Haemoglobin 2

Globin – conformation of the molecule, types of chains, interactions between the chains. Haem – structure, binding of oxygen, arrangement in oxygenated and deoxygenated state, binding to globin chain. Haemoglobin (Hb) – binding 2,3-BPG, Bohr effect, allosteric interactions, saturation curve, physiological and abnormal types of haemoglobin. Glycation of proteins.

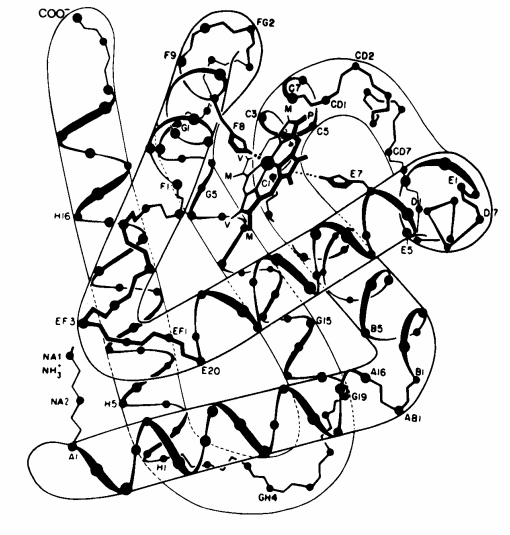
Haemoglobin

M_r(tetramer) = 64 000
Concentration in blood: 2.15–2.65 mmol/L (tetramer)
Binding of oxygen: at totals saturation 4 mol O_2/mol Hb
Saturation of Hb with oxygen: arterial blood ~ 0.97
venous blood ~ 0.73

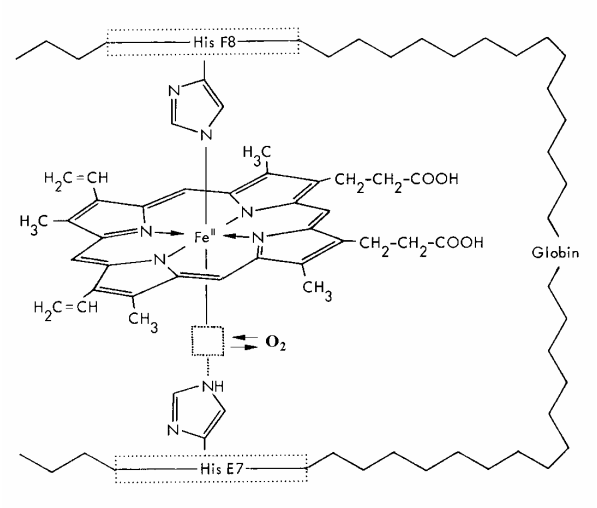
Average values of pO_2:
Alveoli of the lung: 13–15 kPa
Arterial blood: 9–13 kPa
Mixed venous blood: > 5 kPa
(Critical value for hypoxia 3.5 kPa)

1. Give the mass and molar concentration of Hb related to the Hb monomer.
2. Calculate the maximal volume of oxygen that can bind to 1 g of Hb. (1.4 mL)
3. When isolated haem reacts with oxygen, the oxidation of Fe^{2+} to Fe^{3+} occurs. Describe the reaction of oxygen with haemoglobin. Explain the difference.

Secondary and Tertiary Structure of Globin Chain



Structure of Haem



4. Characterize the secondary and tertiary structure of globin chain.
5. Characterize the structure of haem and its bindings to the globin chain.

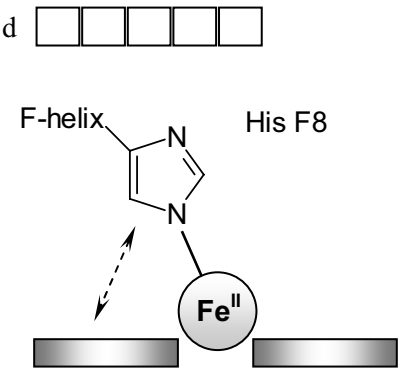
Binding of Oxygen to Haem

Electronic configuration of Fe^{2+} (*complete*)

$_{26}Fe^{2+}$ $1s^2$.....

