Hemoglobin & Myoglobin

Myoglobin (Mb)

Overview

- Myoglobin is an intracellular heme protein found in most cells.
- It functions mainly to store oxygen and facilitate oxygen diffusion in muscles, especially in heart and skeletal muscle.
- It binds the oxygen released by hemoglobin (Hb) and makes it available to muscle cells when needed.

Structure

- Myoglobin consists of a **single polypeptide chain** of 153 amino acids, attached to a **single heme group.**
- About 80% of the myoglobin molecule is in the form of α-helices.
- It has **eight α-helical segments**, named A–H.
- Each α-helix is terminated by a proline residue (which breaks α-helices) or by β-bends and loops.
- These eight helices fold into a globular (spherical) structure, forming a pocket (cradle or box) that holds:

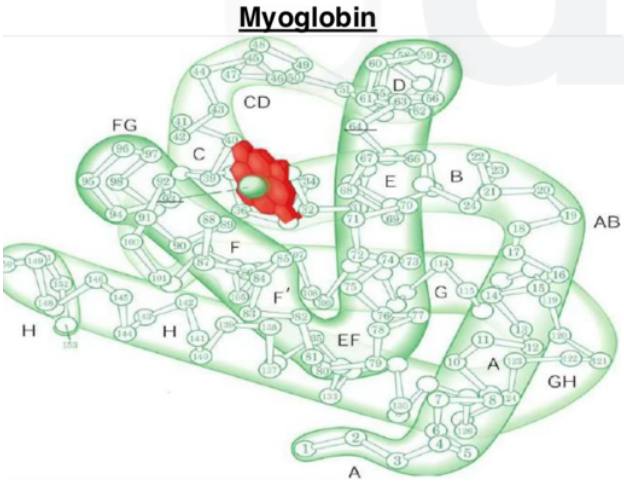
1. The heme group, and
2. The oxygen-binding site.

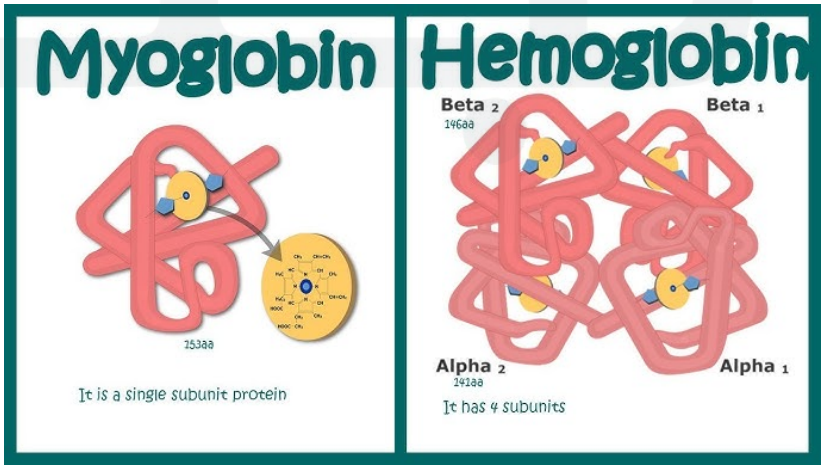
- The heme group lies between helices E and F.

Functions of the Polypeptide Chain

The polypeptide of myoglobin serves three essential roles:

1. Holds the heme group in the correct position.
2. Provides a pocket where oxygen can bind properly.
3. Protects the heme iron (Fe^{2+}) from being oxidized to Fe^{3+} (which would make it unable to bind oxygen).





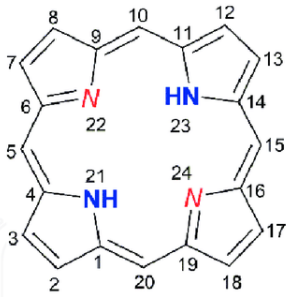
Structure of Heme in Myoglobin and Hemoglobin

