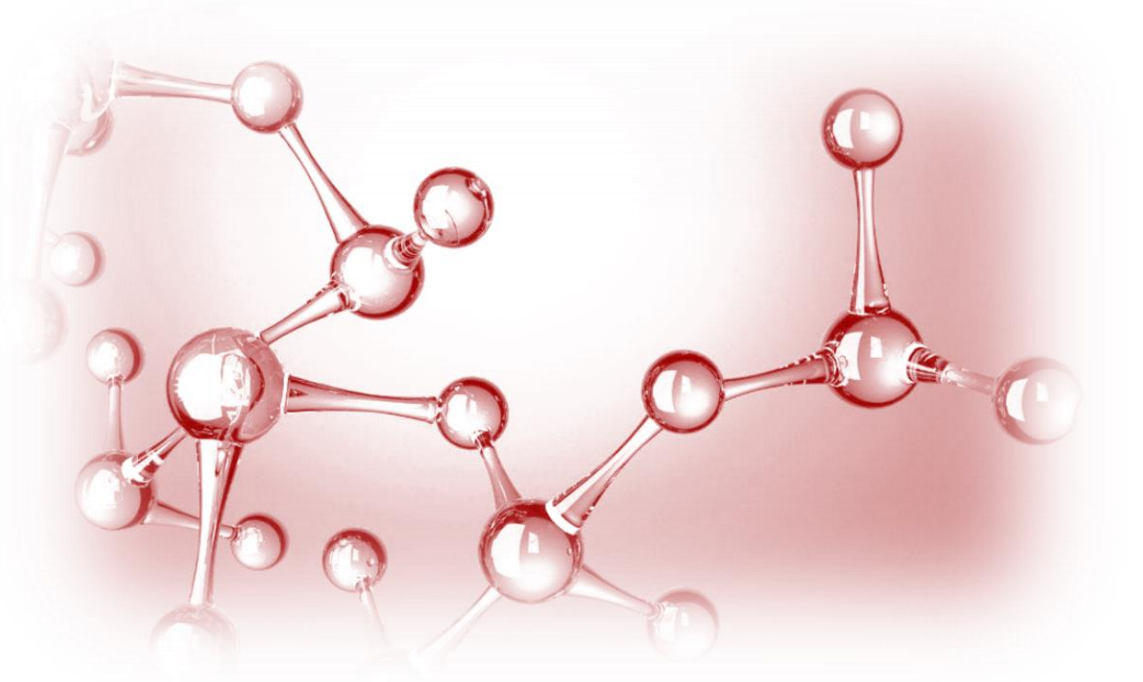
Med Learn

Dr.Ahmad Al-Qawasmi

Biochemistry

Sheet 10

Globular proteins

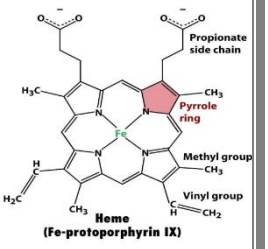


❖ Globular proteins

- Examples ➔ **Myoglobin and hemoglobin** → they are hemoproteins

- **Hemoproteins:**
  - ➢ a group of specialized proteins <u>containing heme</u> as a tightly bound **prosthetic** group
  - ➢ Both **myoglobin and hemoglobin** are hemoproteins
  - ➢ Heme has many functions → dictated by the protein's environment

- **Heme Group:**



  - ➢ It is a <u>prosthetic group</u> (a non- protein group covalently attached to a protein)
  - ➢ Heme group belongs to **porphyrins family** (it has a ring structure)
  - ➢ Heme is a **flat** (planar) & rigid molecule
  - ➢ It has <u>4 cyclic groups</u> known as **pyrrole rings** with a **Ferrous ion (Fe^{+2}) in the middle**
    - ✓ Every pyrrole ring has a **nitrogen**, all the <u>4 nitrogen binds with the **Iron**</u>
    - ✓ Every ring has **2 side chains** that are exposed to the outside (methyl/vinyl/propionate)
  - ➢ Heme is synthesized by several steps → having different types of porphyrins → until getting heme
  - ➢ Heme is also called **Fe-Protoporphyrin IX**
  - ➢ Upon absorption of light, heme gives a deep red color

