University of Jordan
Faculty of Medicine

Medical Committee
The University of Jordan

Introduction to

BIOCHEMISTRY

Lecture #: (.....13.....)

■ Sheet ☐ Slides ☐ Other

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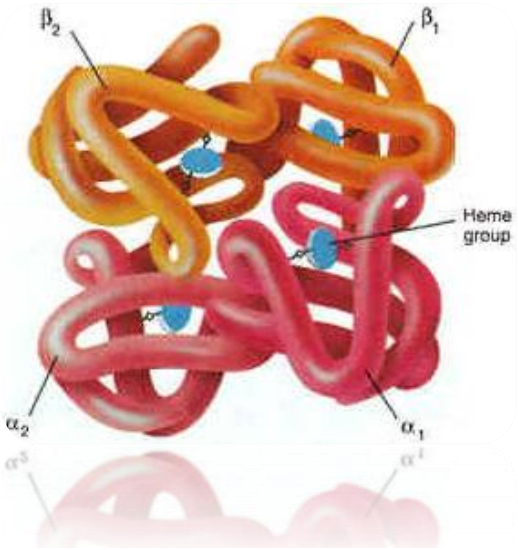
Lecture 17:

Protein Structure-Function Relationship

We will talk about two proteins, Hemoglobin and Myoglobin; because they are very important to understand how structure relates to function:

Hemoglobin:



is tetramer protein (consist of 4 subunits); α 2 β 2

- <u>The way how Hb gathers itself:</u>
  α is gathered with β in one dimer +
  α is gathered with β in one dimer →
  then both dimers gather together through noncovalent interactions.
  Between the dimers it is electrostatic, salt-bridges mainly, it also has hydrogen bond and noncovalent interactions.
  *notice that every subunit has a heme.
- <u>Why Hb is 4 subunits and why Myoglobin is 1 subunit?</u>
  because of their different functions
  Hb : transport oxygen , Myoglobin: storage
- **Myoglobin** : is one polypeptide chain, 1 subunit, composed of 153amino acids
  Hemoglobin: 2 different subunits, α (141 a.a) and β (146 a.a)
- From the structures of myoglobin, α and β subunits of Hemoglobin , you can realize that most of the amino acids are similar in the three of them, which make sense; because they more or less do perform binding to oxygen. But they are different in a certain and these differences create the difference in functions.
- <u>Affinity of myoglobin towards oxygen in very high while affinity of hemoglobin towards oxygen low as a protein. Why?</u>
  It has a high affinity because it is only one subunit, if you want to change the affinity you have to introduce more subunits, so the relation in between the subunits creates the difference in affinity.
- <u>How do we measure the **affinity**?</u>
  By how much pressure of oxygen is needed to saturate the protein.
  If you need a low amount of the material with low pressure to saturate the protein then the affinity is high and vise versa.
  As you can see in the comparison in between them, myoglobin has a very high affinity with around **1-2 torr** you achieve the 50% saturation while we need around **26 torr** to saturate **hemogloben.**

Physiological explanation: Hb transfers oxygen through the blood, so the affinity should be lower than myoglobin, because Hb gives its oxygen for myoglobin, myoglobin inside the tissues abstracts oxygen from Hb, so if the affinity of myoglobin isn't higher, the oxygen won't be transported from the blood to the tissues.

- Hemoglobin shows ***positive cooperativity***.

  1- To do cooperativity you should have more than one subunit.

  2- It has 4 subunits, each one contain a heme. Heme binds to oxygen, so the total number of oxygen that can bind the heme is 4.

  When the first oxygen binds to the heme in the first subunit, it makes it easier for the 2nd subunit to bind, which make it much easier for the 3rd subunit and much much easier for the 4th one. (when you increase the pressure the first subunit till the first subunit is saturated you need less pressure to saturate the 2nd one).Till it reach 50% saturation, below 50% saturation saturation it is easier for Hb to lose oxygen and above 50% saturation it is easier for Hb to gain oxygen.

- Why Hb is designed to have the cooperativity?