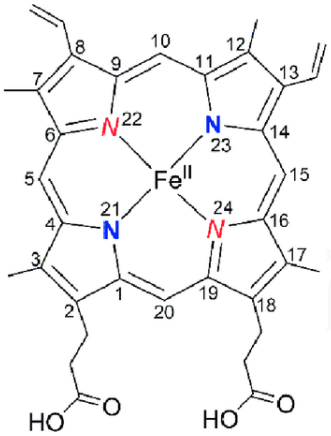
Heme Composition

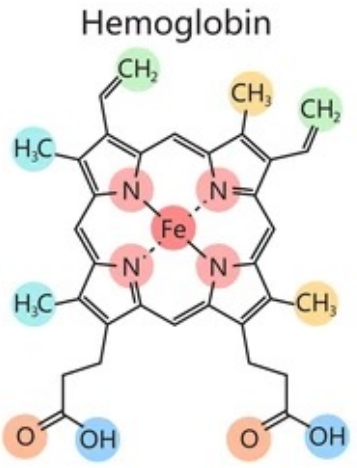
- Both myoglobin and hemoglobin contain a heme group.
- **The heme structure is identical in both proteins.**
- Heme is a complex made of:
- A porphyrin ring, and
- A ferrous iron (Fe^{2+}) atom at its center.

Porphyrin Structure

- Porphyrins are organic compounds made of four pyrrole rings interconnected by **α-methylene bridges (-CH=).**
- Each pyrrole ring consists of four carbon atoms and one nitrogen atom arranged in a ring.
- The Fe^{2+} binds to the nitrogen atoms of the pyrrole rings and also interacts with oxygen when present.







Hemoglobin (Hb)

Overview

- Hemoglobin is a globular protein found in red blood cells (RBCs).
- Each human RBC contains about 270 million hemoglobin molecules.
- A single hemoglobin molecule consists of:
- **Four polypeptide chains**: two α (alpha) and two β (beta) chains.
- Each chain has a heme group similar to that in myoglobin.

Subunit Differences

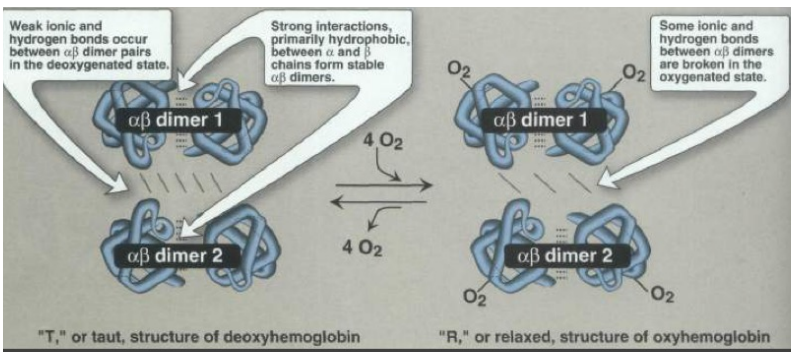
- The α and β subunits differ in their primary structure (amino acid sequence) and are encoded by different genes.
- β-chain = 146 amino acids → shorter than myoglobin (153 residues) because its H helix is shorter.
- α-chain = 141 amino acids → also has a shortened H helix and lacks the D helix.

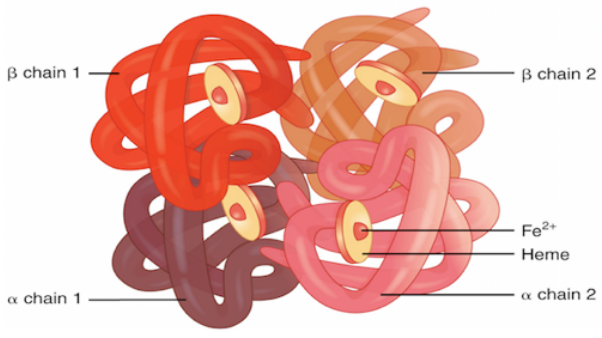
Notable Structural Features	Amino Acids	Protein/Subunit
Full H helix	153	**Myoglobin**
short H + No D helix	141	**α-globin (Hb)**
short H helix	146	**β-globin (Hb)**

