Hematology - Biochemistry

(Numbering is according to the 2nd set of slides on the website)

ONLY EXTRA NOTES

Slide 8

- We can see the repulsion force between the porphyrin ring and proximal histidine, this repulsion causes the histidine to pull the iron slightly above the plane (steric hindrance). Absolutely, this is the case in the deoxy- form only.

Slide 9

- The diagram on the right represents the F helix upon binding of oxygen. Besides the movement of the iron atom into the plane upon oxygenation (the molecule becomes coplanar), the whole helical segment (F) is moved as well, and this movement is transmitted into the remaining parts of the molecule. This is what we meant by the change which is responsible for cooperativity concept.

- Looking at the diagrams on the left, we have an example on similar changes on the quaternary structure of Hb upon oxygenation. We have alpha and beta chains. The tyrosine in alpha chain is bound to aspartate of the beta chain by some electrostatic forces. This is definitely the deoxy- form. After oxygenation, the movements cause those bonds to deform and new bonds are being formed. A change is ultimately going to happen.

Slide 11

- The hydrophobicity of the heme pocket is very important as we learned. If it gets mutated (adding some hydrophilic amino acids for example), a charge separation will take place. This charge separation is due to oxidation (electron transfer) of ferrous into ferric (Fe^{+2} into Fe^{+3}) which - in turn - causes the reduction of oxygen into water. The hydrophobicity in this region prevents this charge separation and prevents oxidation of ferrous and reduction of oxygen, so we will not get any electron transfer (we decrease the dielectric constant) [(1)].

- If there is a mutation here, we will get hydrophilic effects and ferrous will be oxidized into ferric and a super oxide ion will be formed as well. This means that no more oxygen could be bound and subsequently, methemoglobin (HbM which is hemoglobin with a ferric ion and reduced oxygen in the form of water) will be formed.

- Major amounts of HbM are formed by inherited defects in the chains. There might be small amounts happening accidently.

- The mechanism by which we reduce the ferric into ferrous is accomplished by using the enzyme NADH-cytb5 reductase. This enzyme uses an electron from NADH (not NADPH) for that purpose and thus, converts back HbM into normal Hb.

- In cyanide poisoning, the cyanide binds complex 4 in electron transport chain and inhibits it. Here we need certain amounts of HbM as a treatment. This HbM will interact with the cyanide and form cyanomethemoglobin which is not harmful at all. This byproduct will be metabolized slowly and the amounts of ingested cyanide are going to be gradually eliminated.

- In cases of HbM accumulation (due to reductase enzyme deficiency), we treat the patient with methylene blue dye (ideal treatment). This dye acts as an electron carrier that oxidizes an NADPH molecule and reduces the ferric into ferrous. This treatment is not allowed in cases of G6PD deficiency, it can - on the contrary - cause some hemolysin effects. So, a patient with HbM accumulation must have his G6PD enzyme levels checked before undergoing treatment by methylene blue.

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(1): it is the relative permittivity which is what expresses the force between two point charges in the material.

Slide 12

- We'll begin discussing the allosteric effectors.

- Pure Hb means a Hb outside the intracellular medium. Its p50 is about 7-10 mmHg, thus, has a higher affinity.

- CO_2 and BPG are called (negative allosteric effectors). They cause the sigmoidal shape to be shifted to the right (lower affinity).

- Temperature is an important negative effector as well. Its increase produces shift-to-right, increases p50, decreases affinity and increases oxygen unloading toward the tissues. This is actually important in oxygen delivery in the exercising tissues where the temperature and the oxygen demand get higher.

Slide 13

- Decreasing pH produces a shift to the right and an increase in p50. On the other hand, increasing pH causes a shift to the left and an increase in the affinity.

Slide 15

- BPG exists in only catalytic amounts in other cells. However, it's present in considerably large amounts in RBCs even equal to those of Hb (4-5 mMol).

- Notice the 5 negative charges on BPG. This negativity is important in the entrapment of BPG in its site between the two beta chains which have surrounding positive amino acids (the same 3 amino acids are present in the 2 beta chains) and the positive N terminal of the valine plays a role as well. This will create ionic bonds between BPG and the beta chains.

Slide 16

- Notice that the stripped Hb (without BPG) resembles the pure Hb (p50 is about 7-10 mmHG). It takes a hyperbolic shape.

- When the concentration of BPG is increased from 5 to 8 mMol, a shift-to-right takes place as well as an increase in p50 (it will become around 35 mmHg).

- The presence of BPG in required amounts is very important for a proper oxygen delivery inside the tissues.

Slide 17

- 1,3 Bisphosphoglycerate acts as a source of ATP production during glycolysis by substrate-level phosphorylation.

- In RBCs, some of this 1,3 Bisphosphoglycerate is being shunted to form 2,3 BPG (The so-called BPG) and this is catalyzed by the enzyme mutase.

Slide 18

- HbA (adult hemoglobin) constitutes about 90% of our Hb. HbF (fetal) extracts the oxygen from the maternal circulation through the placenta during the early life. Notice that HbF is formed by alpha and gamma chains.

- In HbF, the histidine 143 (one of the 3 positively charged amino acids in beta chains in HbA) is not present but replaced by serine, thus, the binding with BPG is reduced, consequently, the affinity of HbF for oxygen is higher than HbA.

Slide 21

- BPG stabilizes the T state (deoxy- form) of Hb, but upon oxygenation, the BPG is expelled out.

- In hypoxia cases (COPD in which lower amounts of oxygen are taken, and in high altitudes where there are lower oxygen tension levels), the oxygen saturation is less than normal, so we tend to increase the unloading by increasing the amounts of BPG.

- In blood storage in blood banks, the stored blood loses its BPG gradually. So if a patient (usually anemic) receives this blood, the Hb will entrap oxygen because of having high affinity for it. To avoid such a case, we supply the stored blood by supplements (like adenine and changes of protons, phosphate and hexoses) and this will restore ATP (for maintenance of the ionic balance and the survival of RBCs), improve the concentration of BPG (not back to normal though), and increase the storage time.