  - In the lungs, pO2 = 100 torr, above the 50% saturation, so the Hb will be fully saturated and this is how we want it, we want Hb to be loaded with oxygen in the lungs.

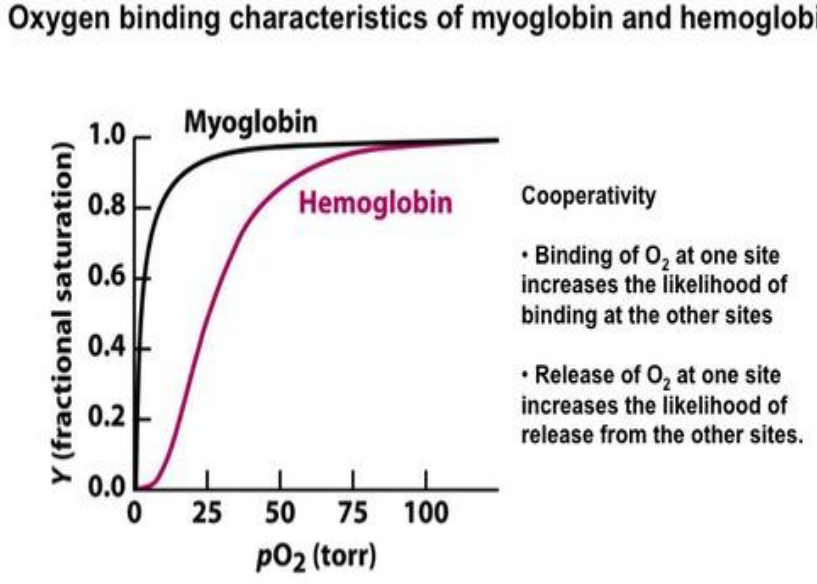
  -In tissues, pO2 = 20 torr, below the 50% saturation, so it is easier to Hb to lose oxygen that to gain oxygen and this is how we want it, we want Hb to release oxygen to the tissues.

- Hb changes its affinity according to oxygen pressure.

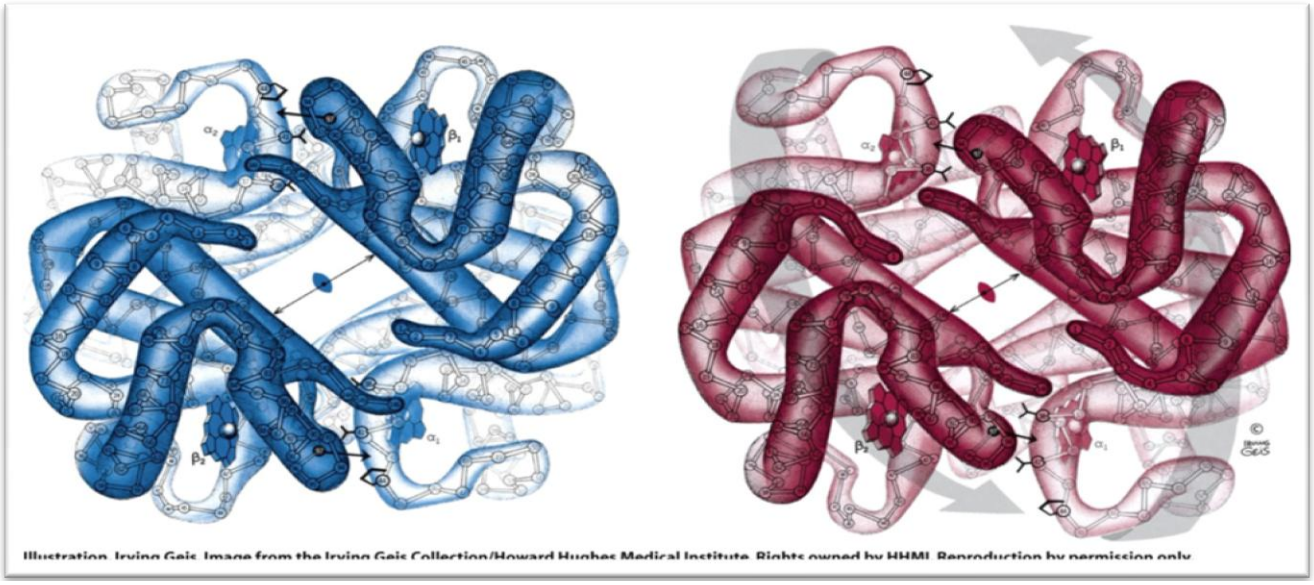
The relation between pO2 and the affinity in Hb is hyperbolic and in myoglobin it is sigmoidal.

