جامعة الملك سعود
King Saud University

"اللَّهُمَّ لا سَهْلَ إلاَّ ما جَعَلْتَهُ سَهْلاً، وأنْتَ تَجْعَلُ الحَزْنَ إذَا شِئْتَ سَهْلاً "

Biochemistry437

Structure and Function of Hemoglobin

Biochemistry Team 437

Color index:
Doctors slides
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Extra information
Highlights

GNT block

Revised By
Hadeel Awartani & Abdullah AlOmar

MED437
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Objectives:

- The structure and function of hemoglobin.
- The factors affecting oxygen binding to hemoglobin.
- Examples of normal and abnormal hemoglobin structures.

Hemoglobin[1] (Hb)

- A Hemeprotein[2] found only in red blood cells.
- Oxygen transport function.[3]
- Contains heme as Prosthetic group.[4]
- Heme reversibly [5] binds to Oxygen

[1] Hemoglobin: is a globular protein, spherical in shape, soluble in water, Tetrameric (has 4 subunits) that can bind to 4 O_2 molecules.

↬ Why do we have a carrier for O_2? Because O_2 is not soluble in water so it won't be able to be carried around in the blood, so the body use hemoglobin to transfer O_2 from the lungs to the tissue.

[2] A protein that has a heme group attached to it.

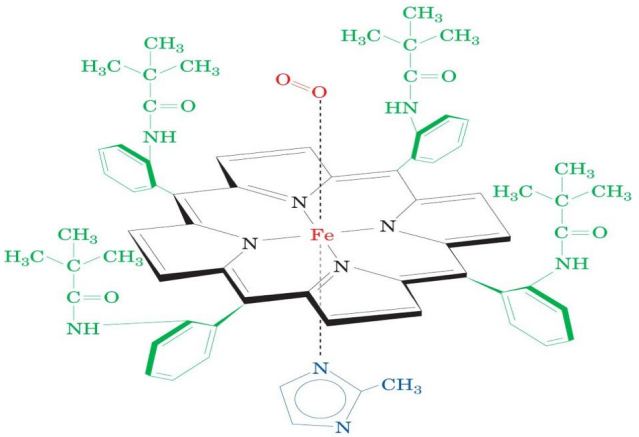
[3] Hemoglobin also carry CO_2 and Protons (H+) from the tissue to the lungs. So it's also considered as a physiological buffer but it's not the main buffer that the body depends on (the main buffer is the Bicarbonate which transport the majority of CO_2).

[4] Some enzymes require a non-protein part to be active (Apoenzyme+Non-protein part= Holoenzyme), the non-protein part could be a Cofactor (metal ions) or a Coenzyme (small organic molecule), the coenzymes are divided into a Prosthetic group (Permanently attached) **e.g.** Heme group and a Cosubstrates (Temporarily attached).

[5] it can bind to O2 and can also release it, which is very important to prevent O2 trapping.

The Heme Group

- A complex of protoporphyrin IX (9) and ferrous iron (Fe^{2+})[1]
- Fe^{2+} is present in the center of the heme
- Fe^{2+} binds to four nitrogen atoms of the porphyrin ring
- Forms two additional bonds with:
  - Histidine residue of globin chain
  - Oxygen



The heme group: Fe^{2+} - porphyrin complex with bound O_2

- Each Hemoglobin contains 4 globin subunits, each subunit is attached to a Heme group, and every Heme group can carry one molecule of O_2.
- Heme group structure consists of a protoporphyrin ring and an iron molecule in the center.
- The iron molecule must be in the **ferrous state (Fe^{2+})**
  When the Heme group contains ferrous iron, the iron is able to bind to 6 molecules:
  - 4 Pyrole nitrogens of the porphyrin ring
  - 1 Histidine of the globin chain
  - 1 Molecular O_2

In case the iron was in the ferric state (Fe^{3+}), it can only make five bonds, so it cannot bind to oxygen. This is the case in Met-hemoglobin. “Abnormal hemoglobin”

