L2-Hemoglobin Structure and Function

1st Year-College of Medicine
Hematology Module-Biochemistry
Semester II
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Objectives

- Definition of the Hemoglobin (Hb)
- Describe the basic structure of Hb
- Determine the functions of Hb
- Identify the factors affecting Hb-oxygen transporting function
- Define the haemoglobinopathies
**Definition** Hemoglobin (Hb) is a member of hemeproteins group which are specialized proteins that contain heme as a tightly bound prosthetic group and constituting 1/3 of the red blood cells.

**Synthesis begins in proerythroblast** (Immature RBCs)
- 65% at erythroblast stage
- 35% at reticulocyte stage

Hb is composed of Two Parts
- **Heme** & **Globin** that are held together

**Synthesis of Hemoglobin (Hb)**
- Heme & globin are produced at two different sites in the cells: **Heme in mitochondria**
- **Globin in polyribosomes**
- Well synchronized
Heme synthesis occurs mainly in mitochondria by a series of biochemical reactions starting from simple building unit; glycine and succinyl-CoA-regulatory enzyme is δ-aminolaevulinic acid (ALA) –B6 is cofactor which is stimulated by erythropoietin.
Heme group is Protoporphyrin ring with an iron in the form of ferrous ion (Fe++) in center. The iron is held in the center of the heme molecule by bonds to the four nitrogens of the porphyrin ring. The heme Fe++ can form two additional bonds, one of these positions is coordinated to the side chain.
of a histidine residue of the globin molecule, whereas the other position is available to bind oxygen molecule.

Note: Mature red cell does not contain mitochondria

**Globin Group and Hb Structure**
Various types of normal globin chain combines with heme to from different hemoglobin such as HbA, HbA2, HbF,...., etc.

Note: Types of globins are differ in amino acids compositions and sequences
Functional (normal) globin chains (like alpha \(\alpha\), beta \(\beta\), gamma \(\gamma\) and delta \(\delta\)) are polypeptide chains. Hb is tetrameric molecule; composed of four polypeptide chains held together by non covalent (weak) interactions such as hydrogen bonds. The Hb types, of which, the major Hb in adults HbA can be envisioned as being composed of two identical dimers, \((\alpha\beta)1\) and \((\alpha\beta)2\), HbFetal-HbF(\(\alpha2\gamma2\))-referred to Quaternary structure.
Polypeptide chain

Polypeptide chain

Polypeptide chain

Heme groups
Adult Hemoglobin

**HbF**  
α2γ2  
0.5-0.8%

**Hb A2**  
α2δ2  
1.5-3.2%

**Hb A**  
α2β2  
96-98%

Structure

Normal%

Globin synthesis, starts at 3rd week of gestation

![Graph showing globin synthesis across prenatal and postnatal ages](image)
Functions of Hemoglobin: The main function of Hb is to transport oxygen O2 from the lungs to the cells of the body and also to reverse transport CO2 and H+ from the tissues to the lungs.

Reaction of Hb & oxygen is
Oxygenation not oxidation
One Hb can bind to four O2 molecules
Less than 0.01 sec required for oxygenation.
Factors Affecting Hb Function—Oxygen-hemoglobin dissociation curve (O2 carrying capacity of Hb) at different PO2

Sigmoid shape-heme-heme interaction

Binding of one molecule of O2 with Hb facilitate the second molecule binding, therefore the affinity binding of the fourth O2 is 300 greater than the affinity binding of the first O2.

\( P 50 \) (partial pressure of O2 at which Hb is half saturated with O2) 26.6 mmHg
O$_2$ Binding to Hb shows positive cooperativity-heme-heme interaction - Hb binds four O$_2$ molecules

O$_2$ affinity increases as each O$_2$ molecule binds

Increased affinity due to conformation change

Deoxygenated form = T (tense) form = low affinity

Oxygenated form = R (relaxed) form = high affinity

Y is O$_2$ saturation with Hb
The normal position of curve depends on

Concentration of 2,3-DPG (2,3 BPG)
H⁺ ion concentration (pH)
pCO₂ in red blood cells & Hb structure

1. Right shift (easy oxygen delivery); low affinity
to O₂ at low pO₂ as at tissues
   High 2,3-DPG
   High H⁺
   High CO₂
   HbS

2. Left shift (difficult oxygen delivery); high affinity
to O₂.
   Low 2,3-DPG
   HbF

2,3-DPG: intermediate product of glycolysis pathway of
   glucose metabolism; 2,3 Diphosphoglycerate.
Hb has sigmoidal $\text{O}_2$ binding curve
Hb high affinity for $\text{O}_2$ at high $p\text{O}_2$ (lungs)
Hb low affinity for $\text{O}_2$ at low $p\text{O}_2$ (tissues).