-sigmoidal: the curve has S shape

- hyperbolic: with a small increase in X axis you will get a high increase in the Y axis.



- Other things affect Hb function:
  When they crystallized Hb they saw that the de-oxy Hb has a different structure than oxy Hb, if you measured the distance in between the dimers in each one, it will be longer in the deoxygenated Hb. Hb have a certain degree of movment in relation of the dimers together and this is affected by the presence or absence of oxygen.



Bohr Effect:

stated that with increasing the amount of protons [H^+], hemoglobin affinity will get lower .

1- <u>The effect of [H^+]</u>

Once you increase the [H^+] (higher pH)-> increase the protonation of amine groups on the blobin chain especially **His** ^{146} → it carries one more +ve charge → it made a salt bridge and bind to **Asp** -> this salt bridge will preserve the structure of Hb on the de-oxygenated form → lower the affinity.

Metabolism increases the acidity in the tissues -> Hb loses the oxygen.
In the lungs less [H^+] -> Hb gains oxygen

2- <u>The effect of CO_2</u>

the main recourse of CO_2 is crips cycle.

- Through the buffering system:

$\uparrow CO_2 \rightarrow \uparrow H_2CO_3 \rightarrow HCO_3^- + H^+$

you will shift the equilibrium to the right → $\uparrow[H^+]$ → $\downarrow$pH → less O_2 affinity

-Directly

CO_2 can bind to the free amino group of the β subunit of hemoglobin -> creates the ***carbamate*** (nitrogen bonded to an acidic group COO-) it is negatively charged it can bind an alpha residue (Arg) which is positively charged -> carbamate creates this electrostatic attraction preserving the Hb in the deoxy form.

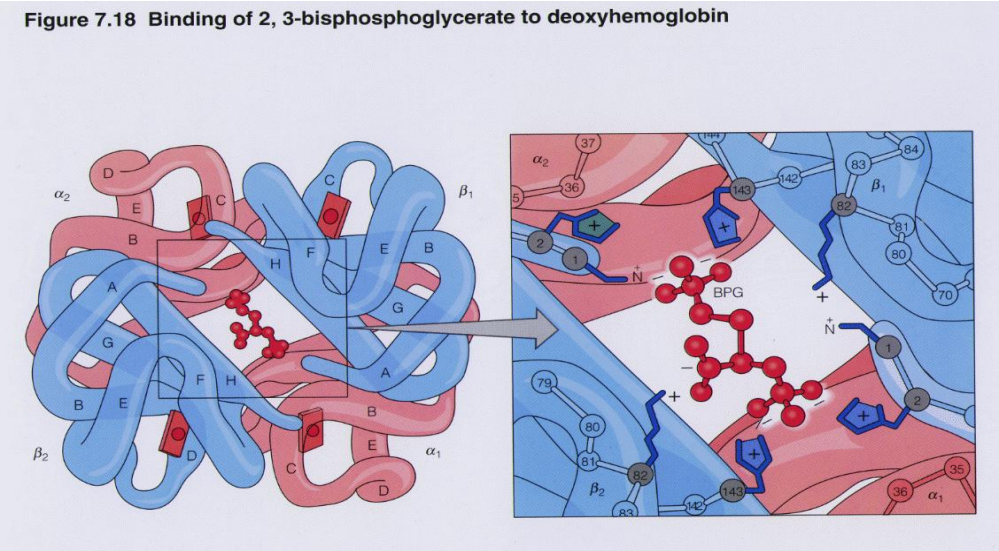
* CO_2 does not bind Hb through iron, but on the free amino group.
What bind the iron on the heme? O2, CO, cyanide, azide