Types of Hemoglobin

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Normal Hb
Can carry O_2

Form	Chain composition	Fraction of total hemoglobin
HbA	$\alpha_2\beta_2$	90%
HbF	$\alpha_2\gamma_2$	<2%
HbA_2	$\alpha_2\delta_2$	2%–5%
HbA_{1c}	$\alpha_2\beta_2$-glucose	3%–9%

- Major HB in adults
- Major HB in fetus, only 2% in adults
- Another form in adults, 2 to 5% only
- HbA with glucose attached to it

Abnormal Hb
Cannot carry O_2

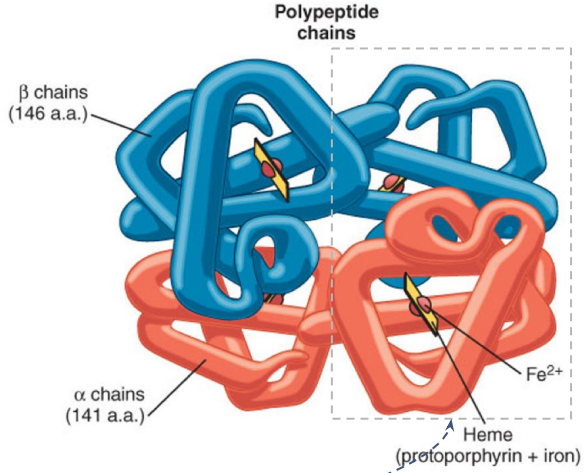
Hemoglobin bound to something else instead of O2

- Carboxyhemoglobin
- Methemoglobin
- Sulfhemoglobin

We will discuss each type in details in a different slide.

Hemoglobin A (HbA)

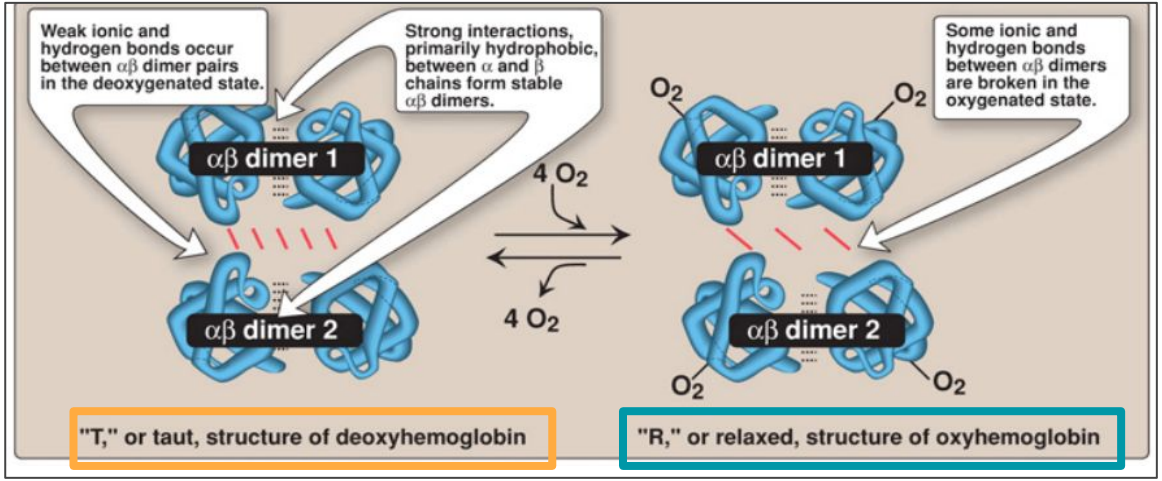
- Major Hb in adults (90%)
- Composed of four polypeptide chains:
  - Two α and two β chains
- Contains two dimers of αβ subunits.[1]
- Held together by non-covalent interactions[2]
- Each chain is a subunit with a heme group in the center that carries oxygen
- A Hb molecule contains 4 heme groups and carries 4 molecules of O_2



1 A dimer is made of one α and one β chains.

2
- The bond between the two subunits in one dimer is called an intradimer bond
- The bond between the 2 dimers are called interdimer bonds
- The intradimer bonds are much stronger than the interdimer bonds, which allows the movement of the 2 dimers

HbA Structure



As we said, the intradimer bonds are very strong and stable hydrophobic bonds, but between the dimers "interdimer" the bond is a weak hydrogen bond allowing for flexibility of movement.