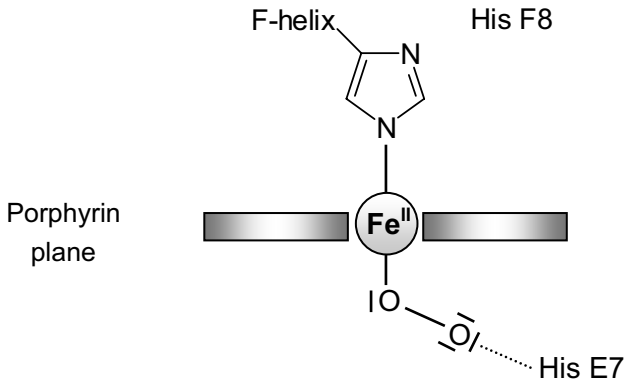
High spin state (the number of coordination 5)

3d



Low spin state (the number of coordination 6)

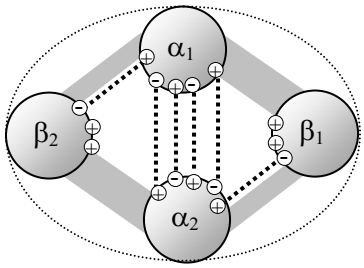
3d



6. What change in haem structure is triggered by binding of oxygen?
7. What change in deoxyHb subunit conformation results from it?

Quaternary Structure of Haemoglobin

T-Conformation



$2\ H_2O + 2\ CO_2 \leftarrow 2\ H_2CO_3$ R-Conformation

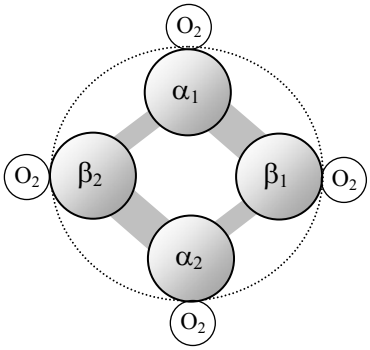
Carbonic anhydrase

Lungs

$2\ H^+ + 2\ HCO_3^-$

$4\ O_2$ $2\ H^+ + 2,3\text{-BPG}$

$4\ O_2$ $2\ H^+ + 2,3\text{-BPG}$



$2\ H^+ + 2\ HCO_3^-$

Tissues

Carbonic anhydrase

$2\ H_2O + 2\ CO_2 \rightarrow 2\ H_2CO_3$

8. Describe the main types of non-covalent interactions between haemoglobin subunits in oxygenated and deoxygenated state.
9. Give the formula of 2,3-bisfosfoglycerate and mark its binding in the T-form of Hb.
10. What is the principle of the Bohr effect?
11. Explain, why is the affinity of Hb to oxygen decreased in the presence of 2,3-BPG.

Dissociation of Haemoglobin

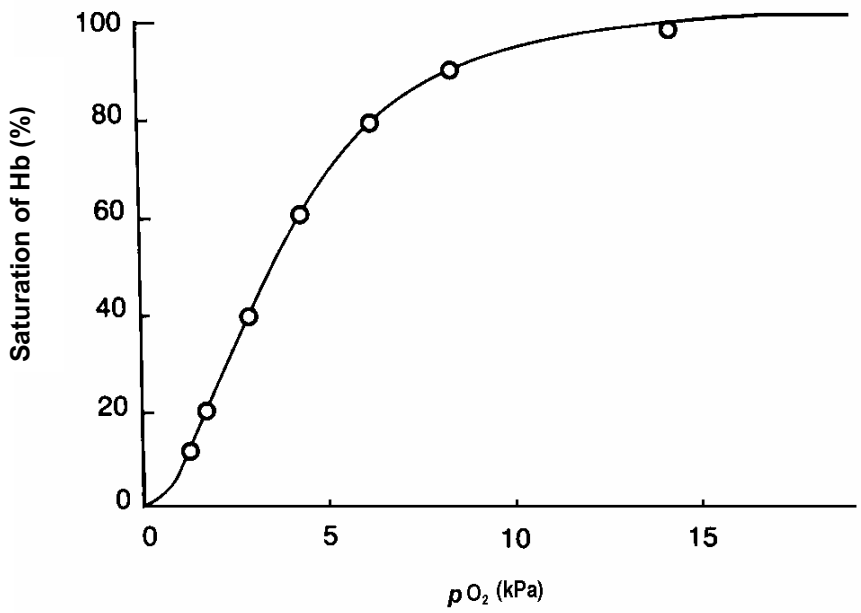
$$HHb \rightleftharpoons Hb^- + H^+ \qquad pK_A \sim 7.8$$

