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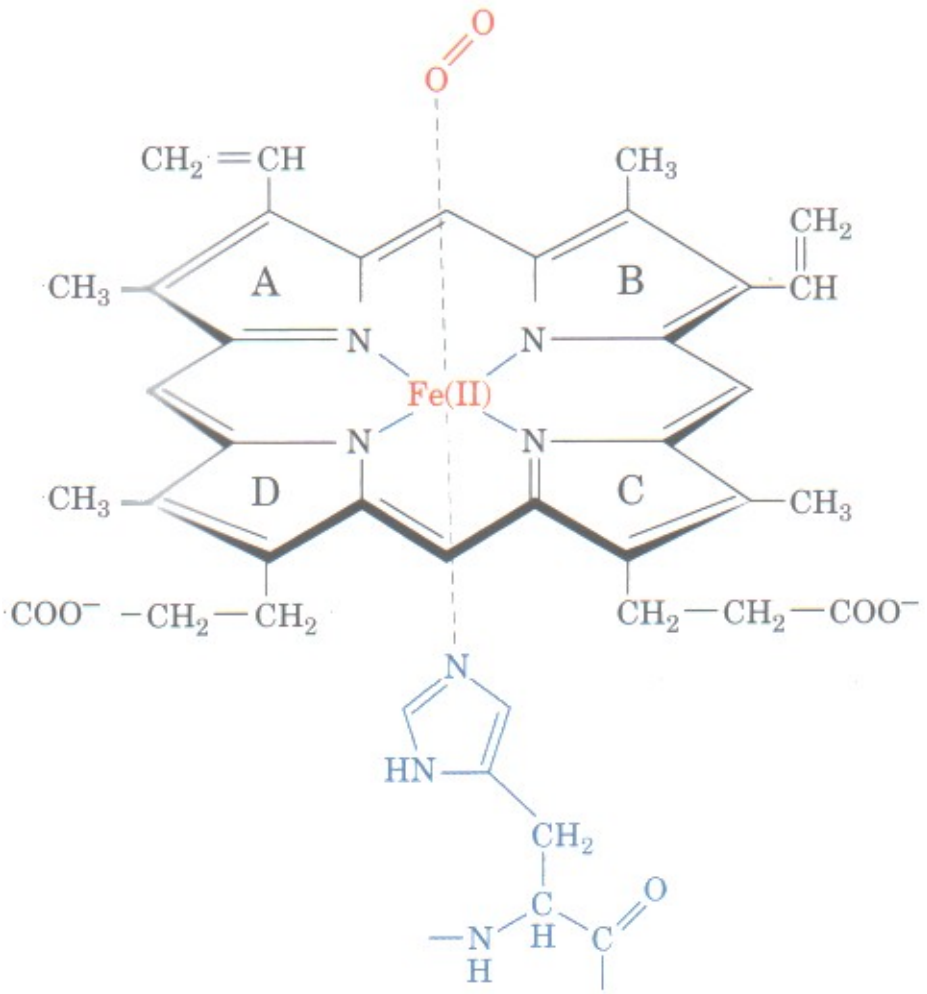
Chapter 10: Hemoglobin



Voet & Voet: Pages 320-353

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Hemoglobin Function

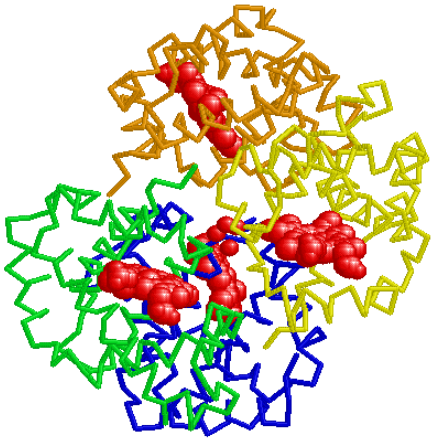
Larger aerobic (oxygen utilizing) organism require an O_2 transport system to deliver sufficient O_2 to tissues

- **Dissolved O_2 diffusion is only sufficient for very small organisms or cells (< 1mm thick)**
- **O_2 solubility is too low (eg. ~0.1 mM in blood plasma) to support metabolism**

Hemoglobin serves as the primary O_2 transporter in vertebrates

- **Invertebrates may have a hemoglobin-based O_2 transport system or an alternative system based upon either hemocyanin or hemerythrin**

O_2 binding to Hemoglobin increases the amount of O_2 in solution enough to support metabolism