How will this affect the structure of hemoglobin?

When oxygen binds to hemoglobin, it causes a conformational change that breaks some of the weak hydrogen bonds between the dimers, making the structure more relaxed.

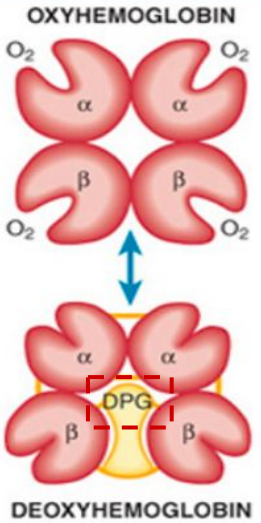
So that means we have 2 forms of Hba,

- "T" or taut structure of deoxyhemoglobin "no O2 attached to it"
- "R" or relaxed oxyhemoglobin "when O2 is attached to it"

In its deoxygenated state the compound is very taut due to the ionic and hydrophobic bonds between the dimers, however when it binds with oxygen the oxygen breaks some of these bonds leaving the compound in a relaxed state.

Forms of Hb

T-Form (Taut)	R-Form (Relaxed)
• The deoxy form of Hb • Taut form (in tissue) • The movement of dimers is constrained • Low-oxygen-affinity form (Promotes release)	• The oxygenated form of Hb • Relaxed form (in the lungs) • The dimers have more freedom of movement • High-oxygen-affinity form



Affinity of Hb to O2 and the role of DPG:

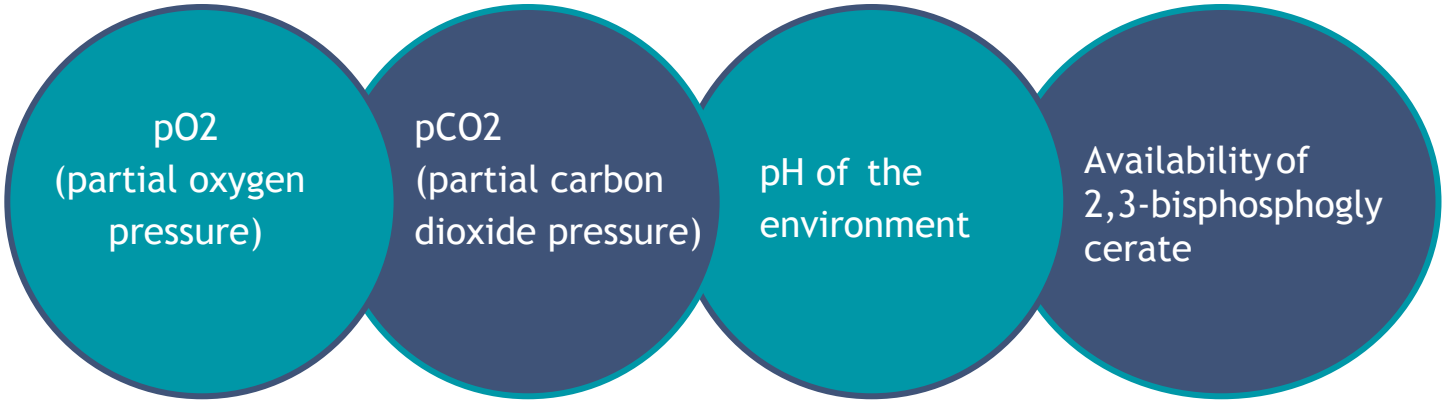
- The environment at the level of the tissue (low PO_2 or high PCO_2) favors the stabilization of deoxy hemoglobin in order for the oxygen to release.
- DPG "2,3 bisphosphoglycerate" or "diphosphoglycerate" is a molecule made by the shunt in the pathway of glycolysis in the RBCs. This molecule binds to the deoxy form of Hb and stabilizes it as well. This allows the release of O_2.
- In case of deficiency of DPG, the Hb can bind to oxygen but can't release it, resulting in the trapping of O_2 in the Hb

