# Hematology

## BIOCHEMISTRY

☑ Sheet ☐ Slide ☐ Handout

Number: **1**

Subject: **Introduction to globular hemeproteins**

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Price:

# Globular hemeproteins
# Hemoglobin and Myoglobin

(During this system we will take 8-9 biochemistry lectures and here is an overview of what we will study through them ).

## Objectives :

- ✓ **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.
- ✓ **Hemoglobin is an allosteric protein**: we will study the physiological role of allosteric effectors and how do they affect the function of hemoglobin.
- ✓ **Hemoglobin is a tetramer consisting of 4 chains of 2 different kinds** ( 2 alpha and 2 beta chains) : For hemoglobin to function properly it has to be tetramer and has two kinds of chains .
- ✓ **Concept of molecular diseases and Molecular bases: why fetus has its own hemoglobin**? And what is the molecular base of that ?
- ✓ **Different types of normal hemoglobin**: we are more interested in those which are polymorphic.
- ✓ **Abnormal hemoglobin:** including abnormalities in structure and quantity.
- ✓ **Myoglobin and hemoglobin normal structure.**
- ✓ **Hemoglobin role in transport of oxygen, carbon dioxide and proton, and the effect of PO2, PH, temperature and 2,3-BPG on the function of hemoglobin.**
- ✓ **Bohre effect** and its molecular bases
- ✓ **Normal derivatives of hemoglobin**
- ✓ **Thalassemia**
- ✓ **Heme metabolism**
- ✓ **Iron metabolism**

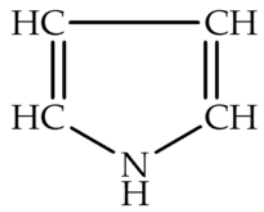
- ✓ **Metabolism in mature RBC and related genetic disorders**: Maturation of RBC ( biochemically known as semi dead cell) by losing its organelles and the several remaining metabolic pathways that should be present to help the cell to live as long as 120days.

## Globular hemeproteins

- We have many hemeproteins other than Hemoglobin and Myoglobin such as cytochromes and catalase enzyme.
- The globular hemeprotein is composed of 2 parts:
  ***{prosthetic group (heme group) found in catalytic site + apoprotein}***
- **Globin** is the apoprotein in case of Hemoglobin and Myoglobin.
- **Heme is capable of performing various functions .The apoprotein part provides an environment of 3 dimensional structure that dictate the role and function of heme.** Here are some examples:

| Cytochromes | Catalase | Hb and Mb |
|---|---|---|
| Heme functions as an electron carrier that is alternately oxidized and reduced | Heme is a part of the acive site of the enzyme that catalyses the breakdown and hydrolysis of hydrogen peroxide | Reversibly binds oxygen for transport issues |

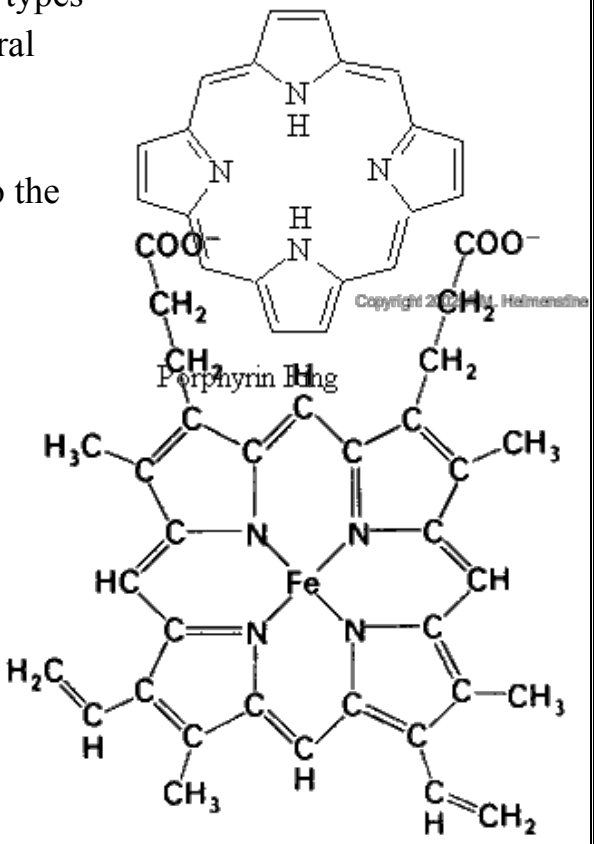
## Structure of heme



Pyrrole ring

- ✓ Heme is composed of one iron molecule and a porphin (porphyrin) ring, to understand heme structure, lets review porphins:

- ✓ porphin essentially consists of **four pyrrole** rings joined together by **four methine (=CH—) bridges.**
- ✓ There are variety of substituents and different types of moieties in porphin which give rise to several types of porphins . **Porphin 9** (AKA protoporphyrin IX ) is the one we care for.
- ✓ protoporphyrin IX has 4 isomers according to the way its substituents are arranged. Out of these 4 , **isomer 3** is the one that is involved in heme structure.
- ✓ The arrangement of substituents in isomer 3 is as follows: **methyl - vinyl- methyl- vinyl- Methyl-propionyl-** ***propionyl- methyl***
- ✓ 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 of heme is able to form 6 bonds. It is coordinately bound to the four nitrogens of the porphyrin ring giving rise into **ferroprotopophyrin** ( the other name for it is heme). Those four bonds can be distinguished by resonance.




**Heme**
(Fe-protoporphyrin IX)

## Strcuture of apoprotein in Hemoglobin and Myoglobin

- Hemoglobin is a **tetramer** ( four polypeptide chains ) while Myoglobin is a **monomer** ( single polypeptide chain that is structurally similar –not identical- to the individual polypeptide chains of tetrameric Hemoglobin ).
- **Why Hemoglobin is tetramer and Myoglobin is monomer?**
  Hemoglobin has on its surface several complementarity groups that establish hydrogen bonds, hydrophopic bonds and electrostatic bonds that are complementary with each other and as a result four subunits are associated.

This complementarity helps Hemoglobin to overcome the hydration of water. Such complementarity isn't present enough in MYOGLOBIN and that's why it is present as a single monomer.

- If you were to examine the secondary and tertiary structure of the alpha and beta chain and even myoglobin you will notice a lot of similarity despite of the fact they are being derived from different genes and the variation we have in amino acid sequence.

## Secondary structure of beta chain of Hemoglobin

- ✓ It is composed of **146** amino acid residues.
- ✓ **70-75%** of its structure is **helical** .
- ✓ The helical segments are terminated by the common terminators of helices which are:
    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).

- ✓ The alpha helical sigments are labeled from **A to H** (**<u>8 helical segments</u>**). Each region separating two helices from each other is named by two letters starting from N –terminal until reaching the C-terminal as follows: **NA - AB- BC- CD- DE- EF- FG- GH- HC**
- ✓ To determine the location of certain amino acid residue you either:
    1. Go by simple numbering: 1,2,3, …. , 146
    2. Number each helical sigment by its own.
       There are two important residues we need to know their location. **Histidine of F segment** which is known as ***<u>proximal histidine</u>*** (**F8\Beta92**) and **histidine of E segment** which is known as ***<u>distal histidine</u>*** (**E7\Beta63**). They are classified as proximal and distal based on their proximity to heme.