- **Iron**
  - ➢ It presents in the **Fe^{+2} (ferrous)** state **<u>not</u>** in the **ferric state (Fe^{+3})**
  - ➢ If it is in the **ferric** state, we call it **Hemin** ➔ Incapable of normal O_2 binding → No O_2 transport
  - ➢ Fe in the ferrous state (Fe^{+2}) can form **6 bonds**:
    - ✓ <u>4 with the nitrogen</u> of the pyrrole rings
    - ✓ <u>1 with N of the imidazole in Proximal His</u> → 5th coordination
    - ✓ <u>1 with O_2</u> → 6th coordination
    - ✓ One (known as the fifth coordinate) with the nitrogen of a histidine imidazole (known as proximal His)
    - ✓ One with O2 (the sixth coordinate)

❖ Functions of myoglobin and hemoglobin

- **Myoglobin** ➔ **storage** of O2 in <u>muscles</u>
  - ➢ During periods of oxygen <u>deprivation</u>, oxymyoglobin releases its bound oxygen

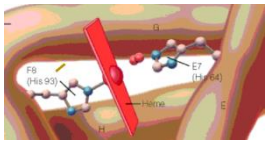
- **Hemoglobin** ➔ **transport** of O2 and CO2 & blood **buffering (Due to His residues)**
  - ➢ Hemoglobin transports O2 to cells to use it in Cellular respiration and combustion reactions

❖ Myoglobin

- A **monomeric** protein (1 subunit) has a heme group (interior) → found in the **muscle** tissue
- The tertiary structure (overall structure) ➔ **8 α-Helices** (designated from A-H) connected by short non-helical regions

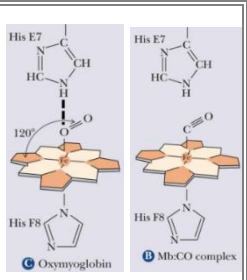
- It can present in 2 forms:
  - ➢ **Oxymyoglobin** → Oxygen-**bound** form
  - ➢ **Deoxymyoglobin** → Oxygen-**free** form (not bound to oxygen)

- In Myoglobin & other polar other globular protein, amino acids with polar R-groups are exposed on the surface (hydrophilic), while those in the interior are predominantly hydrophobic



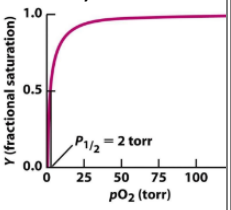
  - ➢ Except for **2 histidine residues present in helix E & F** ➔ Known as E7 & F8
    - ✓ E7 → The 7th residue in Helix E / F8 → the 8th residue in Helix F
    - ✓ F8 Histidine → is also called proximal His → binds to iron
    - ✓ E7 Histidine → is also called Distal His → It is a gate that opens and closes as O_2 enters the hydrophobic pocket to bind to the iron of heme → then **distal His forms H-bond to O_2**

- So, **proximal His binds always to Fe**, and the distal His stabilizes the interaction (O_2-Fe) by H-bonds
- In low pO_2 or smoking conditions → CO will bind to Fe (Mb:CO complex) **irreversibly preventing the binding of O_2**
- Myoglobin-heme interaction is stabilized by **hydrophobic attractions** between the hydrophobic pocket of the protein with Heme
  - ➢ The heme group stabilizes the tertiary structure of myoglobin



- The **hydrophobic region** surrounding the Heme group **prevents** the oxidation of iron from Fe^{+2} to Fe^{+3} so when O_2 is released, the iron remains in the Fe(II) state and can bind another O_2

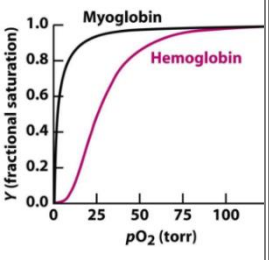
- Myoglobin binds O_2 with high affinity → so can bind to O2 in very low concentration (sensitive)
- **P50:** oxygen partial pressure (indicates concentration) required for 50% of all myoglobin molecules to be saturated
- P50 for myoglobin ~2.8 torrs or mm Hg
- Given that **O2 pressure in tissues** is normally 20 mm Hg, it is almost fully saturated with oxygen at normal conditions
- The Mb-O_2 follows a **hyperbolic saturation curve**