Quaternary Structure of Hemoglobin

Subunit Interactions

- Hemoglobin's four chains form two identical dimers:
- **Dimer 1:** $\alpha_1\beta_1$
- **Dimer 2**: $\alpha_2\beta_2$
- Within each dimer, the α and β chains are held together tightly by **hydrophobic interactions**, with additional ionic and hydrogen bonds.
- Between dimers ($\alpha_1\beta_1$ and $\alpha_2\beta_2$), the interactions are weaker, consisting mainly of **ionic bonds** (salt bridges) and **hydrogen bonds.**
- A salt bridge (also called a salt bond) is a relatively weak ionic bond between oppositely charged groups.**





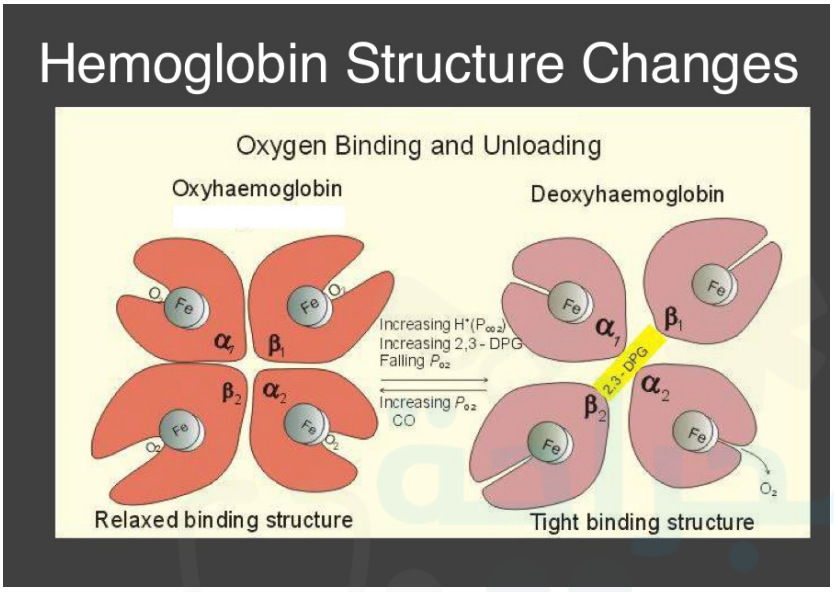
T and R Forms of Hemoglobin

T (Tense) Form – **Deoxyhemoglobin**

- The T form is the deoxygenated form of Hb.
- In this form, the two αβ dimers are held together by many ionic and hydrogen bonds, which **restrict** movement of the polypeptide chains.
- Therefore, **the T form has low affinity for oxygen.**

R (Relaxed) Form – **Oxyhemoglobin**

- When oxygen binds, some of the ionic and hydrogen bonds between the dimers are broken.
- This allows the subunits more **freedom** to move, forming the **R (relaxed) state, which has a high affinity for oxygen.**



Red Blood Cells (RBCs)

- RBCs are biconcave disks, a shape that provides:
- Large surface area for gas exchange.
- Flexibility to squeeze through narrow capillaries.

Carbon Monoxide (CO) and Hemoglobin

- **CO binds to the same site on heme as oxygen (O_2).**
- Affinity for CO is much **higher** than for O_2.
- When CO binds, hemoglobin cannot carry oxygen, leading to hypoxia and tissue death.
- Treatment: Administer pure oxygen to displace CO from Hb.

Methemoglobin Formation

- For oxygen binding, the iron in heme must be in the ferrous state (Fe^{2+}).
- Reactive oxygen species (ROS) can oxidize $Fe^{2+} \rightarrow Fe^{3+}$, forming methemoglobin, which *cannot* carry oxygen.

Reduction System in RBCs

RBCs have a system to reduce Fe^{3+} back to Fe^{2+}, consisting of:

1. **NADH** (from glycolysis) – supplies electrons.
2. Cytochrome b_5 reductase (methemoglobin reductase).
3. **Cytochrome b_5** – directly transfers electrons to methemoglobin.

Mechanism:

$Fe^{3+} + e^- \rightarrow Fe^{2+}$

Cytochrome b_5 donates the electron and is then regenerated by cytochrome b_5 reductase using NADH.