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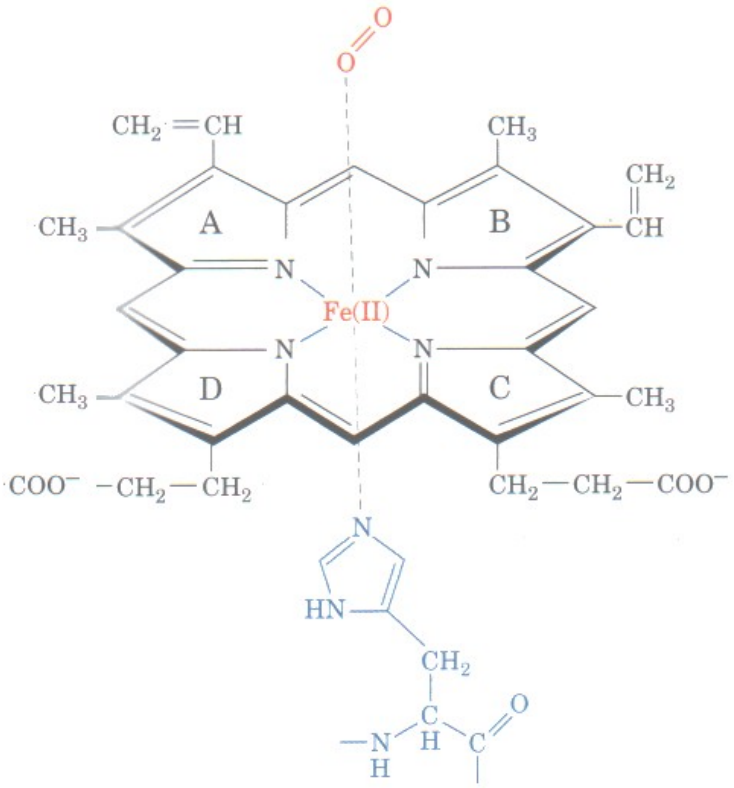
Heme



Myoglobin and each subunit of hemoglobin bind a single heme group

- **gives rise to characteristic red color of blood**
- **site of O_2 binding**
- **same group that is present in cytochromes and catalases**

Heme is a porphyrin derivative containing 4 pyrrole rings linked by methylene bridges and an Fe atom

- **Technically, the heme of hemoglobin is 'protoporphyrin IX' with a bound ferrous (Fe^{2+}) ion**
- **Fe generally remains in the 2^+ oxidation state regardless of O_2 binding**

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Quantifying Binding

Reversible binding of a protein (P) and ligand (L) can be described by an equilibrium expression characterized by an equilibrium association constant, K_a (M^{-1})

- **In cells, the [ligand] is typically far larger than [protein]**

$$P + L \rightleftharpoons PL \qquad K_a = \frac{[PL]}{([P][L])}$$

Equilibrium can be expressed as the fraction of ligand binding sites occupied by ligand

$$\theta = \frac{(binding\ sites\ occupied)}{(total\ binding\ sites)} = \frac{[PL]}{([PL]+[P])}$$

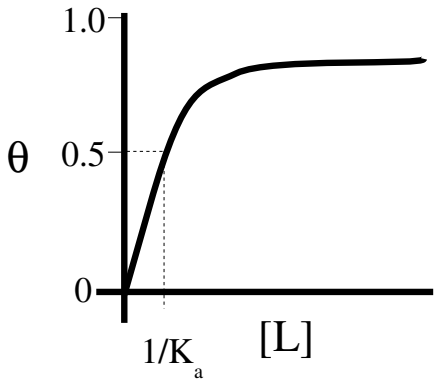
Substituting K_a [L][P] for [PL] and rearranging

$$\theta = \frac{(K_a[L][P])}{(K_a[L][P]+[P])} = \frac{(K_a[L])}{(K_a[L]+1)} = \frac{[L]}{([L]+\frac{1}{K_a})}$$

Determining K_a (or K_d)

Plotting θ versus [L] yields a hyperbolic curve

- **At θ = 0.5; the equilibrium equation yields [L] = ($1/K_a$)**



θ can be followed spectroscopically by detecting conformation differences associated with ligand binding

K_d; the dissociation constant is the reciprocal of K_a and is often used in its place

Why?
a) the equilibrium equation is (slightly) simplified
b) units are concentration

