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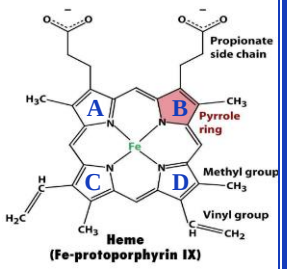
Biochemistry

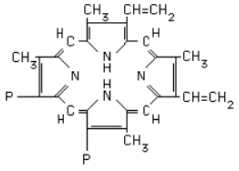
Globular proteins

❖ Globular proteins

- **Hemoproteins:** A group of specialized proteins containing heme as a tightly bound prosthetic group
  - **Prosthetic group:** a **tightly (covalently)** bound, specific **non-polypeptide organic** (vitamin, sugar, or lipid) or **inorganic** (such as a metal ion) which is required for the biological function of proteins
- Some types of Hemoproteins:
  - Myoglobin and hemoglobin: Storage and transport of O_2
  - NOS and Cytochrome P450: Used in the oxygenation reactions
  - Cyt c and Cyt b_5: They transfer electrons in the Electron transport chain of the mitochondria
  - Sensor proteins: Sense the amount of heme and gases (such as CO) in the blood

- **Heme Group**
  - It is a prosthetic group that consists of protoporphyrin IX attached to iron (Fe^{+2})
  - Porphyrin is a flat (planar) molecule consisting of **4 pyrrole rings**
    - ✓ Each pyrrole ring has **2 side chains** that are exposed to the outside, where one side chain is a methyl and the other can be a vinyl or propionate group
    - ✓ Each pyrrole ring has a nitrogen, all the **4 nitrogen binds with the Iron**
  - Iron presents in the **Fe^{+2} (ferrous) state** not in the ferric state (Fe^{+3})
    - ✓ In the ferrous state (Fe^{+2}) iron can form 6 covalent bonds (4 bonds with N atoms of the pyrrole rings, 1 with N of the imidazole in Proximal His (5^{th} coordination) and 1 bond with O_2 (6^{th} coordination))
  - Heme is a **hydrophobic** molecule
  - The protein environment dictates the function of the heme
  - Upon absorption of light, heme gives a **deep red color**

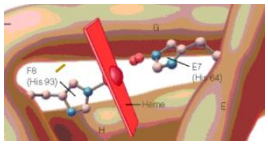




❖ Myoglobin (Mb)

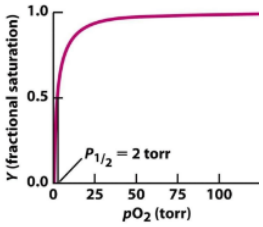
- It is a **monomeric** protein, mainly found in the **muscle** tissue (skeletal muscles)
- The tertiary structure 8 α-Helices (designated from A-H) connected by short non-helical regions
- It can present in 2 forms:
  - Oxymyoglobin: Oxygen-bound form
  - Deoxymyoglobin: Oxygen-free form (not bound to oxygen)

- In Myoglobin and 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
- **Globin fold** is a hydrophobic O2 -binding pocket which contains the heme group
  - The heme group is bound **covalently** to the myoglobin
  - The propionate groups form **electrostatic interactions** with the polar amino acids on the surface of the myoglobin
  - **Hydrophobic interactions** between the heme and the globin pocket stabilizes the tertiary structure of myoglobin, and this hydrophobic environment prevents the oxidation of iron from Fe^{+2} to Fe^{+3}



- The only exception is 2 histidine residues present in helix E & F, known as E7 & F8
  - **E7:** The 7^{th} residue in Helix E and it is also known as the **distal histidine** which represents a gate that opens and closes allowing the entry of O_2 to the hydrophobic pocket, and it also stabilizes the interaction with oxygen by H-bonding with it
  - **F8:** the 8^{th} residue in Helix F and it is also known as the **proximal histidine** which binds to iron

- Myoglobin stores O_2 in the muscles
- Myoglobin binds O_2 with **high affinity**
  - **P50** is the oxygen partial pressure required for 50% of all myoglobin molecules to be bound with oxygen ($P50_{myoglobin}$ ~ **2.8** torrs)
  - At the normal O_2 pressure (pO_2) in tissues (20-40 mm Hg), so Mb is almost fully saturated with oxygen
  - During **hypoxia**, pO_2 drops suddenly causing quick **release of O_2**
  - The Mb-O_2 follows a **hyperbolic saturation curve**

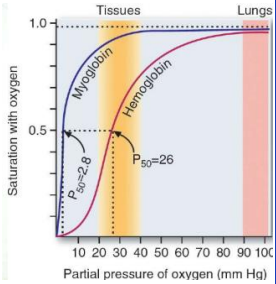


❖ Hemoglobin (Hb)

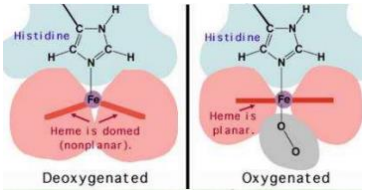
- Hemoglobin is a **hetero-tetramer** that is made of 4 globin subunits (2 alpha, 2 beta)
  - It consists of 2 αβ-protomers
  - Each subunit consists of multiple α-helices (α subunits have **7** helices with 141 A.A & β subunits have **8** helices with 146 A.A), with a heme group in the interior of the protein
- **Hydrophobic interactions** between α and β subunits stabilize the αβ-dimer
  - Hydrophobic amino acids are not only present in the interior of the protein, but also on the surface
- **Electrostatic interactions** (salt bridges) and **hydrogen bonds** exist between the 2 different αβ-dimers