Binding of Oxygen to Myoglobin and Hemoglobin

Allosteric Effects

- Hemoglobin's oxygen-binding is influenced by several allosteric effectors, including:
- pO_2 (oxygen partial pressure)
- pH
- pCO_2
- 2,3-bisphosphoglycerate (2,3-BPG or 2,3-DPG)
- "**Allosteric**" means that binding at one site affects another site *on the same molecule*.
- **Myoglobin's** oxygen binding is **not** influenced by these effectors.

2,3-Bisphosphoglycerate (2,3-BPG or 2,3-DPG)

Function

- 2,3-BPG is a three-carbon intermediate of glycolysis.
- It is a key regulator of oxygen binding to hemoglobin.
- **Low oxygen levels** (low pO_2) in tissues stimulate **increased** synthesis of 2,3-BPG in RBCs.

Mechanism of Action

- 2,3-BPG **binds** to partially deoxygenated hemoglobin, **lowering its affinity for O_2.**
- This promotes oxygen release to the tissues by stabilizing the deoxy (T) form of Hb.

Binding Site

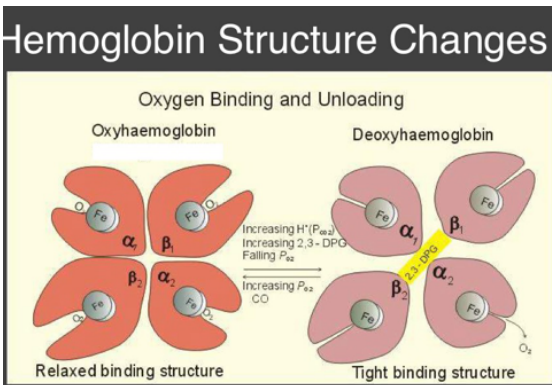
- The binding site for BPG lies within the central cavity of the hemoglobin tetramer.
- BPG is negatively charged and binds to positively charged amino acids (mainly histidine and lysine) on the **β**-chains.
- This cross-links the β subunits, helping stabilize the deoxy form.
- Thus, O_2 and BPG are mutually exclusive — BPG binds only when O_2 is released.

Specific Amino Acid Interactions

- Phosphate groups of BPG form **ionic bonds** with:
- N-terminus of amino acids at carbons 1 and 2, and
- Histidine at position 143, and
- Lysine at position 82 of β-chains.

Change in Hemoglobin Structure During Oxygenation

- When Hb becomes oxygenated:
- The central cavity of Hb becomes narrower.
- Therefore, 2,3-BPG cannot fit inside the cavity of oxyhemoglobin.
- **This explains why BPG binds only to deoxyhemoglobin.**



Fetal Hemoglobin (HbF)

Structure

- Fetal hemoglobin (HbF) differs from adult hemoglobin (HbA) in its **polypeptide chain composition.**
- In HbF, the β-chains of adult hemoglobin are replaced by γ (gamma) chains.
- Each γ chain contains 146 amino acids, **similar in length to the β chain** but slightly different in sequence.
- Therefore, the structure of fetal hemoglobin is written as $\alpha_2\gamma_2$, whereas adult hemoglobin is $\alpha_2\beta_2$.

Binding of 2,3-Bisphosphoglycerate (2,3-BPG)

- **2,3-BPG binds less effectively to fetal hemoglobin than to adult hemoglobin.**
- This difference is due to an amino acid substitution in the γ chains:
- In adult β chains, Histidine (positively charged) is present at position 143.
- In fetal γ chains, this Histidine is replaced by Serine (a polar but uncharged amino acid).
- As a result, HbF lacks two positive charges in the central BPG-binding cavity, reducing its ability to bind 2,3-BPG strongly.

Effect on Oxygen Affinity

- Because 2,3-BPG binds less tightly to HbF, the fetal hemoglobin remains in a higher-affinity (R) state for oxygen.
- Hemoglobin without 2,3-BPG is almost fully saturated with O_2 even at low pO_2 levels.
- Therefore, **fetal hemoglobin (HbF) has a higher affinity for oxygen than adult hemoglobin (HbA)**.

