Chem 431A-L19-F'07
admin: Online quiz deadline
Chapt 4 Monday, Nov5
no exceptions. If your computer
didn't work, tough!

Last lecture:

1) Saturation curves for Mb and Hb

2) Hill coefficient – what it means

Today: group quiz

1) Cooperativity and Allostery of Hb

2) structure of Hb (two αβ dimers)

2) Bohr effect. CO_2 transport. 2.3-BPG

3) MWC and KNF models of Hb.

Hb changes from strong binding protein at high O_2 to weakly binding at low P_{O2}. sigmoidal curve. cooperative mechanism. Archibald Hill (1910): assumed 1 step: $Hb + nO_2 \rightarrow Hb(O_2)_n$ (ie infinite cooperativity). $\Rightarrow \theta = (P_{O2})^n / \{(P_{50})^n + (P_{O2})^n \}$ known as Hill eqn. n= # subunits. can show by rearrangement: $\theta/(1-\theta) = (P_{O2})^n /(P_{50})^n$	Hill plot based on taking logs of both sides: $\text{Log}\{ \theta/(1-\theta) \} = n\log P_{O2} - n\log P_{50}$. plot y = mx + b => where slope is n (an integer representing the number of subunits). actual graph is diff: sometimes 1 sometimes 3. for ideally cooperative Hb, n = 4. Note that experimentally. n ≤ # monomers

Compare Mb and Hb: Hb tetrameric: 2 α and 2 β subunits. Actually 2 dimers: $\alpha_1\beta_1$ and $\alpha_2\beta_2$. Interactions: Hphobic, H-bonds and ion-pairs. Where both α and β are struc. similar to Mb. An α β dimer is hard to separate by usual usual denaturation. The 2 dimers interact between them. This interaction changes upon binding of O_2.	**T and R states:** T= "tense" state, stabilized by more salt bridges. R= "relaxed" state. See figures 5-8 to 5-10. T state has lower affinity for O_2 while R has greater affinity to O_2. T state is favored when there is low pO_2 while R is favored when there is high pO_2.

That means that when pO_2 is high (as in the lungs), Hb likes to bind O_2 . But when pO_2 is low (as in respiring tissues), Hb likes to release O_2.

Cooperativity: Hb binds O_2 cooperatively. In absence of O_2, Hb is in the T, low affinity state. In the lungs, there is lots of O_2 and Hb-O_2forms which shifts its equilibrium to the R state. R state is also high affinity (to O_2) state. Sigmoidal θ vs pO_2 curve shows cooperativity (see Fig5-12)

Allostery: The binding of a ligand to one site affects its binding at another site. Ligand can be O_2 as in Hb. Or, inhibitors or activators.

Homotropic: modulator = normal ligand;
Heterotropic:modulator ≠ normal ligand

2 models: MWC (Monod, Wyman and Changeaux) or "concerted model"
and sequential model KNF (by Koshland, Nemethy and Filmer) *fig 5-15

MWC or concerted or symmetry model:
1) an allosteric protein is an oligomer of symmetrically related subunits
2) Each subunit can exist in 2 conformational states designated R and T; these are in equilibrium
3) Ligand can bind to a subunit in either conformation. Only the conformational change alters the affinity for the ligand.
4) The molecular symmetry of the protein is conserved during the conformational change.

Koshland et al model, sequential model
1) ligand-binding induces a conformational change in the subunit to which it binds.
2) cooperative interactions arise through influence of those conformational changes on neighboring subunits
3) Conformational changes occur sequentially.

(Reduces to the MWC model if the interactions between subunits is strong).
Both features are observed in Hb – O_2 binding.
** look at the conformational changes.

Hb transports H^+ and CO_2 . both pH and [CO_2] affect O_2 binding by Hb
Carbonic anhydrase: $CO_2+H_2O \rightarrow H^+ + HCO_3^-$.
Raising [H^+] and lowering pH.

Go over *Bohr effect*. (Fig. 5-16)
Hb binds O_2 and H^+ *inversely* to each other like:

$$\mathbf{H}\text{-}Hb^+ + O_2 \Leftrightarrow Hb\text{-}O_2 + H^+$$

H^+ binds to the His^{146} HC3 residue (C-terminal aa) of the β subunit.

CO_2 also binds as *carbamate* group to N-terminal's α amino group of all 4 subunits inversely to O_2 binding.

$CO_2 + H_2N\text{-subunit} \rightarrow {}^-O_2C\text{-}NH\text{-subunit} + H^+$
(carbaminohemoglobin)

Another ligand:(*heterotropic* allosteric modulation):

1 CO_2^-

2 H C-PO_4^{2-}

3 H_2C-PO_4^{2-}

2,3 *bisphosphoglycerate* (2,3-BPG) binds to the (+charge sidegroups of) central cavity between the β subunits of the T state. It's binding to Hb has inverse relation to O_2binding:

Hb-BPG + O_2 ⇔ Hb-O_2+ BPG

BPG lowers affinity of Hb to O_2 (fig 5-17)
Absence of BPG makes it behave like Mb

Fetal Hb contains: $\gamma_2\beta_2$ not $\alpha_2\beta_2$ as in adults. $\gamma_2\beta_2$ has a lower affinity for 2,3- BPG and therefore higher affinity for O_2. Fetus needs to extract O_2 from the moth3r's Hb and therefore must have a lower affinity for O_2 than the Hb of the mother.

Sickle cell anemia:

Due to single aa substitution: a Val instead of a Glu in the position #6 of the β chain. Consider the hydrophobic vs ionic R group substitution: the "sticky Hϕ patch" causes the Hb to aggregate into strands when in Hb in the deoxy form. Need to avoid vigorous exercise.