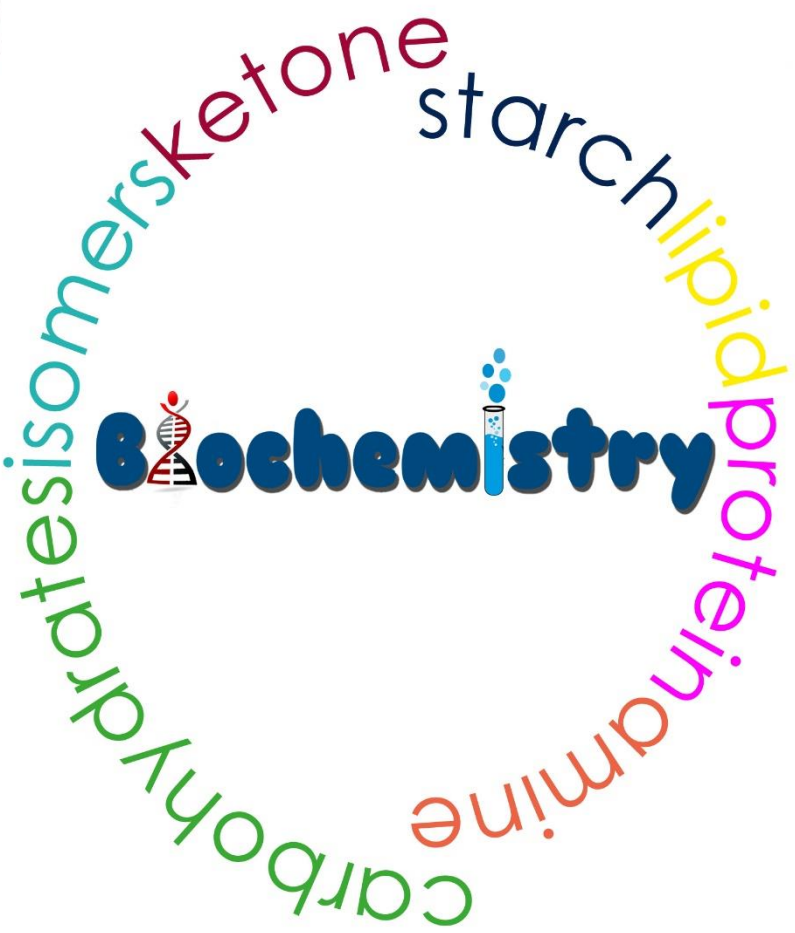


◉ Sheet ○ Slides

Subject :	Hemoglobin and purification
Done by :	Ahmad Maswadeh
Corrected by :	Saleh Al_jbour
Number :	7

Revision:

Hemoglobin

Hemoglobin is a tetramer; it composed of 4 peptide chains (4 subunits), each subunit is composed of a protein chain tightly associated with a non-protein heme group. A heme group consists of an iron (Fe) ion (charged atom) held in a heterocyclic ring (that has atoms of at least two different elements as members of its ring), known as a porphyrin. This porphyrin ring consists of four pyrrole molecules (heterocyclic aromatic organic compound, a five-membered ring with the formula C_4H_4NH), with the iron ion bind in the center, which is the site of oxygen binding. Fe may be either in the Fe^{2+} or in the Fe^{3+} state, but ferrihemoglobin (Fe^{3+}) cannot bind to oxygen, thus iron must exist in the +2 oxidation state to bind to oxygen. On the other hand, myoglobin is composed of only one subunit with a heme group attached to this subunit.

- The purpose of having a protein made up of more than one subunit is to change in between each other and give **different functions**; stimulation and inhibition. So this is why quaternary structure proteins exist.

- How these subunits are connecting between each other?
  If they interact covalently, we are restricting the function and movement, so it is mainly **non-covalent** interactions such as H-Bonds, Ionic interactions, electrostatic interactions (salt bridges), hydrophobic interactions (mainly interior), and hydrophilic interactions (mainly exterior).However, you can **rarely** find <u>hydrophobic regions such as the binding sites of the subunits on the outside</u> and <u>hydrophilic regions such as histidine (proximal and distal) inside the protein.</u>

a-subunit + B-subunit = aB-dimer (hydrophobic interactions)

