HEMATOLOGY
&LYMPH SYSTEM

Biochemistry sheet

Number	1
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Globular hemeproteins

Hemoglobin and Myoglobin

Structure- function relationship in proteins: Studying globular hemeproteins give us a good example about structure -function relationship. The first studied hemeprotein was myoglobin and the relationship between each part of its structure and its physiology was best understood. After that, other proteins were studied in great detail but hemoglobin and myoglobin always remain the best two molecules were structure- function relationship is best understood.

Globular hemeproteins

A hemeprotein is a metalloprotein (composed by 2 portions: a heme prosthetic group found in the catalytic site, and an apoprotein portion) we have other hemeproteins than Myoglobin and Hemoglobin such as cytochromes and catalase and so we have variety of functions done by the heme which is dictated by the 3 dimensional structure that is the provided by the apoprotein portion of the hemeprotein.

Examples:

Cytochromes	Catalase	Hb and Mb
Heme functions as an electron carrier.	Heme functions in the breakdown and hydrolysis of H_2O_2	Reversibly binds oxygen for transport issues

Heme structure:

Heme is composed of one iron molecule and a porphin ring.

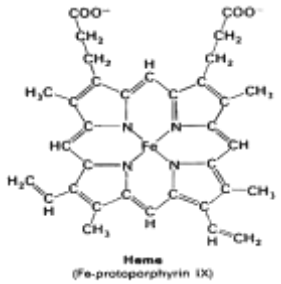
Porphins review:

* A Porphin is a tetrapyrrole joined together by a methine bridge

* There are several types of porphins according to the substituents and types of moieties that are involved in the structure.

* **Porphin 9** (protoporphyrin IX) has 4 isomers according to the arrangement of its substituents , we are concerned in the **3rd isomer** as it is the one that is involved in the heme structure.

*The arrangement of substituents in isomer 3 is as follows: **methyl - vinyl- methyl-vinyl- Methyl-propionyl- propionyl-methyl.** (in the picture)



***** Isomer 1 is found in our cells but we don't need it and the arrangement is as follows: **methyl - vinyl- methyl- vinyl- Methyl- propionyl- methyl- propionyl.**

*Iron can make 6 bonds, so when it binds to the 4 nitrogens of the porphyrin ring it gives rise to ferroprotoporphyrin (heme), but if the oxidized iron (ferric) binds it gives rise to hemin. Those four bonds can be distinguished by resonance.

Strcuture of apoprotein portion in Hb and Mb:

Hemoglobin is a tetramer (four polypeptide chains 2 alpha and 2 beta) while Myoglobin is a monomer (single polypeptide chain that is structurally **similar – not identical** to the individual polypeptide chains of tetrameric Hemoglobin).

Secondary structure of beta chain of Hemoglobin (apoprotein part):

*It is composed of 146 amino acid residues.

*75-80% of its structure is helical, 8 helical segments designated by A -> H ,The region between the A and B helix is called AB, between B and C it is called BC… etc and between and the N and C terminus it is called NA, HC respectively.

How to determine the location of a certain amino acid?

1) By simple numbering: 1,2,3,4,5…, 146
2) Refering to the number of the amino acid in each segment: A10, B12… etc.

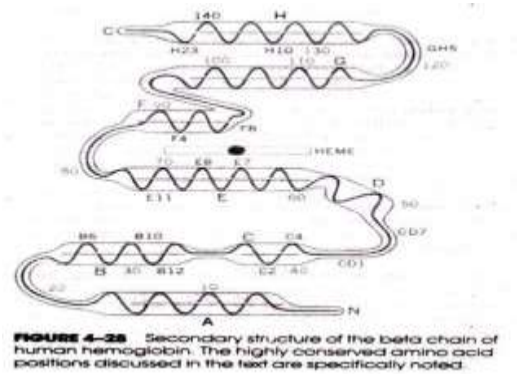
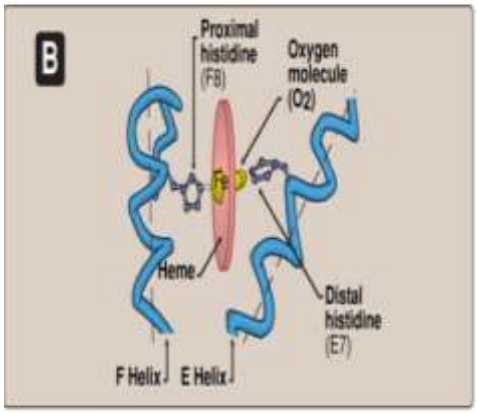


FIGURE 4–28 Secondary structure of the beta chain of human hemoglobin. The highly conserved amino acid positions discussed in the text are specifically noted.