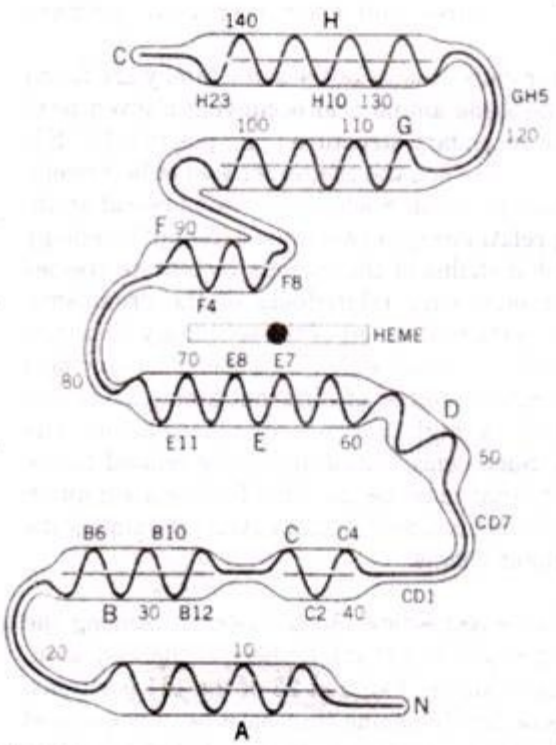



**FIGURE 4–28** Secondary structure of the beta chain of human hemoglobin. The highly conserved amino acid positions discussed in the text are specifically noted.

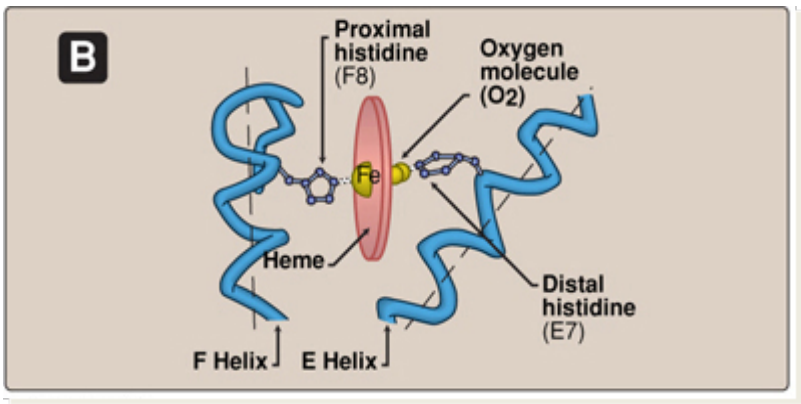
## <u>Secondary structure of alpha chain of Hemoglobin</u>

- It is composed of **141** amino acid residues (shorter than beta)
- **70-75%** of its structure is **helical**
- **<u>7 helical sements</u>** labeled from **<u>A to H with no D</u>** segment
- *Proximal histidine* : F8\ alpha 87
- *Distal histidine* : E7\ alpha 58

## Myoglobin secondary structure

- ✓ Composed of **153** amino acid residues
- ✓ Shows wide similarity with beta and alpha secondary structures of Hemoglobin.

## Binding site of heme




- The secondary structure of alpha, beta and MYOGLOBIN folds forming a globular **compact** structure
- The heme pocket is located between F and E helix
- The iron of heme is coordinately bound by the fifth coordinate bond to proximal histidine ( binds iron directly)
- Distal histidine doesn't bind directly to the iron and this has a role in *stabilizing the binding of oxygen to the ferrous iron*.
- During oxygen transport, Oxygen binds coordinately to the iron establishing the sixth coordinate position.
- Heme group sits in a **crevice** which is lined with **non polar hydrophobic** amino acids. (Notable exceptions are the two *proximal and distal histidine* residues which are *polar* ).The hydrophobicity of heme pocket is very important to:
    1. **Maintain the iron in the ferrous state** (reduced state) because the iron doesn't bind oxygen in the ferric state. If any mutation occurs replacing the hydrophobic amino acids in heme pocket with hydrophilic ones water will be able to enter the pocket and oxidize iron to ferric state so no oxygen will bind.
    2. **Permits the reversible binding of oxygen molecule**.
- 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.