Hemoglobin Function

- Carries oxygen from the lungs to tissues
- Carries carbon dioxide and protons from tissues back to the lungs
- Normal level (g/dL):
  - Males: (14-16)
  - Females: (13-15)

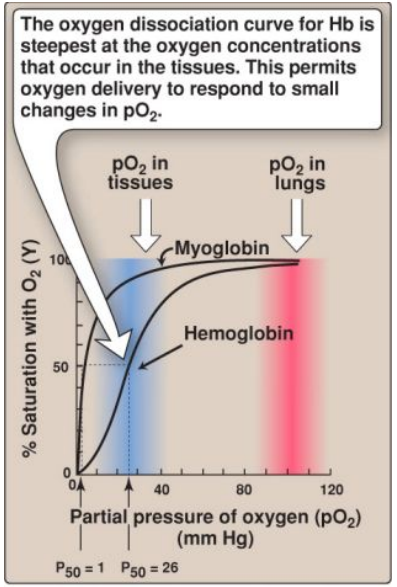
Factors affecting its oxygen binding "affinity"

There are four allosteric factors affecting heme's oxygen binding, they are:



Oxygen Dissociation Curve

- The curve is **sigmoidal**
- Indicates cooperation of subunits in O2 binding
- Binding of O2 to one heme group increases O2 affinity of others.
- Heme-heme interaction
- As the PO2 increases, saturation increases as well.



Oxygen dissociation curve:

- It is a curve that shows the relation between Hb saturation and the partial pressure of O2 of the blood
- Percent Saturation depends on how many O2 molecules are bound to Hb, for example Hb bound to one O2 molecule is 25% saturated, if 2 molecules it is 50% saturated and so on. " we have different degrees of saturation"
- The shape of the curve is sigmoidal "it has a lag phase and a steep phase" which indicates the presence of cooperative binding.

Heme-heme interaction:

- One hemoglobin can bind to 4 molecules of O2, by cooperative binding
- This happens when the binding of the first O2 molecule facilitates the binding of the second molecule "makes it faster". And the binding of the first and second facilitates the third and so on.
- The last molecule binding is the fastest.
- This is called heme-heme interaction
- The same principle of binding and is applied in release of oxygen as well.

- At the lung: very high partial pressure of O2, Hb saturation is 100%
- At the tissue: low partial pressure, Hb saturation is reduced "O2 was released"

P_{50}

- P_{50} (mmHg): the pressure of the O2 at which Hb is 50% saturated with O2.
- P_{50} values can be used to describe the change in affinity of Hb to oxygen.
- Indicates affinity of Hb to O_2:
  - High affinity ⇨ slow unloading of O2 (low P50 value)[1] if the O_2 affinity is too much it wont release O_2
  - Low affinity ⇨ fast unloading of O2 (high P50 value)[1]
- Lung pO2 is 100 mmHg ⇨ Hb saturation is 100%
- Tissue pO2 is 40 mmHg ⇨ Hb saturation is reduced
- Hence O2 is delivered to the tissues.

[1] Changes in p50 indicate change of Hb affinity of O2:
- If the p50 value is decreased "Hb will reach 50% saturation at a lesser PO2 than normal" means the affinity for oxygen is increased and the curve will shift to the Left.
- Increase in p50 "Hb will achieve 50% saturation at a higher PO2 than normal" means the affinity is decreased and the curve will shift to the right.