There are 2 important residues we are going to point at which are histidine of F segment which is known as proximal histidine (F8\Beta92) and histidine of E segment which is known as distal histidine (E7\Beta63). They are classified as proximal and distal based on their proximity to heme.

*The helical segments are terminated by the common terminators of helices :

1) The presence of proline whose five membered ring cannot be accommodated in an alpha helix. Four of the helices at least are terminated by proline.



2) Beta bends and loops stabilized by hydrogen bonds and ionic bonds (also termed electrostatic interactions or salt bridges).

Secondary structure of alpha chain of Hemoglobin (apoporotein part):

*It is made up of 141 amino acid residue.

*7 helical segments (A -> H), but without the D segment.

*Proximal histidine : F8\ alpha 87.

*Distal histidine : E7\ alpha 58.

Myoglobin structure (apoprotein part):

*Composed of 153 amino acid residues.

*Shows wide similarity with beta and alpha secondary structures of Hemoglobin.

*Myoglobin is a globular compact molecule (45 ×35 × 25 Angstrom)

*75% helical in structure with 8 helical segments.

*4 helices are terminated by proline

*Interior consist of hydrophobic residues except for proximal and distal histidine (basic amino acid with a positive charge)

Binding site of heme:

*The secondary structure of alpha, beta and Myoglobin folds forming a globular compact structure

*The heme pocket which is located between the F and E helix.

*As we mentioned before Iron makes 4 bonds with the nitrogens of the porphyrin ring, the fifth bond is made with the proximal histidine, and the last bond with the oxygen.

*Distal histidine doesn't bind directly to the iron and this has a role in **stabilizing** and the binding of oxygen to the ferrous iron.

*the heme pocket or crevice is lined with hydrophobic residues (except for the distal and proximal histidine residues) that are necessary in:

1) Stabilizing and binding to the hydrophobic heme, and maintaining the iron in its ferrous Fe^{+2} state (reduced).

2) Permits the reversible binding of oxygen molecule.

*any replacement in those residues will result in an abnormal hemoglobin because the iron will be oxidized into ferric and it no longer can bind to the oxygen.

*Loss of electrons by ferrous iron is rare and we have certain mechanisms in our body to reverse the reaction if it happens.

*The 2 Propionate substituents of the heme are hydrophilic so they are directed toward the exterior surface away from the hydrophobic heme pocket.

Comparison between Mb and Hb:

The primary, secondary and tertiary structures of Hb chains and Mb show close resemblance in the three dimensional structure.

*If we take different Mb from different species and sequence it we will find 83 amino acid residues in each species, and when comparing those sequences with the alpha and beta chains of the Hb we will notice that there are 15 similar amino acid residues.

*These reisdues are called **invariant** because they can't be changed due to their importance in proper oxygen binding, and they include the proximal, distal histidine and the hydrophobic residues in the heme pocket.

*Many of the residues that are changed are conservative (means that you change an amino acid with another amino acid that belongs to the same group, so if it wasn't in the active site and it function to stabilize the 3D shape only, nothing will occur).

Quaternary structure of Hemoglobin:

*Structure and function of Hemoglobin tetramer (two dimers each dimer consists of one alpha and one beta chain) is more complex than Myoglobin.

*Oxygen binding to Hemoglobin is regulated by allosteric effectors while Myoglobin is not.

*Hemoglobin binds to the oxygen in a cooperative manner expressed by the sigmoidal shape of the oxygen dissociation curve, whereas the myoglobin exhibits a hyperbolic oxygen dissociation curve.

*Hemoglobin transports H^+, CO_2 in addition to O_2 (each Hemoglobin molecule transports 4 oxygen molecules 1 oxygen per heme group), but Myoglobin transports oxygen only.

*Hemoglobin binds large amount of oxygen (98%) directly but it is responsible directly and indirectly for transport of about (80-90%) of carbon dioxide (10-15% directly).