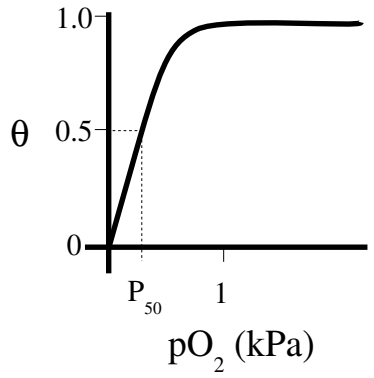
$$\theta = \frac{[L]}{([L] + K_d)}$$

Oxygen binding to myoglobin

Must modify (slightly) equilibrium binding equation as O_2 is a gas

- **Partial oxygen pressure (pO_2) is easier to measure than dissolve oxygen concentration ($[O_2]$)**
- **P_{50} is the concentration at which half the binding sites are filled (K_d)**

$$\theta = \frac{[L]}{([L]+K_d)} = \frac{pO_2}{(pO_2+K_d)} = \frac{pO_2}{(pO_2+P_{50})}$$



Partial pressure of O_2 is pressure of O_2 above solution

O_2 binds tightly to myoglobin with a P_{50} of 0.26 kPa

(Note: oxygen is ~21% of the 101.3 kPa gas pressure)

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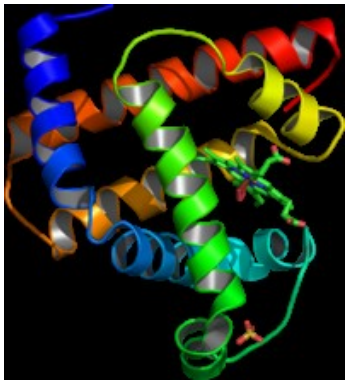
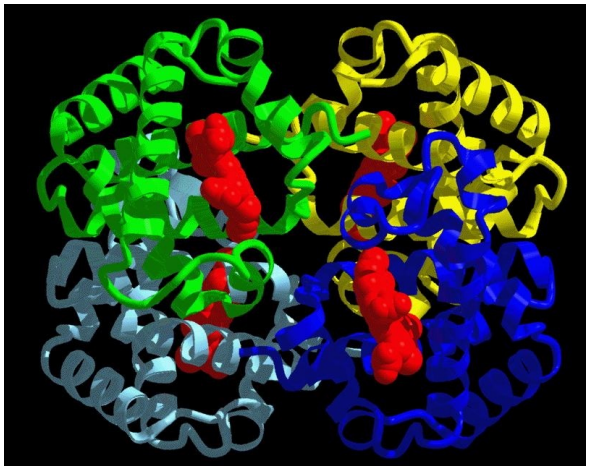
Hemoglobin



- **Myoglobin: Comparatively insensitive to small changes in physiological [O_2]**
  - **Suited to storage**
  - **O_2 bound under physiological conditions**
- **Hemoglobin (Hb) is an $\alpha_2\beta_2$ oligomer and a homologue of myoglobin**
  - **It carries almost all oxygen in animals**
- **Interaction between the Hb subunits modulate its binding affinity allowing it to respond to small changes in physiological [O_2]**
  - **Suited for oxygen transport**
  - **Binds/Releases O_2 under physiological conditions**



Cooperativity

X-ray analysis show Hb exists in two states

- **R (relaxed) state with high affinity for O_2**
- **T (tense or taut) state with low affinity for O_2**

Low [O_2] favors the T state

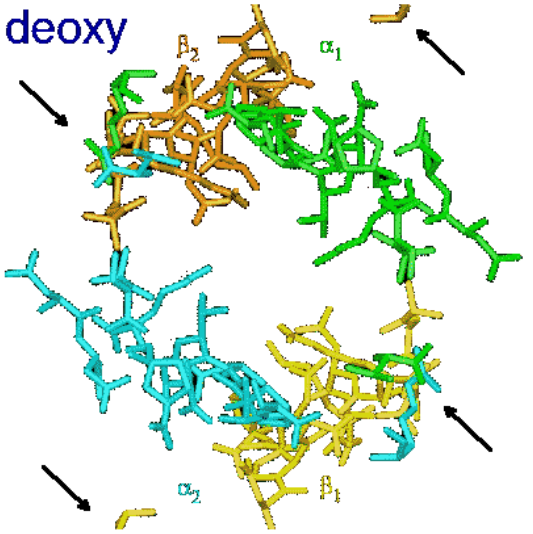
- **Requirement for O_2 release at sites needing oxygen**

Binding of O_2 to one of the Hb sites triggers a conformational change to the R state

