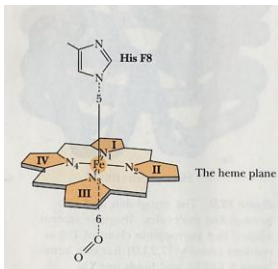


# HEMOGLOBIN AND MYOGLOBIN

## I. OXYGEN CARRIERS

- A. Why do we need oxygen carriers?
- Cannot carry enough in blood to meet metabolic demand
  - Oxygen is very reactive – oxidizes
  - Oxygen cannot diffuse very easily (we have thick skin)
- B. Properties of a good oxygen carrier
- Binds oxygen at a high  $[O_2]$
  - Doesn't oxidize cellular components
  - Gives up oxygen on demand
- C. Hemoglobin and Myoglobin
- Cooperativity
    - Hemoglobin needs to have high affinity to bind  $O_2$  in the lungs, but low affinity to unload to myoglobin
    - Sigmoidal curve: represents weak-binding state at low  $P_{O_2}$  and strong-binding state at high  $P_{O_2}$
  - Hemes
    - The heme binds  $O_2$ , not the protein
    - Function of protein: provides crevice – keeps heme from oxidizing
      - absence of protein: ferrous atom ( $Fe^{2+}$ )  $\rightarrow$  ferric state ( $Fe^{3+}$ )
      - heme buried in hydrophilic environment of protein:  $O_2$  binding does not result in oxidation

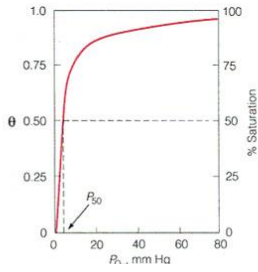


\* 5<sup>th</sup> bond = helix F8, residue 93 in Mb, residue 92 in the  $\beta$ -chain of Hb and residue 87 in  $\alpha$ -chain of Hb  
\*\* 6<sup>th</sup> bond = helix E7, His 64 for Mb, His 63 in b-chain and 58 in a-chain

- Heme structure
  - Each polypeptide of protein is made from 8 residues  $\rightarrow$  6 helices – A, B, C, D, E, F
  - $Fe^{2+}$  has 6 coordinating bonds
    - 4 bonds = nitrogens from tetrapyrrole ring system
    - 5<sup>th</sup> bond - Helix F binds to Fe at terminal Histidine molecule (His F8)\* = **proximal histidine**
    - 6<sup>th</sup> bond – deoxygenated: empty, histidine residue from helix E\*\* hovers; oxygenated: oxygen bonds here
  - Oxygen binds to Fe at  $120^\circ$  angle  $\rightarrow$  easily removed

## II. MYOGLOBIN

- A. Physico-chemical properties
- 153 amino acids – single polypeptide chain
  - Very compact: globular structure  $\rightarrow$  little empty space for solution to get in
  - Tertiary structure: 8 alpha helices (A-H), 4 helices terminated by proline residues
  - About 75% is in alpha helical structure
  - Polar side chains on outside of protein  $\rightarrow$  interact with solution
  - Myoglobin = storage protein  $\rightarrow$  mainly in skeletal muscle
  - High  $O_2$  affinity – does not change with concentration
  - Monomer  $\rightarrow$  no cooperativity



### B. Oxygen binding to Mb

i.  $\theta = (pO_2)/(p50 + pO_2)$

1.  $\theta$  = fraction of Mb sites bound to O<sub>2</sub>
2. p50 = O<sub>2</sub> partial pressure for half-saturation

ii.  $\theta/(1-\theta) = pO_2/p50$

1. take logs of this equation → linear graph → no cooperativity

## III. HEMOGLOBIN

### A. Structure

#### Differences in Hb and Mb

- Mb is a storage protein – binds O<sub>2</sub> avidly, dissociates slowly
- Mb is not cooperative
- Mb is 1 polypeptide