♦ The saturation curve

- Hemoglobin has a **lower affinity** to O_2 than myoglobin
  - **p50** of hemoglobin is approximately **26** mm Hg
  - At 100 mm Hg (in the lungs) hemoglobin is 95-98% **saturated** (oxyhemoglobin)
  - As the pO_2 **falls** (in the tissues), oxygen is **released** into the cells (**unsaturated,** deoxyhemoglobin)
- The curve has a **sigmoidal shape**
  - A sigmoidal curve indicates that the protein has different structures
  - The binding of the 4 oxygens is gradual with positive cooperative
- Hemoglobin is an **allosteric protein**
  - Allosteric protein: A multi-subunit 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 by changing its structure slightly



- Hemoglobin exists in two allosteric forms, T-state and R-state
  - **T-state** is also known as the "taut" or "tense" state and it has a low-binding affinity to oxygen causing the release of oxygen in the tissues
  - The **R-state** is known as the "relaxed" state and it has 500 times higher affinity to oxygen than T, causing the binding of oxygen in the lungs
- Binding of O_2 causes slight conformational changes in hemoglobin (**0.4 Å long and 15º degrees only**), converting it from the low affinity T-state to the high affinity R-state
- How does the structure change by the binding of O_2?
  - 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, due to the hydrophobic heme is repelled by the proximal His



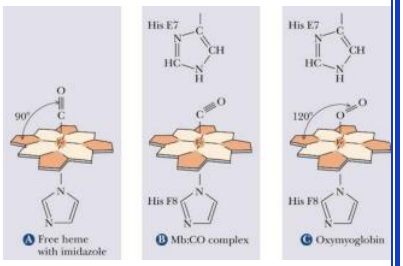
- ✓ When oxygen binds to an iron atom, distal His forms H-bonds with O2 causing the structure to become **planar** and the iron moves into the plane of the heme pulling proximal histidine (F8) along with it

- ➢ Breakage of the electrostatic bonds
  - ✓ These changes in tertiary structure of individual hemoglobin subunits breaking the electrostatic interactions at the other oxygen-free hemoglobin chains, changing the quaternary structure of Hb

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**)
  - ➢ Oxygen is a homotropic effector (the allosteric modulator is the substrate itself)
  - ➢ Also, the release of one oxygen makes it easier for the next oxygen to be released
- **Homotropic allosteric regulator/effector:** Effector and ligand regulated by the effector are the same molecule (e.g., O2 binding affects subsequent O2 binding)
- **Heterotropic allosteric regulator:** Effector and ligand are different molecules (H^+ or BPG binding affects O_2 binding)
- **Positive allosteric interaction:** effector binding increases affinity for ligand
- **Negative allosteric interaction:** effector binding decreases affinity for ligand

- This is a **protective mechanism**
  - ➢ Isolated Heme has a higher affinity (thousands of folds) to bind CO than O_2
  - ➢ When heme is bound to hemoglobin its affinity toward CO decreases dramatically (CO affinity is only 250 times more than O_2)
    - ✓ This decrease is due to the distal His
    - ✓ CO prefers the straight bonding and O_2 prefers the bent bonding
  - ➢ CO occupies 1% of hemoglobin, but 99% if distal His does not exist



- **Smoking** conditions, CO will bind to iron irreversibly preventing the binding of O_2

Past papers

1. **The reason why myoglobin cannot be allosteric is:**

   A. Heme doesn't change shape when it binds oxygen
   B. Myoglobin binds with strong affinity to oxygen
   C. Myoglobin is a muscle-specific molecule
   D. Myoglobin is monomeric
   E. Myoglobin is a conjugated protein

2. **The sigmoidal shape of the oxygen saturation curve of hemoglobin indicates that:**

   A. Hemoglobin is an allosteric protein
   B. Hemoglobin is a hetero-multimeric protein
   C. Hemoglobin is a conjugated protein

D. Hemoglobin has a prosthetic group
E. Hemoglobin is a holoprotein

3. Distal histidine has this significant role in hemoglobin:

A. It stabilizes oxygen binding to heme via the formation of hydrogen bonding with it
B. It covalently links the heme group to hemoglobin
C. It makes the affinity of hemoglobin to carbon monoxide lower than that of oxygen
D. It reduces iron when oxygen is released and iron is oxidized
E. It prevents the entry of carbon monoxide into the heme binding core

4. What is the usual outcome of mutation in the amino acid residues on the surface of hemoglobin?

A. Reduced oxygen binding
B. Protein denaturation
C. Protein aggregation
D. Protein instability
E. Usually nothing major

5. This is how propionate groups of heme molecules are positioned in both myoglobin & hemoglobin

A. They are covalent linked to distal histidine.
B. They are oriented towards the exterior surface of the protein.
C. They are covalently linked to proximal histidine.
D. They are hidden inside the protein.
E. They are linked to one of the internal alpha helices

6. The R conformation of hemoglobin always predominates in which of the following tissue:

A. RBCs
B. Lungs
C. Liver
D. Kidneys
E. Muscles