Hematology

BIOCHEMISTRY

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Number: **2**

Subject: **Structure of hemoglobin**

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Price:

Structure of hemoglobin:

Hemoglobin is a tetramer composed of two (αβ) dimers. Between the polar groups of the two dimers hydrogen bonds occur. When oxygen bind to the deoxy state (T state or tight state) the molecule undergoes a conformational change to a more relaxed state (R form), some of the ionic and hydrogen bonds between the dimers are broken or become weaker allowing movement and change in structure.

Hemoglobin is an allosteric protein. There are two theories for the transformation of allosteric proteins from inactive to active form; one of them is the concerted model of Monod and the other is the sequential model.
Hemoglobin follows the concerted model of Monod:
There is T state (less active) and R state (fully active). When oxygen binds bonds become weaker and some of them become broken.

**The concerted model of Monod* states that we have only two forms, T or R. the equilibrium favors one of the conformational states. in the case of deoxyhemoglobin the T form predominate, while the R form is unstable and is present in minimal amount. When the substrate (positive effectors) is added the equilibrium is shifted to the R form. While binding of negative effectors causes shift to the T form (proportion depends on how much substrate do you have)
So, when oxygen binds we will have some R form, and T form decrease. As more oxygen binds the trend keeps going on.
Some other allosteric enzymes follow the sequential model in which a gradual conformational change takes place.

Cooperativity of hemoglobin:

in deoxyhemoglobin there is steric repulsion between the imidazole of histideine and the pyrole rings. Iron, is situated in the plane of the heme when heme is alone (out of the globin protein). But because of this steric repulsion mentioned above Iron bulges.

When oxygen binds to iron it pulls iron back to the plane of heme (iron becomes coplaner with heme). Iron is bound to imidazole, which is part of the F helix. So the movement is transmitted to the F helix which cause the movement of the neighboring subunits. This is how this movement is initiated and transmitted to neighboring subunits. And that explains how hydrogen bonds between the (αβ) dimers are broken in the oxygenated state.

Binding of oxygen should be reversible (oxygen should bind to Hb at high oxygen tension (lungs) but it should also be released in low oxygen tension (peripheral tissue))

$Fe\text{-}O_2 \rightarrow Fe^{3+} + O_2^-$ THIS REACTION SHOULD NOT HAPPEN!!

Fe^{3+} : ferric

O_2^- : superoxide (a free radical—dangerous!)

We are protected against it and ferrous iron is preserved by the hydrophobic environment of the heme pocket so no charge separation takes place (if the pocket was hydrophilic then we will have water, which can separate charges- remember from school chemistry that when you dissolve NaCl in water they separate into Na^+ and Cl^- and that is because water molecules surround these particles and keep them separated) However This reaction happens but only to a small extent.

If a mutation occurs in the hydrophobic pocket and water was allowed in charge separation will take place and we will have ferric iron.

- Remember : C is the 3rd litter of the English alphabet; so Fe^{3+} is ferric iron
- Ferrous iron is the one that can bind and carry oxygen so remember Fe^{2+} carries O_2 "Fe+Two carries O two!"

Hemoglobin+ferric iron(instead of ferrous)=**Methemoglobin** and it does not bind oxygen. Methemoglobin increases in the blood when exposed to oxidizing drugs, or if you have a mutation in the heme pocket (HbM) as mentioned earlier on.

NADH-cytb5 reductase enzyme protects us from methemoglobin accumulating in the blood through reducing the ferric iron back to ferrous and therefore methemoglobin to hemoglobin. This enzyme-as its name implies- needs NADH to function. Newborns have only half of the amount of the enzyme compared to adults. That is why newborns should not be given some of the oxidizing drugs. Rare deficiency of this enzyme is present around the world and these patients should be careful not to take oxidizing drugs.

Methemoglobinemia is treated with methylene blue to reduce methemoglobin back to hemoglobin. Methylene blue needs NADPH to work (Remember that G6PD enzyme is the source of NADPH, an enzyme of high incidence of deficiency in our area). If the patient was also G6PD deficient give ascorbate- which is also a reducing agent but is less effective.