- i. Primary, secondary and tertiary structures are same as Mb

- ii. More than 1 subunit → quaternary structure:

1. 2  $\alpha$  and 2  $\beta$  subunits; cooperativity in binding and release of O<sub>2</sub>
  - a.  $\beta$  subunits: 146 residues, identical (same gene)
  - b.  $\alpha$ : 141 residues, differ by 1 or 2 genes
2. In urea, Hb dissolves into dimers of  $\alpha/\beta$  →  $\alpha$ - $\beta$  interaction is stronger than  $\alpha$ - $\alpha$  or  $\beta$ - $\beta$
3. Tetramer is globular molecule: spherical

- iii. Subunits are 2.5nm apart → cooperativity is not due to heme-heme interaction; affinity of O<sub>2</sub> varies with concentration (also with pH, CO<sub>2</sub>, 2,3 biphos. - see c)

### B. Conformational states

- i. Deoxy/Oxy Hemoglobin

1. Deoxy: molecule is very rigid, large cavity in center
2. Oxy: when exposed to O<sub>2</sub>, molecule loosens, rotates, cavity becomes smaller

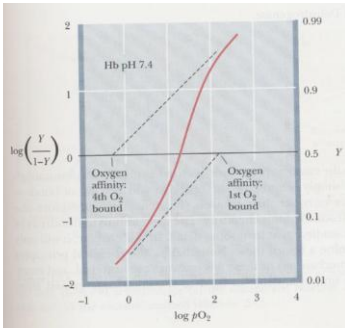
- ii. Graph – sigmoidal curve → logs of affinity: 2 tangents – when 1<sup>st</sup> O<sub>2</sub> bound, 4<sup>th</sup> O<sub>2</sub> bound → demonstrates cooperativity – 2<sup>nd</sup> binds 9x faster, 3<sup>rd</sup> 36x, 4<sup>th</sup> ~100x

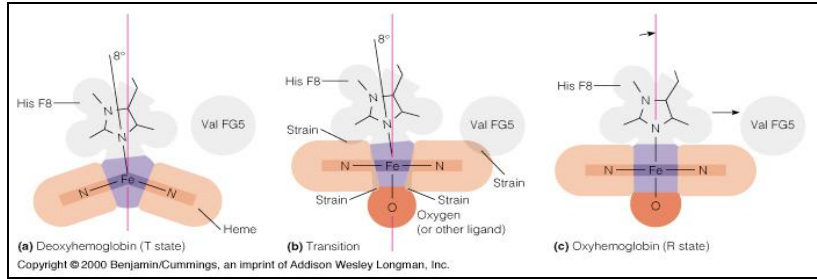
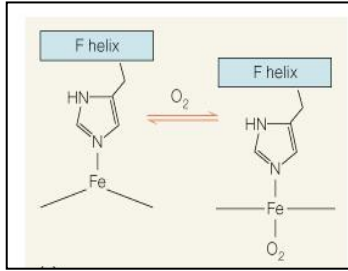
- iii. Salt bridges of Hb –

1. charge-charge interactions between termini and other residues in deoxy state:
  - a. C terminus of  $\beta$ 2 (146 His) with Asp of  $\beta$ 2, helix C of  $\alpha$ 1 (Lys40)
  - b.  $\beta$ 1 and  $\alpha$ 2 have same interactions (due to symmetry)
  - c. N term of  $\alpha$ 1 with C term of  $\alpha$ 2; C term of  $\alpha$ 1 with N term of  $\alpha$ 2
  - d. C term of  $\alpha$ 1 with Asp126 of  $\alpha$ 2; inverse
  - e. Tyr140 of  $\alpha$ 1 h bonds to COOH of Val93 of  $\alpha$ 1; also in  $\beta$
2. When O<sub>2</sub> binds, these interactions are disrupted and Hb relaxes, permitting sliding and rotating to assume oxy conformation