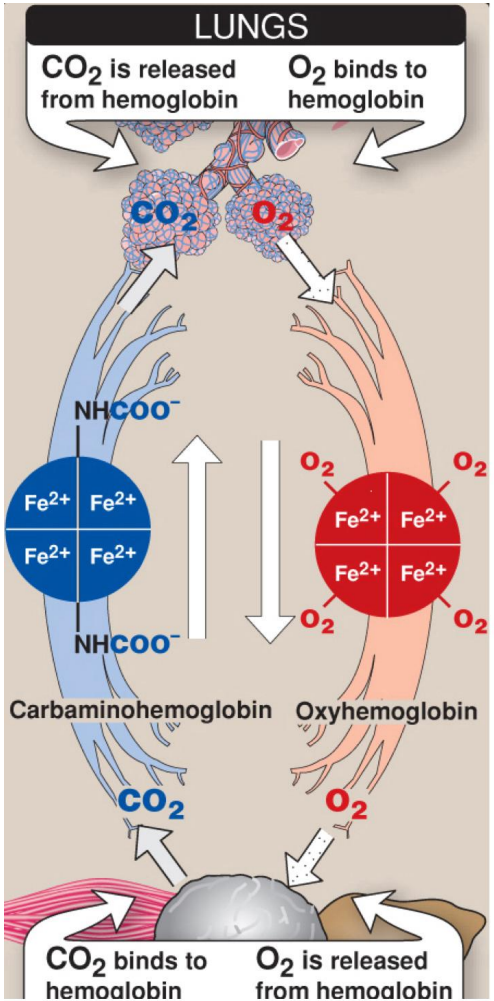
The Bohr effect

At the lungs:

- Protons are unloaded, and the bicarbonate in the blood interact with the proton so that gives carbonic acid that will dissociate to CO2 and exhaled out.

At the tissues:

- Oxyhemoglobin releases O2 and binds to some molecules of CO2, we call it carbaminohemoglobin
- Carbaminohemoglobin can also carry protons.
- When protons or CO2 bind to hemoglobin, they stabilize the deoxygenated hemoglobin and reduce the affinity of Hb. "will be discussed next slide"



- It is the shift of the ODC "oxygen dissociation curve" to the right in response to an increase in pCO_2 or a decrease in pH.
- It describes the Effect of ph and pCO_2 on:
  - Oxygenation of Hb in the lungs
  - Deoxygenation in tissues

The Bohr effect

In the tissues:

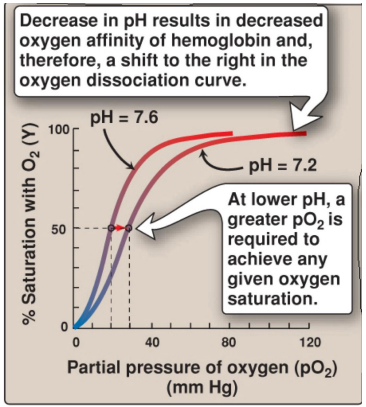
- Tissues have lower pH (acidic) than lungs. Because when tissue is actively metabolising the production of CO_2 is increase, this leads to proton generation
  Due to two reactions:

$$CO_2 + H_2O \rightleftharpoons H_2CO_3$$
$$H_2CO_3 \rightleftharpoons HCO_3^- + H^+$$

- Protons reduce O2 affinity of Hb causing easier release into the tissues.
- The free Hb binds to two protons

In the lungs:

- Protons are released and react with HCO^{3-} to form CO_2 gas ($HCO^{3-} + H^+ \Rightarrow CO_2 + H_2O$).
- The proton-poor Hb now has greater affinity for O_2 (in lungs)
- The Bohr effect removes insoluble CO_2 from blood stream.
- Produces soluble bicarbonate.



In case of increase CO_2 and protons "decrease in PH" in the environment:

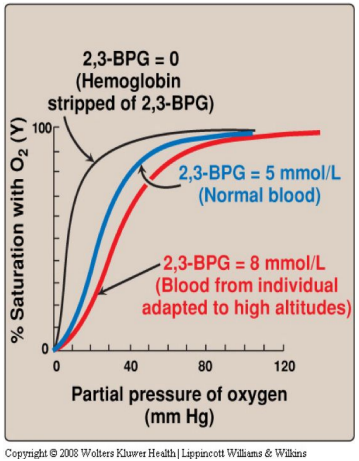
- Deoxy Hb is stabilized
- Affinity of Hb to oxygen is reduced
- Curve shifts to the right and the p50 is increased (bohr's effect)
- Oxygen is released from Hb to tissue "unloaded"