2,3BPG: 2,3-Bisphosphoglyceric acid

a metabolic intermediate in carbohydrate metabolism.
(10 steps pathway to convert Glucose → pyruvate)

Bis → indicates that the two functional groups are bonded to different places
but bi: they are bonded to the same place
Phospho → 2 phosphate groups with 2 negative charges
glyceric → ate: negative -> very acidic

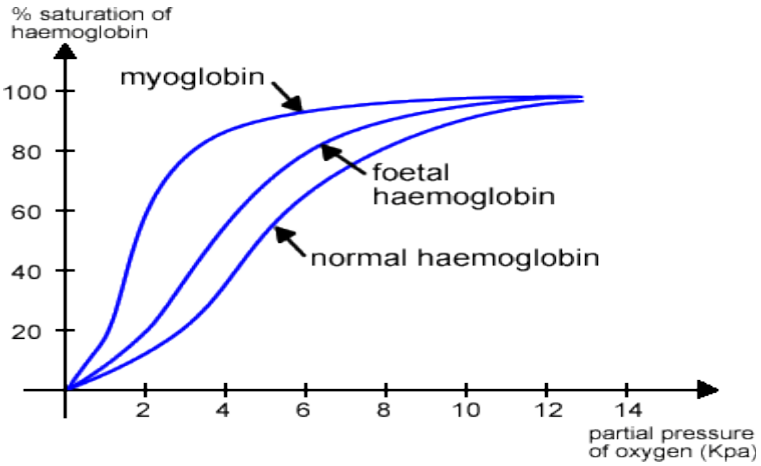
This molecule is inside the hemoglobin (in the space between the subunits). RBCs get their major source of energy from glycolysis which means BPG is present in high concentrations in RBCs. So, it should always bind hemoglobin.



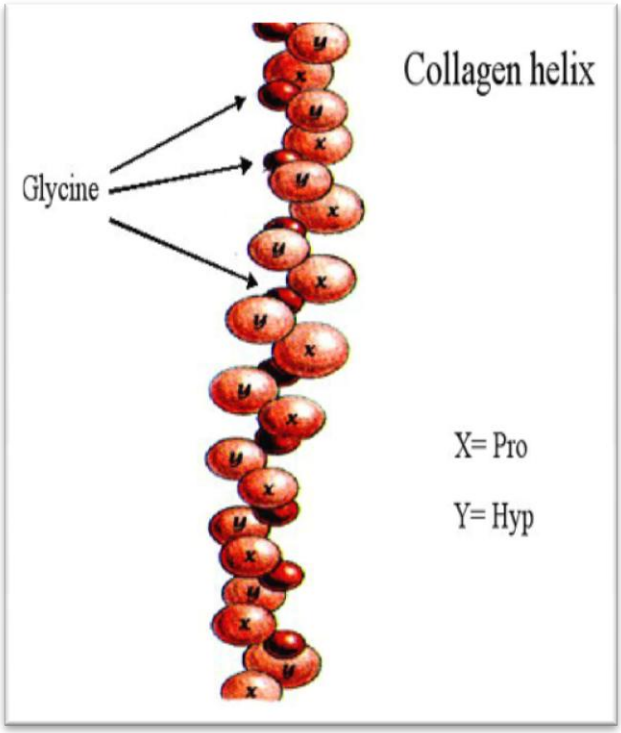
In the space in between the subunits, there are amino acids that are positively charged lining it, the R groups are projecting towards the space, so they are binding BPG and it is what gives Hb its cooperative behavior. If you have remove BPG this is how the saturation of Hb will be. Once you put BPG you are decreasing the affinity of Hb.

