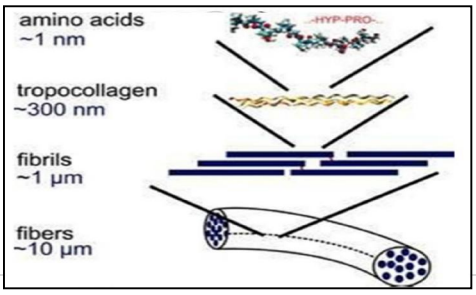
1-Following cellular release of protocollagen, 5 of them polymerize into a **microfibril**, which are connected with each other via aldehyde links.

2-Microfibrils align with each other forming larger collagen **fibrils**, which are strengthened by the formation of covalent cross-links between lysine residues.

3-Microfibrils assemble into collagen **fibers**.

Note1: the fiber structure can be observed under the light microscope ,but fibrils can be observed under the electron microscope.

Note2:The cross linking of collagen molecules increases with age so the tissue becomes more rigid , that's why when we choose meat, we like it to be young (More tinder).



☺ Good luck "الله ما بينسى عباده"