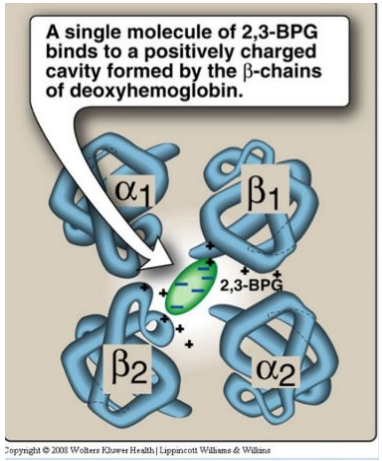
Availability of 2,3 bisphosphoglycerate

- Binds to deoxy-hb and stabilize the T-form
- When oxygen binds to Hb, BPG is released
- This is specially important for people living in high altitudes

At high altitudes:

- RBC number increases
- Hb conc. Increases
- BPG increases





High attitude and oxygen affinity

In hypoxia and high altitude:

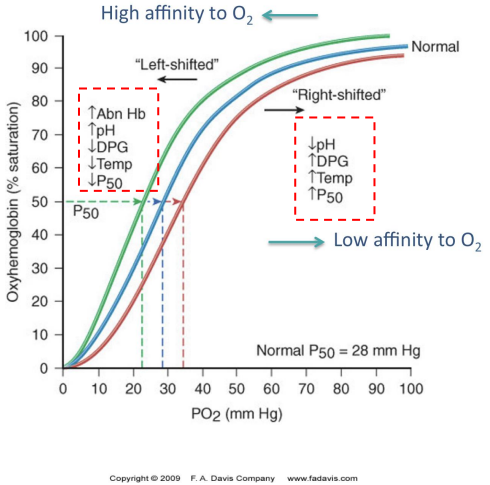
- 2,3 BPG levels rise
- This decrease oxygen affinity of hemoglobin
- Thus increase oxygen delivery to tissue

High oxygen affinity

High oxygen affinity is due to:

- Alkalosis
- High levels of hemoglobin F
- Multiple transfusion of 2,3 DPG- depleted blood

- Alkalosis favors low delivery of oxygen
- HbF: has Higher affinity compared to HbA so it can take oxygen form HbA (mother's Hb)
- Compromised individual unable to produce 2,3 DPG or an Individual who got multiple blood transfusions and the blood was not enriched with 2,3 DPG — chances of oxygen high affinity



HbA1c

- HbA undergoes non-enzymatic glycosylation
- Glycosylation depends on plasma glucose levels
- Hba1c levels are high in patients with diabetes mellitus
- Measures the amount of glucose present in the blood
- Glucose binds to Hb spontaneously (no enzyme, no energy required)
- Helps in monitoring the maintenance of sugar level because Hb stays in RBCs and the life span of the RBC is 3 months.

Fetal hemoglobin (HbF)

- Major hemoglobin found in the fetus and newborn
- Tetramer with two α and two γ chains
- Higher affinity for oxygen than HBA
- Transfer oxygen from maternal to fetal circulation across placenta
- It has higher affinity for oxygen so the fetus can take oxygen from the mother's hemoglobin.

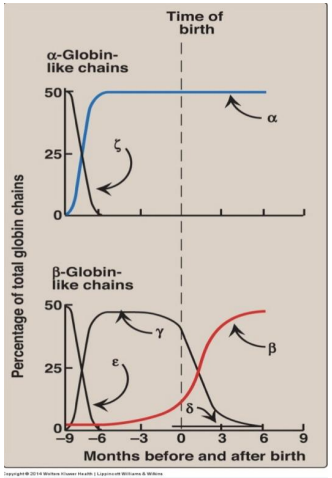
α-Globin-like chains :
Initially there is ζ like chains for a short duration then α-globin chains will appear.

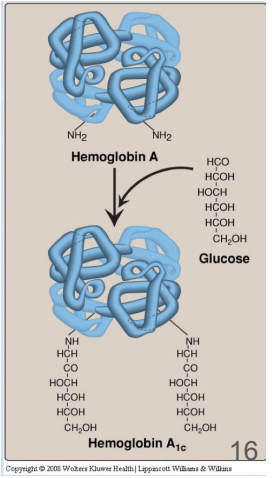
β-Globin-like chains :

- Initially ε-globin like chains appears only in the first trimester
- Then gamma chains stays until after birth and its level decreases in adults
- Delta chains appears just before birth

HbA2

- Appears shortly before birth
- Constitutes ~2% of total hemoglobin
- Composed of two α and two δ globing chains





Abnormal hemoglobins

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Unable to transport oxygen due to abnormal structure.