$$HHbO_2 \rightleftharpoons HbO_2^- + H^+ \qquad pK_A \sim 6.2$$

12. Which of the two forms of haemoglobin (Hb or HbO_2) is stronger acid?
13. Which of the amino acids is responsible for acid base properties of haem at physiological pH?

Saturation of Haemoglobin by Oxygen

Saturation curve of haemoglobin



14. Mark areas corresponding to the pO_2 in alveoli of lungs and mixed venous blood in the graph. What is the saturation of Hb in % at these pressures?
15. Complete the saturation curve for myoglobin into the graph. Explain the differences in character of the both curves. Which of the both proteins binds oxygen more tightly?
16. The binding of oxygen to haemoglobin has cooperative character. Explain it.
17. On the saturation curve for Hb mark changes resulting from:
    a) lowering of the pH
    b) decrease of pCO_2
    c) decrease of 2,3-BPG concentration
    d) increase of temperature

Types of Human Haemoglobin

Type	Structure	Proportion of the total Hb in adults
HbA_0	$\alpha_2\ \beta_2$ (partly HbA-Glc)	
HbA		~ 97 %
HbA_1	$\alpha_2\ \beta_2$ (glycation on terminal $-NH_2$ group of β-globin)	
HbA_2	$\alpha_2\ \delta_2$	~ 2.5 %
HbF	$\alpha_2\ \gamma_2$	~ 0.5 %

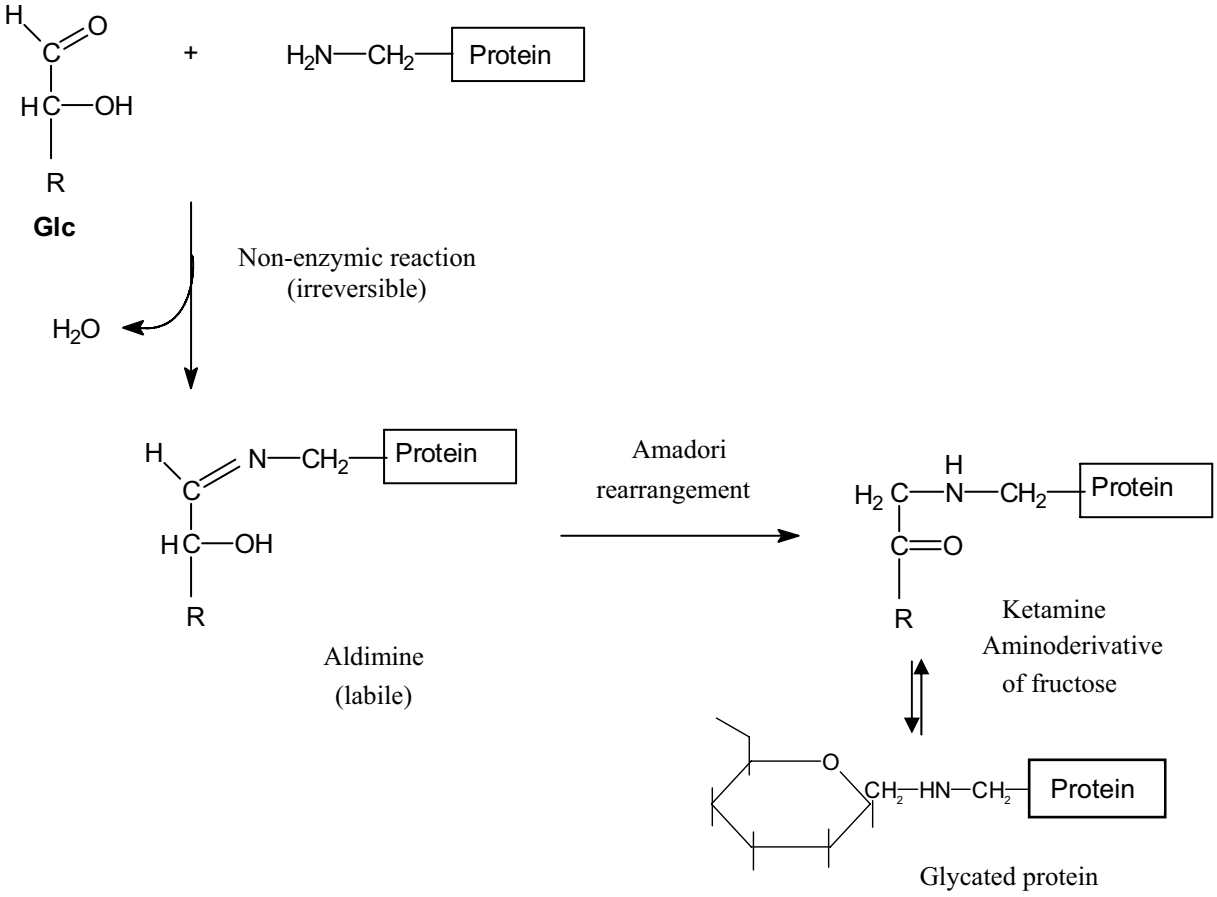
18. Compare the affinities of Hb and HbF to oxygen. What is the cause of this difference? What is its significance?