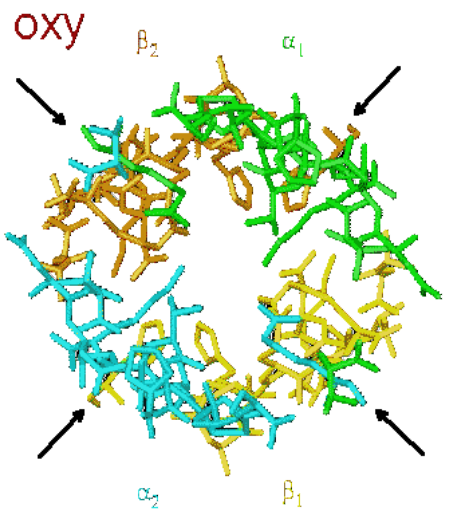
- **Allows all four sites to rapidly fill at higher [O_2]**

Cooperativity – binding at one site alters the affinity of similar sites of other subunits

T



R



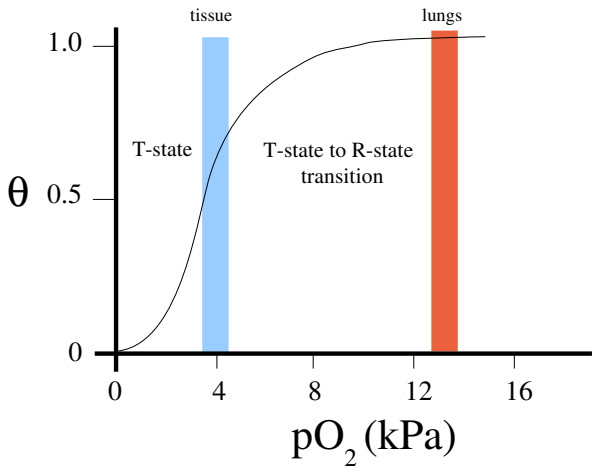
Cooperativity and Hb function

pO_2 in the lungs is ~13.3 kPa and in tissue is ~4 kPa

- Proteins with hyperbolic binding curves bind O_2 efficiently in the lungs but do not efficiently release it in tissue

Cooperative proteins have sigmoidal binding curves

- Arises from low affinity binding at low pO_2 and higher affinity binding at high pO_2



< 4 kPa, Hb is largely in the T-state and deoxygenated (less than 0.5 fractional saturation)

> 4 kPa, the T to R-state transition results in Hb rapidly filling with O_2 (~0.9 fractional saturation at 8 kPa)

> 10 kPa, Hb is saturated with O_2

Note: Complete release of O_2 would be more efficient (ie. At 4kPa) BUT organisms may not be able to survive brief O_2 starvation

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Allosteric proteins

Allosteric proteins bind ligands at one site, undergo a conformational change and the binding properties of another site on the same protein are altered

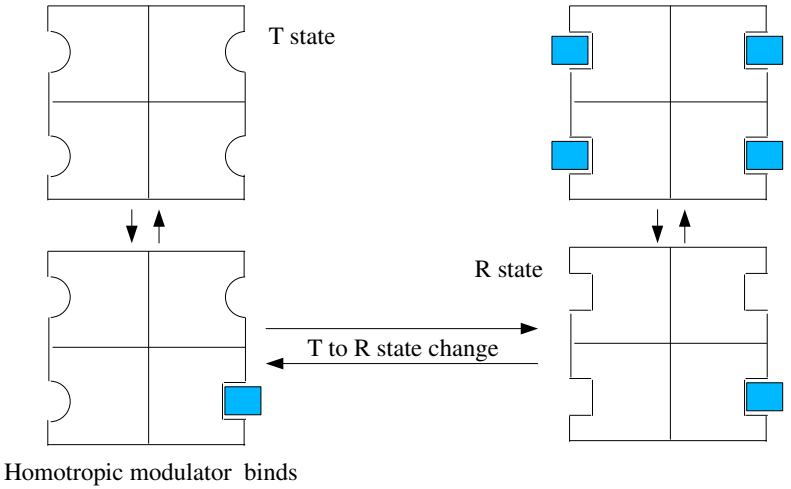
- **Cooperative proteins (*eg* Hb) are a special case of allosteric proteins**
- **All cooperative proteins are allosteric, but not all allosteric proteins are cooperative**

Ligands that induce conformational changes in allosteric proteins are referred to as modulators

- **Modulators may be *inhibitors* (induce less-active forms) or *activators* (induce more-active forms)**