Met-Hb

Contains **oxidized Fe3+** ferric form (~2%) that cannot carry oxygen.

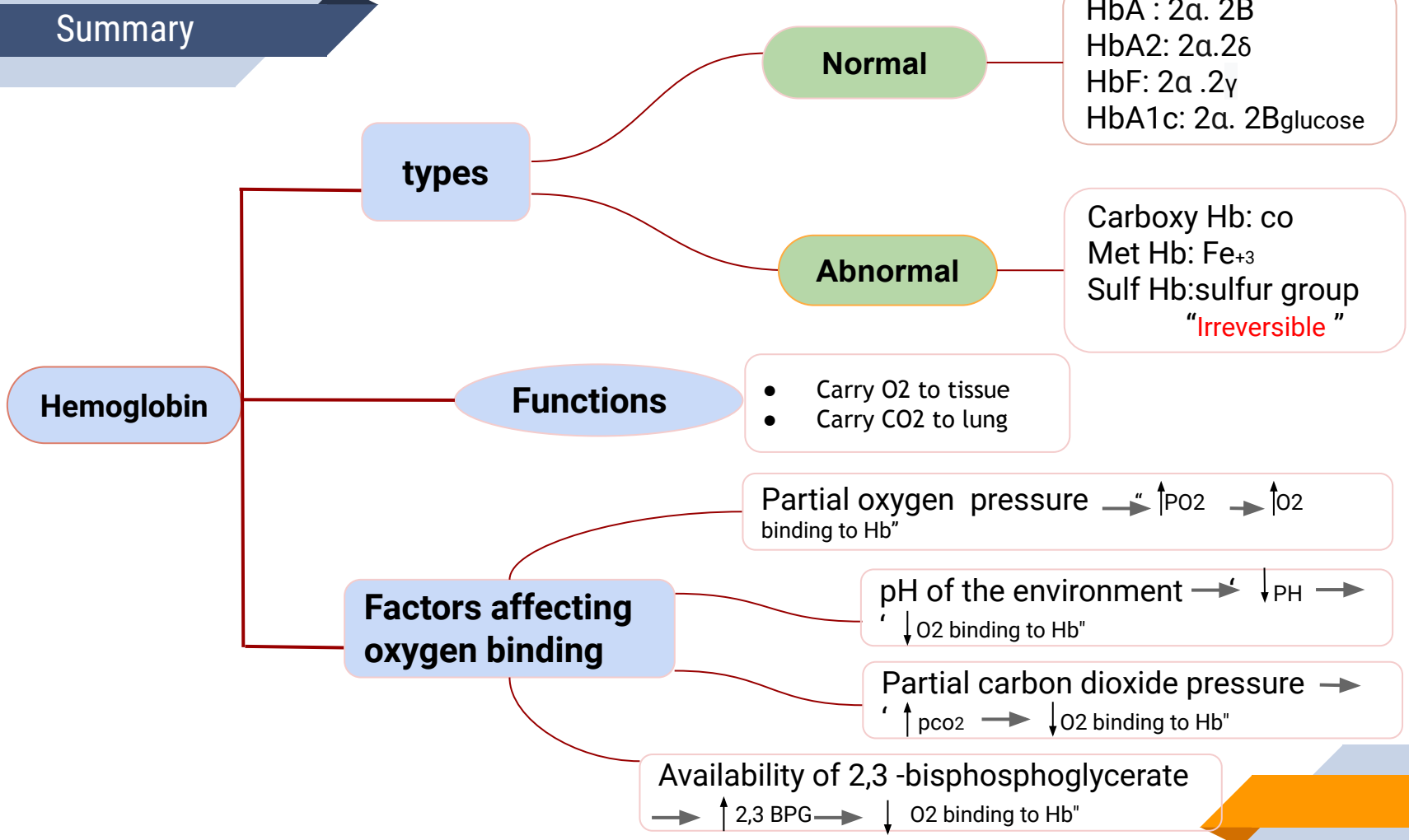
Carboxy-Hb

CO replace oxygen and binds 220x tighter than oxygen (in smokers).