Cyanide poisoning;

Cyanide is a deadly poison because it inhibits complex IV (of the electron transport chain in the mitochondria) Cyanide loves ferric iron (Fe^{3+}) And by the way complex IV has ferric iron in it and that is why it hits it. Oxidative phosphorylation is essential for life. So

when someone is poisoned with cyanide you give them a drug which converts hemoglobin to methemoglobin . i.e. we create MetHb in purpose! So cyanide binds methemoglobin forming cynomethemoglobin in the blood before reaching tissues. And you SAVE the patient's life. YOU ARE THE HERO BECAUSE YOU UNDERSTAND BIOCHEMISTRY WOHOO!!!

Hemoglobin allosteric effectors: CO2, BPG (2,3-bisphpsphoglycerate), protons, temperature.

A shift to the right allows for unloading of oxygen in tissues without decreasing the saturation in the lungs (always reaches 100%).
- Because saturation happens in the lungs where there is very high partial oxygen pressure (pO_2) so allosteric effectors has no effect. These allosteric effectors show their effect on lower pO_2 (in peripheral tissue) increasing or decreasing oxygen delivery to tissue.

Left shift = increased O_2 affinity (less available to tissue)	Right shift: decreased O_2 affinity
↑pH ↓temp. ↓2,3-DPG	↓pH ↑temp. ↑2,3-DPG

- Erythrocytes have the enzyme mutase which produces 2,3-bisphosphoglyerate from 1,3-bisphosphoglycerate (a high energy compound and a source of ATP)in glycolysis. So this compund is present considerably higher in erythrocytes than in other cells. 2,3-BPG (has 5 negative charges) binds in a pocket formed by the two βchains of deoxygenated hemoglobin A. These βchains have several positive charges to interact with the negative charges of 2,3-BPG.
- 2,3BPG normal molar concentration inside the cell is around 4.5millimolar and that is approximitly that of hemoglobin.
- Fetal hemoglobin has two alpha and two gamma chains (instead of the beta chains); one of the differences between gamma and beta chain is that a histidine residue in the beta chain is replaced for a serine residue on the gamma chain and this causes a weaker binding to 2,3-BPG. The result is that HbF has a higher affinity for oxygen than maternal HbA, allowing transplacental passage of oxygen from mother to fetus.

Summary:

- 2,3-BPG is an important regulator of oxygen binding, it is the most abundant organic phosphate in the RBC. It is synthesized from an intermediate of glycolysis. Hb has high affinity to oxygen without BPG. BPG stabilizes theT state, t is increased in response to chronic hypoxia whether it is caused by obstrective lung diseases (COPD, emphysema, etc) or being at high altitudes (low PO_2→lower oxygen carrying capacity→adapt by increasing BPG so increasinf delivery)
- Pyruvate kinase deficiency; causes a hemolytic anemia→ lower oxygen carrying capacity→replenish by increasing 2,3-BPG
- Blood could be stored for 30 to 42 days in the blood bank.Tranfused blood was noticed to need few hours to restore depleted 2,3-BPG. If the patient is severly ill transfused blood will be useless (or might even compromise the patient)because there is yet no BPG so delivery of oxygen is very low. So RBCs trap oxygen instead of delivering it. Now, stored blood is supplemented with phosphate, hexose sugar and adenosine to avoid depletion of BPG.

CO_2 Transport;

Most of the CO_2 is transported as bicarbonate(diffuse from the cell to the plasma→to the RBC; there is an enzyme called *carbonic anhydrase* that catalyzes the formation of carbonic acid→become bicarbonate, which, in its turn dissociates to the plasma . However, some CO_2 is tranported by hemoglobin from the tissues where it is generated as end products of metabolis to be expired by the lungs, Some CO_2 interact covelantly with the hemoglobin to form a carbamino derivetive. Deoxyhemoglobin is more reactive than oxyhemoglobin. The difference accounts for about 15% of the CO_2 transported. The terminal amino group interavts with CO_2 forming the carboammino derivetive.