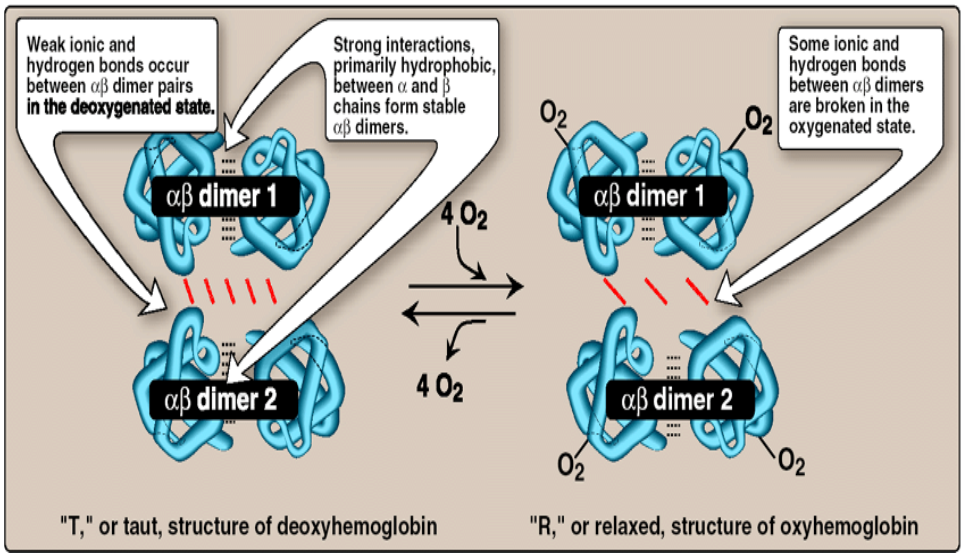
Dimer + dimer = hemoglobin (salt bridges, ionic interactions and H-bonds)

Deoxyhemoglobin→Oxyhemoglobin (breaking bonds between the dimers)

Two states for hemoglobin:

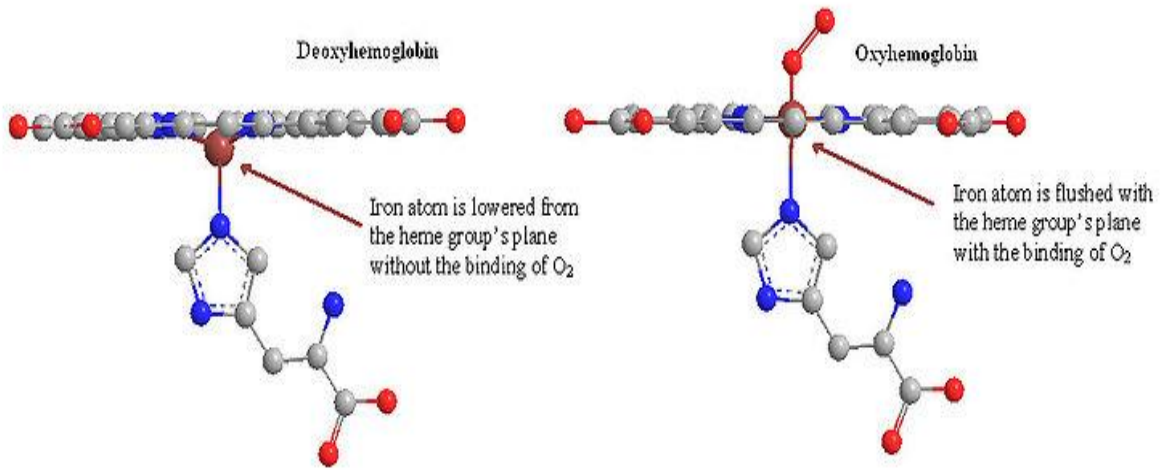
1- Deoxyhemoglobin (T tense) low affinity

2- Oxyhemoglobin (R relaxed) high affinity



- Hemoglobin can depend on the behavior, so it can be classified as an allosteric protein. **Allosteric** means that the binding site is far away from the function, like the remote control, you are changing the action of something away from you, and apparently it must have more than one subunit. In hemoglobin, binding oxygen with the first subunit causes a change in the second and third and fourth subunits, whereas myoglobin is not allosteric because it has only one subunit.

- How does this occur mechanistically?
  Firstly, the hemoglobin in the lungs has low affinity (T) before binding to oxygen, but because of an external factor (The high pressure of oxygen and concentration of oxygen in the lungs 100 torrs) the Oxygen binds to the first subunit.
  Now, the allosteric behavior starts; by changing the structure to make the other subunits have high affinity (R).
  Mechanistically:
  T-state: in deoxyhemoglobin the iron atom of the heme group out of the plane (angled) like dome-shaped.
  R-state: in oxyhemoglobin the iron atom of the heme group becomes into the plane (straight) **- a conformational change happens-** after it was angled with 0.4A (angstrom), pulling the proximal histidine (F8), which pulls the polypeptide chain bonded to it, which make change in the whole subunit, which make change in the other subunit, that change the dimer then the whole hemoglobin will change (**very small change in the structure will affect the properties of molecule).**



Purification

We took that: amino acids→peptides→proteins (how structure relates to the function)

- How can we see these proteins? How can we extract it from cells?

  The first step is **Homogenization** which means cell lysis, degrading the cell membrane, so the contents and the medium become homogeneous.

- How can we achieve homogeneous? How can we break up cells?

  1- Detergents: chemical solutions such as alcohol can disrupt the membrane.

  2- Potter–Elvejhem homogenizer: a test tube with a rod and you keep mechanically do grinding.

  3- Sonication: it can break up things depending on the power of the ultra sonic waves.

  4- Freezing and thawing: repeatedly because of the properties of water.

- The second step is **Differential centrifugation**, it does not break up the cell, and it separates the contents by precipitation.

  Different shapes, densities and molecular weight, and according to these factors we calculate the force, speed and time to precipitate group of proteins.