Sulf-Hb

Forms due to high **sulfur** levels in blood (irreversible reaction).

Summary


Hemoglobin
types
Normal
HbA : 2α. 2B
HbA2: 2α.2δ
HbF: 2α .2γ
HbA1c: 2α. 2Bglucose
Abnormal
Carboxy Hb: co
Met Hb: Fe+3
Sulf Hb:sulfur group
"Irreversible "
Functions
Carry O2 to tissue
Carry CO2 to lung
Factors affecting oxygen binding
Partial oxygen pressure "↑PO2 → ↑O2 binding to Hb"
pH of the environment → "↓PH → ↓O2 binding to Hb"
Partial carbon dioxide pressure → "↑pco2 → ↓O2 binding to Hb"
Availability of 2,3 -bisphosphoglycerate → ↑2,3 BPG → ↓ O2 binding to Hb"

Lippincott Summary

- Hemoglobin A, the major hemoglobin in adults, is composed of four polypeptide chains (two α chains and two β chains, α2β2) held together by noncovalent interactions.
- The subunits occupy different relative positions in deoxyhemoglobin compared with oxyhemoglobin.
- The deoxy form of hemoglobin is called the “T,” or taut (tense) form. It has a constrained structure that limits the movement of the polypeptide chains. The T form is the low-oxygen-affinity form of hemoglobin.
- The binding of oxygen to hemoglobin causes rupture of some of the ionic and hydrogen bonds. This leads to a structure called the “R,” or relaxed form, in which the polypeptide chains have more freedom of movement. The R form is the high-oxygen-affinity form of hemoglobin.
- The oxygen dissociation curve for hemoglobin is sigmoidal in shape (in contrast to that of myoglobin, which is hyperbolic), indicating that the subunits cooperate in binding oxygen. Cooperative binding of oxygen by the four subunits of hemoglobin means that the binding of an oxygen molecule at one heme group increases the oxygen affinity of the remaining heme groups in the same hemoglobin molecule.
- Hemoglobin’s ability to bind oxygen reversibly is affected by the pO2 (through heme-heme interactions), the pH of the environment, the pCO2, and the availability of 2,3-bisphosphoglycerate (2,3-BPG). For example, the release of O2 from Hb is enhanced when the pH is lowered or the pCO2 is increased (the Bohr effect), such as in exercising muscle, and the oxygen dissociation curve of Hb is shifted to the right.
- To cope long-term with the effects of chronic hypoxia or anemia, the concentration of 2,3-BPG in RBCs increases. 2,3-BPG binds to the Hb and decreases its oxygen affinity, and it, therefore, also shifts the oxygen-dissociation curve to the right. Carbon monoxide (CO) binds tightly (but reversibly) to the hemoglobin iron, forming carbon monoxide hemoglobin (Hb CO).

MCQs:

Q1/ Depending on Bohr effect which of the following will reduce the affinity of the Hb to the O2 and cause it release..

A. Low pH
B. Acidity
C. Protons
D. All of the above

Q2/ Which of the following is irreversible?

A. Sulf Hb
B. Met Hb
C. Carboxy Hb
D. None of the above

Q3/ One factor that induces high O2 affinity ?

A. Alkalosis
B. Acidosis
C. Low level of Hb
D. Met Hb

Q4: HbA2 is composed of ?

A. Two α and two δ globin chains.
B. Two α and two γ chains.
C. Four α globin chains.
D. Two α and two β chains.

Q5: Carboxy-Hb is found in:

A. Fetus+newborn
B. Smoker
C. Healthy adult.
D. Sulf users

1- D
2- A
3- A
4- A
5- B

Girls team

- لمياء القويز
- ارجوانة العقيل
- روان المشعل
- رهف الشنيبر
- اروى الجهني
- مجد البراك

Boys team

Team leaders

- رهام الحلبي
- عبد الحكيم العنيق

@biochemistry437

teambiochem437@gmail.com