- iv. Geometric explanation

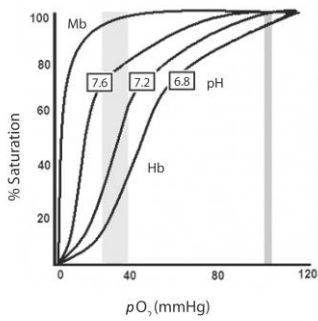
1. Hemes are dome-shaped
2. O<sub>2</sub> binds → pulls Fe down → dome becomes flat → pulls helix





### C. Role of pH, CO<sub>2</sub>, and 2,3 biphosphoglycerate

#### The Bohr Effect

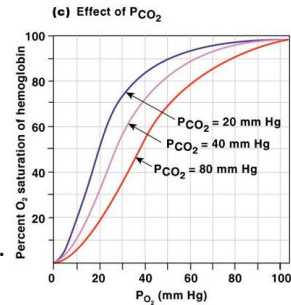


#### i. pH (The Bohr Effect)

1. deoxy Hb exchanges its protons and CO<sub>2</sub> with O<sub>2</sub>
2.  $\text{Hb}(\text{O}_2)_n + n\text{H}^+ \rightarrow \text{Hb}(\text{H}^+)_n + n\text{O}_2$
3. Decreased pH (increased [H<sup>+</sup>]) → decreased ability for Hb to hold O<sub>2</sub> / increased ability to give up O<sub>2</sub> = **The Bohr Effect**

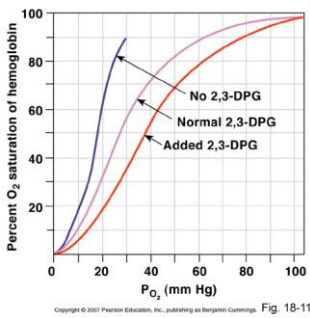
#### ii. Effects of CO<sub>2</sub>

1. Hb carries CO<sub>2</sub> from tissue to lungs
2.  $\text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{HCO}_3^- + \text{H}^+$ 
  - a. Protons facilitate Bohr effect
  - b. HCO<sub>3</sub><sup>-</sup> can bind to the N-terminus groups of chains to form **carbamates** → α/α interaction → deoxy conf. → O<sub>2</sub> released



#### iii. 2,3 biphosphoglycerate (BPG)

1. Cavity between α and β subunits has BPG inside
2. BPG binding to oxy Hb → conf shifts to deoxy → O<sub>2</sub> released
3. Applications:
  - a. At high altitudes, body makes more BPG to achieve this effect (compensates for less O<sub>2</sub> received from atmosphere)
    - i. takes 3-5 days to adjust to new altitude (produce BPG)
    - ii. reversible process
  - b. Stored blood has less BPG → needs to be reconstituted with 5mm BPG so blood will deliver O<sub>2</sub>, not just bind it
  - c. smokers have higher BPG –CO ties up some Hb → less O<sub>2</sub> available
4. CO<sub>2</sub> and BPG have additive effect → they bind at different sites on Hb



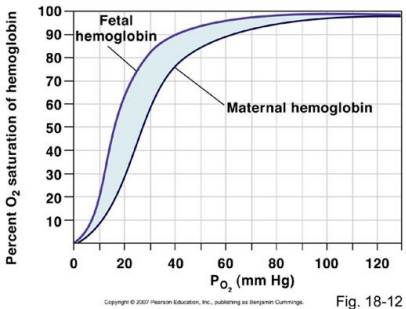
### D. Other factors

#### i. Fetal hemoglobin

1. γ instead of β subunits → doesn't bind BPG as well → quickly turns to oxy → fetal Hb has higher affinity so it can take O<sub>2</sub> from placenta
2. In the γ chain His is replaced by Ser – BPG binds more weakly

#### i. Toxicity of CO

1. Heme pocket can bind other small molecules besides O<sub>2</sub>
2. CO is approximately same size:
  - a. Blocks O<sub>2</sub> from binding