Myoglobin has Hyperbolic curve; high affinity of myoglobin to $\text{O}_2$ at all $p\text{O}_2$.

$Y$ is $\text{O}_2$ saturation with Hb
Modulation of Hb-O2 affinity occurs through change in protein conformation of Hb; the α and β.

2,3 DPG (2,3 BPG), CO₂ and protons H⁺ are allosteric effectors of Hb binding of O₂.

**Bisphosphoglycerate (BPG)**

BPG binds in the cavity between beta-Hb subunits

Stabilizes T-conformation and causes low O₂ affinity to Hb.

Feta Hb (α₂γ₂) has low affinity for BPG, allows fetus to compete for O₂ with mother’s Hb (α₂β₂) in placenta.

Hb-O₂ + 2,3-BPG = Hb-2,3-BPG + O₂
Oxy & deoxyhemoglobin

Oxygenated Hb- **R-shape**-high O$_2$ affinity
Deoxygenated Hb- **T-shape**-low O$_2$ affinity

Oxyhaemoglobin
Deoxyhaemoglobin

Haem
Bohr Effect

Increased concentration of \( \text{CO}_2 \) (Or increased Pco2) leads to decreased pH(increased H+).

\[
\text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{HCO}_3^- + \text{H}^+.
\]

Hb-O2+H+=HbH+O2

Lower pH i.e. higher [H+], or increased Pco2 decreases the affinity of O2 to Hb, permits the easier release of oxygen from hemoglobin (stabilize the T-shape). This occurred in the capillaries of metabolically active tissues than in alveolar capillaries of lungs, where CO2 is released into expired air. Lungs have a higher pH, while tissues have a lower pH. The curve is shift to the right.
As oxygen is consumed, \( \text{CO}_2 \) is released. Carbonic anhydrase catalyzes the below reaction in red blood cells. The \( \text{H}^+ \) generated from this reaction is taken up by the hemoglobin and causes it to release more oxygen. This proton uptake facilitates the transport of \( \text{CO}_2 \) by stimulating bicarbonate formation.

\[
\text{CO}_2 + \text{H}_2\text{O} \rightleftharpoons \text{H}^+ + \text{HCO}_3^- \\
(\text{Hb})-\text{R-NH}_2 + \text{HCO}_3^- \rightleftharpoons \text{R-NH-COO}^- + \text{H}^+
\]

\( \text{R-NH}_2 \) is the N-terminal of globin chains
When Fe(II) goes to Fe(III), oxidized, it produces methemoglobin MHb which is brown and coordinated with water in the sixth position-non functional Hb. Dried blood and old meat have this brown color. Butchers use ascorbic acid to reduce methemoglobin to make the meat look fresh!!

In human body, there is an enzyme methemoglobin reductase that converts methemoglobin to regular hemoglobin.
Hemoglobinopathies

These are a family of genetic disorders (single or point mutation- or deletion) caused by production of a structurally abnormal Hb molecule, synthesis of insufficient quantities of normal Hb, or, rarely both.

1. Sickle cell disease HbS disease-Sickle cell anemia $\alpha_2\beta^s_2$, Life span of RBCs is 20 days, the point mutation is in $\beta$-globin gene-homozygous & heterozygous. Hemolytic anemia, anoxia,..., .

2. HbC, single mutation in $\beta$ chain, is more less severe than HbS.

3. HbSC

4. Thalassemias, these genetic disorders characterized by synthesis defective in either the $\alpha$ or $\beta$. Normally, $\alpha$ and $\beta$ are synthesized in equal quantities. In thalassemias, either: no globin chain synthesis $\alpha^-$- or $\beta^-$-thalassemia- or the globin chain are synthesized in reduced amounts $\alpha+$ or $\beta+$ thalassemias. There is major-thalassemia-severe anemia and minor thalaseemias-less severity.
Normal Hb range level
children Hb: 12-14 gram/dl
Adult male: 14-18 gram/dl
Adult female: 13-17

Below these range is anemia, the subject is anemic.

HbA1c glycated or glysylated Hb (Hbβ-glucose) is glycemic index (indicator of blood glucose level) for 50-70 days
Summary

\( \Delta \text{Hb} \) is a tetrameric molecule; composed of four polypeptide chains. It can be envisioned as being composed of two identical dimers, \((\alpha \beta)1\) and \((\alpha \beta)2\) as in adult one, the HbA. It is composed of two parts; heme and globin. \( \Delta \) The main function of Hb is to transport oxygen \(O_2\) from the lungs to the cells of the body and also to returned \(CO_2\) and \(H^+\) from the tissues to the lungs. \( \Delta \) Hb has sigmoidal \(O_2\) binding curve. 2,3 diphosphoglycerate (DPG), \(CO_2\) and protons \(H^+\) are allosteric effectors of Hb binding of \(O_2\).