- ***Homotropic* modulators are modulators that are identical to the ligand – occur in cooperative proteins (eg. O_2 is a homotropic activator of hemoglobin)**
- ***Heterotropic* modulator are different from the normal ligand (eg. 2,3-bisphosphoglycerate is a heterotropic inhibitor of hemoglobin)**

Cooperativity cartoon



- **Cooperative binding of oxygen was first studied by Hill (1910)**
- **For a protein with n binding sites, the previously discussed equilibrium expressions are:**

$$P + nL \rightleftharpoons PL_n$$

$$K_a = \frac{[PL_n]}{([P][L]^n)} \qquad \theta = \frac{[L]^n}{([L]^n + K_d)}$$

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Hill equation

The fractional occupancy equilibrium expression can be rearranged and converted to a linear form by taking the log of each side:

$$\frac{\theta}{(1-\theta)} = \frac{[L]^n}{K_d} \qquad \log\left(\frac{\theta}{(1-\theta)}\right) = n\log[L] - \log K_d$$

Plotting log (θ/ (1 – θ)) vs log [L] is called the Hill plot

- **The slope of the Hill plot *n* (Hill coefficient), reflect the degree of interaction between binding sites and is represented as n_H**

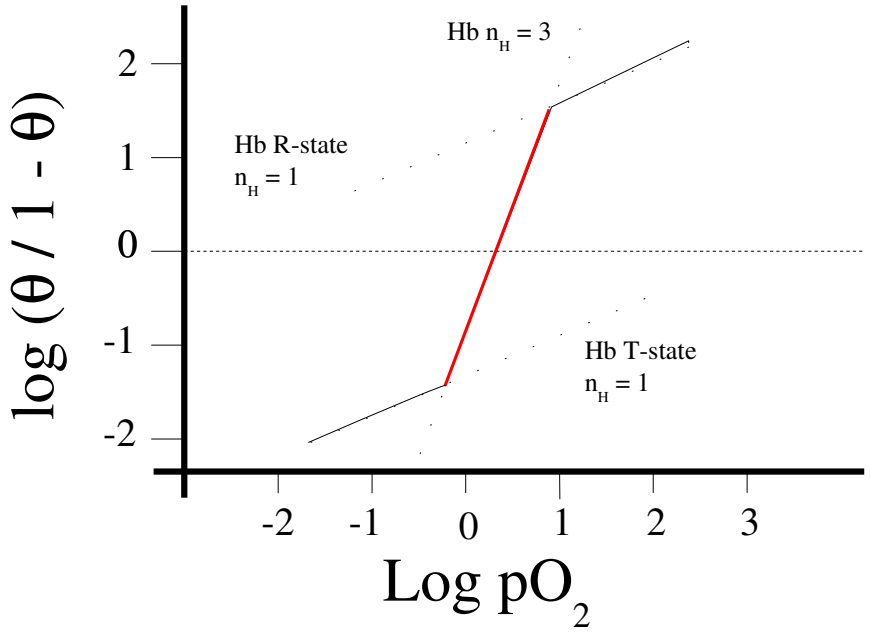
  (Note: n_H only equals *n* if all ligands bind at the same instant)

n_H values range from > 0 to the total number of binding sites, n.

Hill coefficient

$$\log\left(\frac{\theta}{(1-\theta)}\right) = n \log pO_2 - n \log P_{50}^n$$

- $n_H < 1$ negative cooperativity (very rare)
- $n_H = 1$ no cooperativity (typical)
- $n_H > 1$ positive cooperativity (common)
- $n_H = n$ complete cooperativity (never)



Note1: We must modify the equation for Hb by substituting pO_2 for [L] and P_{50}^n for K_d before plotting the data

Note2: P^n_{50} occurs at y=0

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Cooperativity models

Two models proposed to explain cooperative binding

(1) Concerted model – all subunits undergo the conformational change simultaneously

(2) Sequential model – subunit undergo the conformational change one at a time

Difficult to distinguish between the models as the concerted model is a special case of the sequential model (not mutually exclusive)

- **The sequential model becomes the concerted model if the conformational change is sufficiently fast**

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Hb also transports CO_2 and H^+

Hb transports CO_2 from tissues to the lungs and kidneys

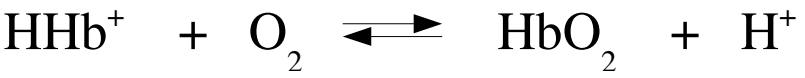
- **Since CO_2 is poorly soluble in water, some is hydrated to HCO_3^- by carbonic anhydrase in a reaction that produces H^+**
- **Hb transports 40% of the H^+ and ~20% of the CO_2 formed in tissues**