## Tertiary structure of Myoglobin

- ✓ 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

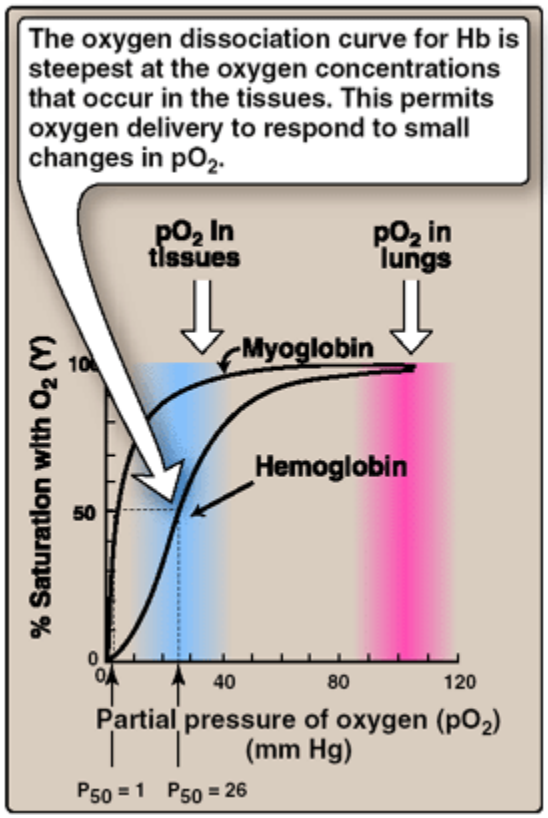
## Comparison between tertiary structure of Myoglobin and Beta chain of Hemoglobin

- ✓ There are lots of similarities and close resemblance in three dimensional structure.
- ✓ If you take Myoglobin from many different sources and then sequence and compare between them, you will notice that **83** amino acids will not differ between those species. Those are called **invariant** residues (they *don't afford changes between species due to their importance to proper functioning of the protein*).
- ✓ Compare the sequences of Myoglobin taken from the sources above with sequences of alpha and beta chain of Hemoglobin from many sources , you will notice that **15** residues are similar to Hemoglobin. They are also called **invariant** residues.
- ✓ Invariant residues include **proximal histidine, distal histidine and the hydrophobic heme pocket**.
- ✓ For the amino acid residues that afford changes between species, many of the changes are **conservative** (*means that you change an amino acid with another Amino acid that belong 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 are **more complex** than Myoglobin.
- Hemoglobin is an allosteric protein that exhibit **cooperativity** and as a result its oxygen dissociation curve is **sigmoidal** while Mg has *no cooperativity* with a *hyperbolic* oxygen dissociation curve
- Hemoglobin isn't only involved in transport of oxygen , it also transports **protons** and **carbon dioxide**. (each Hemoglobin molecule transports 4 oxygen molecules)
- Hemoglobin binds small amount of oxygen directly but it is responsible directly and indirectly for transport of about 80% of carbon dioxide
- Oxygen binding to Hemoglobin is regulated by allosteric effectors while Myoglobin is not.

## Oxygen dissociation curve




- ✓ **P50** is the partial pressure of oxygen needed to achieve half saturation of the binding sites
- ✓ **P50 is similar to Km and Kd in enzymes**
- ✓ P50 for Mb = **1** while it is **26** for Hemoglobin (the *lower p50 the higher the oxygen affinity*)
- ✓ Mb has higher affinity for oxygen than Hb, this is important, because facilitate the transport of oxygen from Hb to MB.
- ✓ 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

- ✓ For Myoglobin there is no proportionality in the increase of binding oxygen with increasing PO2 unlike Hemoglobin and that is why we have the S shape.
- ✓ 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** ( **30-40** 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 po2 : 1 torr = 1mm Hg
  KPa : kilopascal = 7.5 torr

**Extra notes by correction team :**

#note_1: **prosthetic group** is a non-protein chemical compound or metallic ion that's bound tightly to a protein and is required for a protein biological activity to happen. It binds to an apoprotein, converting it to a holoprotein.

#note_2 : **Porphyrins** are a group of heterocyclic compounds, composed of four modified pyrrole subunits interconnected at their α carbon atoms via methine bridges (=CH−). The parent porphyrin is **porphin**, and substituted porphines are called porphyrins.

#note_3 : the oxygen transport sequence is : (atmospheric air→ lungs alveoli→ hemoglobin→ myoglobin→ Complex IV), oxygen is transported from complex with lowest affinity to complex with highest affinity.

يقول رانشو: " ليس عليك أن تكون ناجحا، كن متميزا و سيسعى النجاح خلفك " – الأغبياء الثلاثة

تحية خاصة للأخت و الصديقة الجميلة هبة العمّوري