Derivatives of Haemoglobin

19. Name the derivatives of haemoglobin formed after: a) binding of O_2; CO_2 and CO; b) oxidation.
20. What are the most common causes of CO poisoning? How can be this poisoning detected? What is the first aid in this case?
21. Explain what methaemoglobinemia is and what may cause this disturbance.

Glycation of Haemoglobin

Principle of non-enzymatic glycation



22. Which factors will affect the amount of glycated haemoglobin?

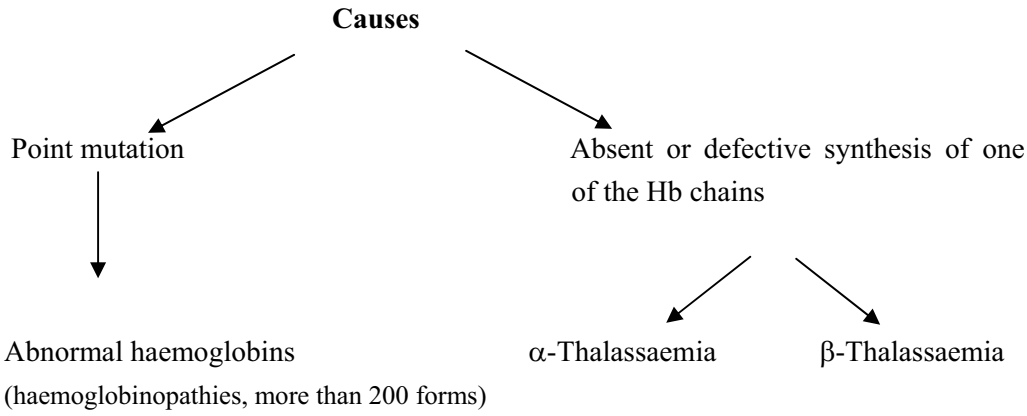
Glycated Haemoglobin

HbA_1 glycation on terminal $-NH_2$ (Val) group of β-chains

4–6 % of the total HbA

HbA-Glc glycation in other sites of Hb: e.g. terminal $-NH_2$ group of α-globin or at ε-NH_2 (Lys) of α, β-globin

Inherited Abnormalities of Haemoglobin Synthesis



Examples: **HbS** $\alpha_2 \beta_2^{6\ Glu \rightarrow Val}$

HbC $\alpha_2 \beta_2^{6\ Glu \rightarrow Lys}$

HbM $\alpha_2 \beta_2^{..\ His \rightarrow Tyr}$ $\alpha_2 \beta_2^{67\ Val \rightarrow Glu}$

23. What is the molecular principle of sickle cell anaemia?
24. What is the cause of sickle shape of erythrocytes?