- An implication of this is **Fetal hemoglobin**.
- The fetus takes the oxygen from his mother through the placenta and umbilical cord.
- <u>Fetal hemoglobin has a higher affinity to O_2 than maternal hemoglobin due to:</u>
  1- α 2 γ 2 has (γ has higher affinity than β)
  2- The positively charged amino acids binding BPG are beta is histidines
  in gama one of the histidines (His ^{143}) is replaced by serene which cannot make electrostatic attraction (salt bridges) with BPG → increases the affinity.

- Myoglobin all the time has higher affinity toward oxygen than hemoglobin.



Collagen:



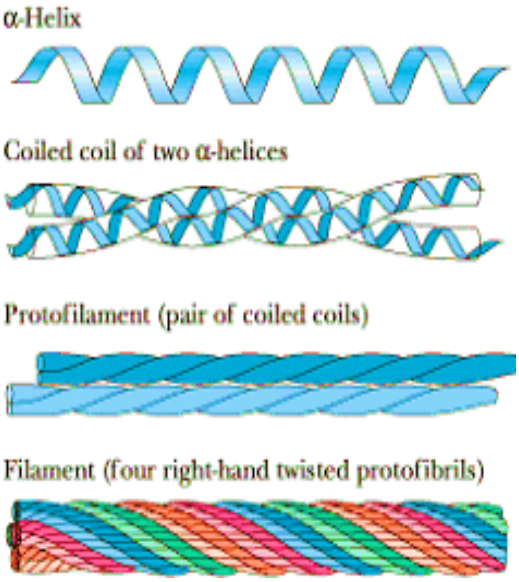
Triple helix → 3 polypeptide chains
**Notice that the 1st one is projecting outside, the 2nd one also but the 3rd one is in between both of them. You don't care what the 1st are and the 2nd amino acids are because they are projecting outward and the space is available, the 3rd one is in between them; so if we want the twist to be really tight, then I have to put an amino acid with a small R group (Glysine).

- **Structure**:
  X1 – X2 (Pro, Pro_{OH}) – Gly
- The 3rd one should be glysine.
- The 2nd amino acid in most of the times is proline because its turns - can create kinks.
- Cross linking in collagen:
  each 3 polypeptides wrap around each other → tropocollagen → tropocollagens cross linked through the proline which can be hydroxylated through *prolyl hydroxlase* to be hyroxyproline, also lysine through *lysyl hydroxylase* to be hydroxylysine.
- For the enzymes to work they need Vitamin C, if there is no Vitamin C they won't function properly then there will be no cross linking and each one is working separately and bleeding is the result.
- Vitamin C can be deficient from the food sp you will have an acquired vitamin c deficiency then it will result in *scurvy*.
- Also the cross linking can be genetically deficient, if the enzymes were genetically deficient creating no cross linking. One of these diseases is *osteogenesis imprefecta* (is a defect in collagen type I formation, no cross linking in collagen type I) mostly

present in bones, patient will present with multiple and frequent fractures, also in the sclera of the eye the collagen will be weak (not tightly connected) so it will show the venous blood behind it giving it bluish color.

Keraitn:

- it is a protein composed of two 2 alpha helices gather →dimer → protofilament →fibril of keratin (filament)
- <u>There are two types of keratin:</u>
  α → in mammals
  β → reptiles and birds
- (α) Has very different subtypes.
  In mammals there is around 30 subtypes
- They are classified as "hard" or "soft" according to the **Sulfur content**; it is mainly present in *cystine*. More sulfur content →harder
- Structure: α-helix, coiled coil



- <u>What they do in the saloons?</u>
  - if you want to perm your hair: you put material can oxidize the cyteine, you are taking out hydrogen, you are bridging the cysteine together→ more disulfide bridges→ increase amount of coiling
  - if you want to make it straight: you are reducing the coiling → separate the disulfide bridges → making thiol group by itself

elastin:

- Protein rich of hydrophobic amino acids as glycine, proline and valine.
- A lot of hydrophobic residues around each other make hydrophobic interactions.
- Cross-linking of elastin occurs through the enzyme lysyl-oxidase producing the Allysine, the pathway for oxidation through lysyl-hydroxylase does not occur in elastin.
  tropoelastin → elastin [lysyl oxidase]

Good luck ☺