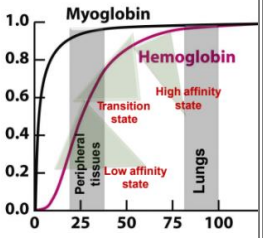
❖ Hemoglobin

- Hemoglobin is a **hetero-tetramer** that is made of 4 subunits (2 alpha, 2 beta)
- Each subunit consists of **multiple α-helices** (α subunits have 7 helices & β subunits have 8 helices), with a **heme group** in the interior of the protein with Fe^{+2}
- The chains (subunits) interact with each other via **hydrophobic interactions**
  - ➢ Therefore, hydrophobic amino acids are not only present in the interior of the protein chains, but also on the surface
- **Electrostatic interactions** (salt bridges) and **hydrogen bonds** also exist **between** the 2 different chains
- Hemoglobin must **bind oxygen** efficiently and become **saturated** at the high oxygen pressure found in lungs (approximately **100** mm Hg) → forming **Oxyhemoglobin (Oxygen-bound)**
- Then, it releases oxygen and become **unsaturated** in tissues where the oxygen pressure is low (about **20** mm Hg) → **Deoxyhemoglobin (Oxygen-Free)**

- **The saturation curve:**
  - ➢ It has a **sigmoidal shape**
    - ✓ Each Hemoglobin bind to **4 O_2** but myoglobin binds only to 1 O_2 so it takes longer time for hemoglobin to be saturated
    - ✓ The binding of the 4 oxygens is gradual with **positive cooperative** (the binding of O2 to the 1st subunit increases the affinity for the 2nd one by breaking electrostatic interactions and so on)



  - ➢ At **100 mm** Hg (in the lungs) → **High affinity state** → hemoglobin is 95-98% **saturated** (oxyhemoglobin)
  - ➢ As the oxygen pressure **falls** in the tissues → **Low Affinity state** → oxygen is released → **unsaturated**
  - ➢ **p50** of hemoglobin is approximately **26** mm Hg (P50 of myoglobin = 2.8)
    - ✓ That indicates that myoglobin is more sensitive for oxygen than Hemoglobin



- Hemoglobin is an **allosteric protein** → have many shapes (conformations) → Have 2 forms **T & R states**

- **Allosteric protein:** A protein where binding of a molecule (ligand) to one part of the protein affects binding of a similar or a different ligand to another part of the protein

- The **T-state** is also known as the "taut" or "tense" state and it has a **low-binding affinity** to oxygen
- The **R-state** is known as the "relaxed" state and it has 500 times **higher affinity** to oxygen than as the T
  - Binding of O_2 causes conformational changes in hemoglobin, converting it from the low affinity T-state to the high affinity R- state
  - In the T-state the 2 αβ dimers are bound to each other by **a lot of interactions (salt bridges)**
  - The binding of O2 → **less interactions between dimers** → R-state

- **How does the structure change by the binding of O_2?**

1) The structure becomes flat pulling the proximal His

- When heme is free of oxygen, it has a **domed** structure and iron is outside the plane of the heme
- When oxygen binds to an iron atom, heme adopts a **planar** structure and the iron moves into the plane of the heme pulling proximal histidine (F8) along with it

2) Breakage of the electrostatic bonds

- This movement triggers changes in tertiary structure of individual hemoglobin subunits → breaking the electrostatic interactions at the other oxygen-free hemoglobin chains

- In **myoglobin**, movement of the helix does not affect the function of the protein

- So, the sigmoidal curve is caused by the **gradual** binding of O_2 → due to the **cooperativity** between the subunits of hemoglobin (**allosteric**)
- The affinity state (T or R) → depends on the pressure of O_2 in the environment

- This is a **protective mechanism**
  - A sudden drop in pulmonary capillary oxygen tension does not affect hemoglobin saturation (such in **high altitudes**), for example:
    - ✓ If pO_2 became 75 or 80 instead of 100 it would remain fully saturated (approximately)
    - ✓ If pO2 became 60 or 50 → it would have a higher effect on the saturation it would be about (60% saturated)