Oxygen dissociation curve

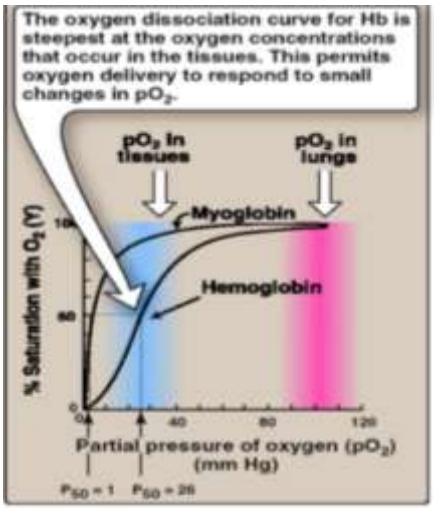
We know that **Km** is the substrate concentration that allows the enzyme to achieve **half Vmax**. In the case of Hb and Mb we use another term called **P50** and it means the partial pressure of oxygen that is required to achieve half saturation it equals **1 torr in and Mb and 26 torrs for Hb** (the lower p50 the higher the oxygen affinity), which means that there is a huge difference in affinity between them.

Mb has higher affinity for oxygen than Hb, this is important, because it facilitates the transport of oxygen from Hb to Mb in the muscles. Myoglobin needs 1 Torr to

get 50% of myoglobin saturated, if the partial pressure of oxygen is more than 1 torr, oxygen easily binds to myoglobin. If the partial pressure is less than 1 torr, oxygen is released from myoglobin. Tissues oxygen pressure 20 torrs (not for memorizing) so myoglobin binds oxygen because the partial pressure of oxygen is higher than 1 torr, remember that myoglobin functions as a storage of oxygen in muscles. At 100 torrs (lungs) hemoglobin is 98% saturated, at tissue's pressure hemoglobin is less the 50% saturated so it releases the oxygen.

*What does cooperative binding mean ?

Cooperative binding of oxygen by the four subunits of Hemoglobin means that the binding of an oxygen molecule at one heme group increases the oxygen affinity of the remaining heme groups in the same Hemoglobin tetramer. In other words the molecule in the beginning exists in an inactive form and it binds the substrate with low affinity. As it binds the substrate it is then transformed to more active form and when it becomes active the curve becomes steep. So although it is more difficult for the first oxygen to bind Hemoglobin, the subsequent binding of oxygen becomes easier and with higher affinity. When first oxygen binds it induces **breakage in hydrogen bonds and salt bridges** and change in the conformation of the neighboring subunits. This conformational change is related to increase in affinity



*In the steep portion notice that any small change in PO2 will lead to great change in percentage of Hemoglobin saturation

* The steep portion lies within the partial pressure of oxygen found in tissues (20-40 mm) so any change in oxygen tension in tissues will produce a great change in Hemoglobin saturation

* Allosteric effectors have their profound effect in the steep region

* Units of PO_2 : 1 torr = 1mm Hg KPa : kilopascal = 7.5 torr

Transitional model from T to R for Hb:

There are two theories for the transformation of allosteric proteins from inactive to active form; one of them is the concerted model of Monod and the other is the sequential model.

Hemoglobin follows the concerted model of Monod, There is T state (tight or less active) and R state (relaxed fully active).

We said that hemoglobin is a tetramer that is composed of two (αβ) dimers, between the alpha and beta chain there are extra bonds (hydrophobic bonds) other than the hydrogen and electrostatic bonds that connect the 2 dimers together. When oxygen binds to the deoxy state (T state), some of the ionic and hydrogen bonds between the dimers are broken or become weaker allowing movement and change in structure, and the molecule undergoes a conformational change to a more relaxed state (R form).

***The concerted model of Monod states that we have only two forms, T or R. the equilibrium favors one of the conformational states**. in the case of deoxyhemoglobin the T form predominate, while the R form is unstable and is present in minimal amount. When the substrate (positive effector) is added the equilibrium is shifted to the R form. While binding of negative effectors causes shift to the T form (proportion depends on how much substrate do you have) So, when oxygen binds we will have some R form, and T form decreases. As more oxygen binds the trend keeps going on. Some other allosteric enzymes follow the sequential model in which a **gradual conformational** change takes place.

All in all, some allosteric enzymes and hemoglobin follow Monod's model (only 2 conformations) while other allosteric enzymes follow the sequential model (gradual conformations).