Physiological Importance

- The fetus depends entirely on the mother's blood for oxygen supply.
- However, the fetal and maternal circulatory systems are separate; they do not mix.
- Gas exchange occurs across the placenta, where maternal and fetal blood come close enough for diffusion.
- Since HbF has a higher oxygen affinity, it can extract oxygen more effectively from the maternal HbA, ensuring efficient transfer of O_2 to the fetus.

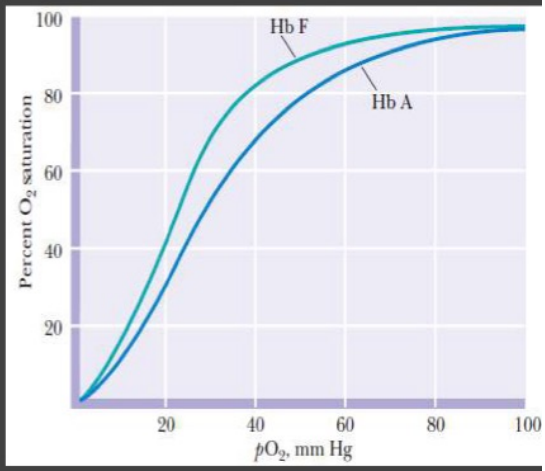


Figure compares the relative affinities of adult Hb (also known as Hb A) and Hb F for O2 under similar conditions of pH and [BPG]. Note that Hb F binds O2 at pO2 values where most of the oxygen has dissociated from Hb A. Much of the difference can be attributed to the low capacity of Hb F to bind BPG

Comparison between Mb and Hb

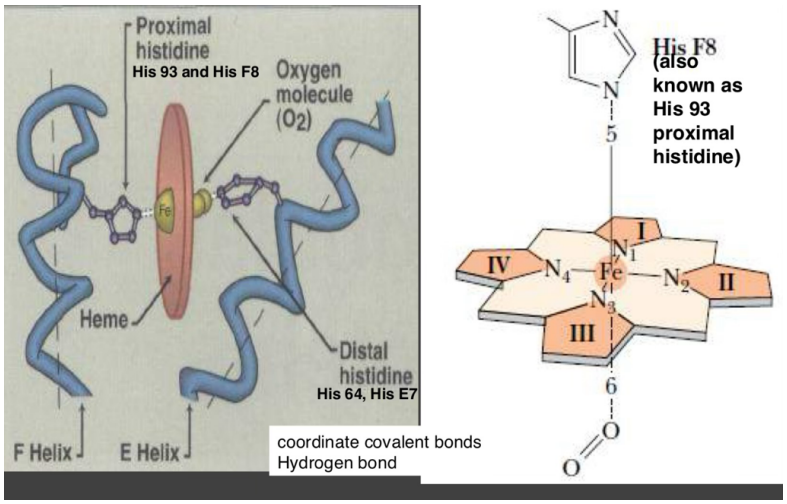
Mb	Hb
In muscle	In RBCs
Reservoir of O_2	Carrier of O_2
No quaternary structure	Has quaternary structure
Can't carry CO_2	Carries CO_2
No cooperativity of O_2 binding	Shows cooperativity
O_2 affinity is higher than Hb	O_2 affinity is lower than Mb

✅ Summary Table

Fetal Hemoglobin (HbF)	Adult Hemoglobin (HbA)	Feature
$\alpha_2\gamma_2$	$\alpha_2\beta_2$	Structure
γ-chain (Serine at position 143)	β-chain (Histidine at position 143)	Chain Difference
Weak	Strong	2,3-BPG Binding
Higher	Lower	Oxygen Affinity
Oxygen uptake from maternal blood via placenta	Oxygen transport in adult tissues	Function

✅ Summary Table

Hemoglobin	Myoglobin	Property
4 polypeptides (2α, 2β) + 4 hemes	1 polypeptide (153 aa) + 1 heme	Structure
Oxygen transport in blood	Oxygen storage in muscles	Function
Cooperative binding, sigmoidal curve	High affinity, hyperbolic curve	O_2 Binding
Affected by pO_2, pH, CO_2, and 2,3-BPG	None	Allosteric Regulation
Red blood cells	Muscle cells	Location



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لَا حَوْلَ وَلَا قُوَّةَ إِلَّا بِاللَّهِ

"من كنوز الجنة"