- b. Has greater affinity → oxy conformation → curve shifts left → harder to release O<sub>2</sub> → blocks respiration

#### IV. DEFECTS IN HEMOGLOBIN STRUCTURE AND DISEASE

- A. Sickle Cell Disease
  - i. General comments:
    - 1. Heterozygous: not serious; homozygous: very serious
    - 2. Malarial parasite's life cycle not continued in sickle cell → malarial regions select for sickle cell allele
  - ii. Mechanism of sickling
    - 1. Mutation in Hb globin gene → change in nucleotide sequence → β<sub>6</sub> Glu becomes Val → significant chemical change to protein
    - 2. Hydrophobic regions of β subunits of two Hb molecules form dimer, then polymer (in deoxy form)
    - 3. Polymers aggregate into elongated tube
    - 4. Tube cannot flow easily through vessels, especially capillaries → blocks transport → more deoxy molecules → more sickling
    - 5. Membrane also gets deformed – loses K<sup>+</sup> → lyses → Hb fibers spill out and get metabolized
      - \*Can detect via electrophoresis: Glu to Val means loss of neg charge
  - iii. Complications
    - 1. Stroke – clotting of vessels in brain
    - 2. Susceptibility to infectious diseases
    - 3. Organ damage and infarction
    - 4. Impaired growth
    - 5. Infertility
    - 6. Renal damage
  - iv. Treatments- need to know?
- B. Other Hemoglobin variants
  - i. Hemoglobin S ..... β<sub>6</sub> Glu → Val
  - ii. Hemoglobin C..... β<sub>6</sub> Glu → Lys – very little consequence
  - iii. Hemoglobin E ..... β<sub>26</sub> Glu → Lys – very little consequence
  - iv. Hemoglobin Constant Spring (α-globin chain that is abnormally long)
  - v. Hemoglobin H .....(β<sub>4</sub>)- high affinity – doesn't deliver O<sub>2</sub> very well
  - vi. Hemoglobin Barts.....(γ<sub>4</sub>) – fetus usually dead before birth
  - vii. Other variants in lecture notes?
- ii. Thalassemias
  - i. Causes
    - 1. One or more genes coding for Hb chains are deleted –called α or β
    - 2. Genes present but mutation causes short polypeptide or frameshift (nonfunctional)
    - 3. Genes present but mutation may affect transcription/mRNA processing – missing/nonfunctional protein
  - ii. Subunits

1. Alpha always present
  2.  $\gamma$  shuts off slowly – 50% present at birth, finally completely shut off
  3.  $\beta$  increases as  $\gamma$  decreases
- iii.  $\alpha$  Thalassemias (normally 4 genes, 2 from each parent)
1. 1 deficient gene (still 3 copies) = silent carriers
  2. 2 def =  $\alpha$  thalassemia trait
  3. 3 = Hem H disease: some  $\alpha_2\beta_2$ , some  $\beta_4$ , mild to moderately severe anemia (too much aggregation)
  4. 4 = hydrops fetalis (blue baby) – death of fetus at or before birth
    - a. Hb is all  $\gamma_4$  (Hb Bart) or  $\beta_4$  (Hb H) – cannot deliver O<sub>2</sub> (loss of cooperativity – binds like myoglobin)
- iv.  $\beta$  Thalassemias (normally 2 genes, 1 from each parent)
1. Minor: 1 copy of gene, thalassemia traits
  2. Major: no copies of gene (or defective copies), must rely on fetal chain ( $\alpha_2\gamma_2$  Hb) – healthy at birth, severe anemia in first years of life 0 require regular transfusions
  3.  $\beta$  thalassemia can also couple with sickling or other variants

minor: $\beta^+\beta$	$\beta^0\beta$
major: $\beta^+\beta^0$	$\beta^0\beta^0$ $\beta^+\beta^+$