Binding* of H^+ (Bohr effect) and CO_2 are inversely related (inhibitory) to the binding of O_2

- **At low pH and high CO_2, the binding of H^+ and CO_2 aid in the release of O_2**
- **Conversely in the capillaries of the lungs, CO_2 is released and the pH rises increasing Hb affinity for O_2**
- ***The Bohr effect (modulation of Hb binding) is an important additional factor explaining why Hb (cooperative) is better suited as an oxygen carrier than Myoglobin***

CO_2 / H^+ and Hb

Complete equilibrium expression for Hb is more formally written as



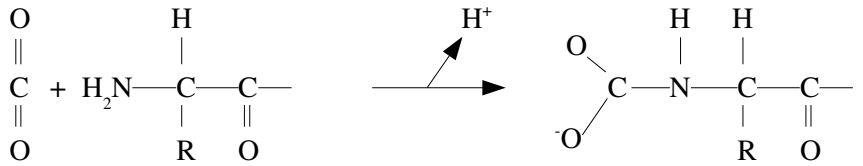
H^+ protonates His146 of Hb (β subunit) in the T state

- **This residue is at the interface between subunits and is directly involved in the Hb T to R state transition**
- **Protonated His146 forms a salt bridge with Asp94 stabilizing the T state**

Several other residues can be protonated with similar effects

CO_2 carbamylates the neutral amino terminal groups of Hb when CO_2 levels are high

- **This reaction releases H^+ and contributes to the Bohr effect**



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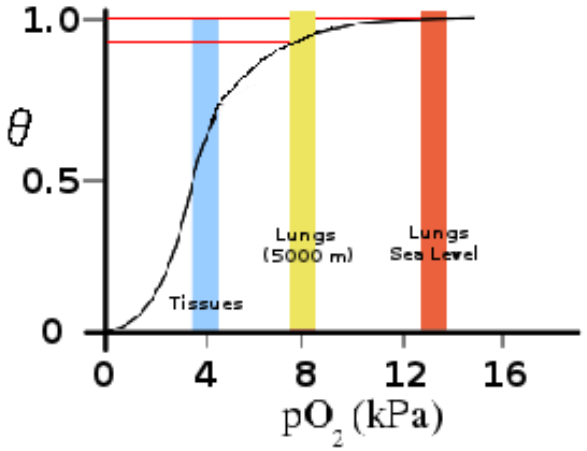
Heterotrophic modulators

2,3-bisphosphoglycerate (BPG) is a heterotrophic modulator of Hb

- BPG binds at a site distant from the O_2 binding site and greatly <u>reduces</u> the affinity of Hb for O_2 under high CO_2 levels
- Leads to increased release of O_2 compared to normal conditions

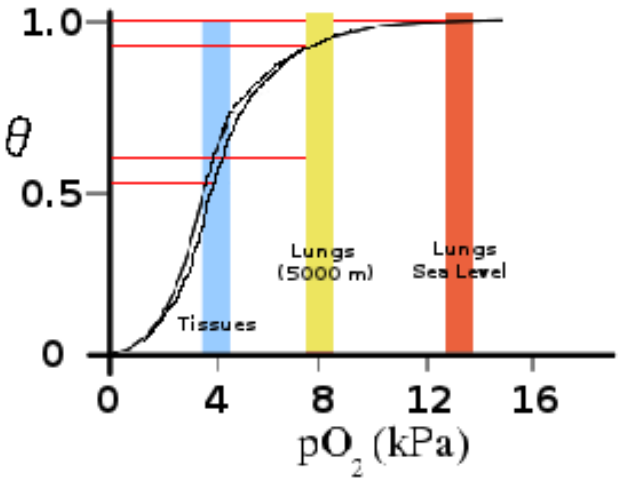
eg **At high altitudes, pO_2 and the fraction saturation of Hb is lower and less O_2 is released to tissues**

- After several hours, BPG binding to Hb promotes release of O_2 at low pO_2 and restores normal levels of oxygen to tissues



Binding Curve showing effect of low pressure

Binding Curve at low pressure in the presence of BPG



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Summary

Hb reversibly binds O_2, H^+ and the heterotrophic regulator BPG

Hb covalently binds CO_2

Each of these ligands and CO_2 affect Hb binding affinity for O_2 by stabilizing either the T or R states