BCH 4054 Spring 2001 Chapter 15 Lecture Notes

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Chapter 15

Enzyme Regulation

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Enzyme Specificity

- Molecular recognition through multiple interactions between substrate and enzyme
  - H-bonds, ionic forces, hydrophobic binding, van der Waals binding
- Lock and Key Model (Emil Fischer)
- Induced Fit Model (Daniel Koshland)
  - Example of hexokinase (See Fig. 15.1)

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Control of Enzyme Activity

- Product accumulation
- Substrate and coenzyme availability
- Synthesis and degradation of enzyme
- Covalent modification
- Allosteric regulation by "effector molecules"
- Specialized controls (zymogens, isozymes, modulator proteins)

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Product accumulation and substrate availability

- Products are generally inhibitors, and as they increase the back reaction increases.
- Substrate availability can be important when the enzyme is not saturated, i.e. substrate is at K_m or below.

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Synthesis and degradation of enzyme

- Control at the level of gene expression
  - **Induction** is the activation of enzyme synthesis
  - **Repression** is the shutdown of enzyme synthesis
- Protein degradation can also play a role sometimes.
- Regulation is relatively slow, and requires considerable resource investment.

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Covalent modification

- A faster means to control rate—don't need to completely synthesize a new protein
- Requires a reaction to activate, another to de-activate.
  - For example, phosphorylation-dephosphorylation (See Fig. 15.2)
- **Converter enzymes** must also be regulated, so interactions can become complex.

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Protein Kinases

- Over 100 known in yeast
- Probably over 1000 in human genome
- Phosphorylation at specific target sequences in proteins
  - Serine, Threonine, or Tyrosine
- Find in many complex regulatory systems, from hormonal stimulation to gene activation to growth regulation.

Phosphorylation and dephosphorylation is not the only method of regulation by covalent modification, though it is probably the most common.

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Zymogens (or "Proenzymes")

- Newly synthesized protein is inactive, and must be activated by proteolytic cleavage.
  - Proinsulin (See Figure 15.3)
  - Chymotrypsinogen (See Figure 15.4)
  - Blood Clotting proteins (See Figure 15.5)

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Isozymes

- Enzymes with the same catalytic activity, but different kinetic properties which are adapted to needs of different tissues.
  - Hexokinase of brain versus glucokinase of liver (low K_m in brain, high K_m in liver)
  - Lactate Dehydrogenase
    - Two "types", muscle (A) and heart (B) lead to five isozymes. (See Fig. 15.6)

The heart type is inhibited by pyruvate, and is kinetically suited to convert lactate to pyruvate, which heart muscle can do. The muscle type is better suited kinetically to convert pyruvate to lactate. Of course the equilibrium position of the reaction would not be affected by either enzyme.

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Modulator Proteins

- Proteins that bind to enzymes and affect their activity.
  - Regulatory subunit of cyclic AMP-dependent kinase (See Figure 15.7)
  - Phosphoprotein phosphatase inhibitor binds to a phosphatase and inhibits it.
  - Lactalbumin binds to a galactosyl transferase in mammary gland, converting specificity toward synthesis of lactose. (See Page 147)

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Allosteric Regulation

- Regulation by "effectors", which usually bear no relation to structure of substrate
- Allosteric means "another site", which refers to the binding site of the effector
- Usually multimeric proteins, with more than one binding site for substrates and effectors
- Kinetic curves are not hyperbolic, but **sigmoid** or **S-shaped.** (See Figure 15.8)
  - Substrate binding is **cooperative**

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Enzymes That Show Allosteric Regulation

- Usually found as first enzyme in a pathway, or at a branch where an intermediate can enter two or more pathways.
- Usually catalyze a step in which ΔG is very negative (i.e. Q/K is < .05)
  - The step is far from equilibrium

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Models Showing Allosteric Kinetics

- A variety of rate laws can lead to sigmoid curves. There must be terms where substrate terms have higher powers., e.g.

$$v = \frac{a[S]^2}{b + c[S]^2} \qquad v = \frac{a[S] + b[S]^2}{c + d[S] + e[S]^2}$$

$$v = \frac{a[S](1 + a[S])^{n-1}}{L + (1 + a[S])^n}$$

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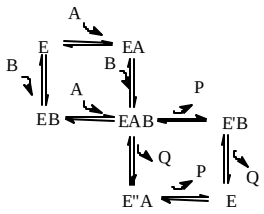
Allosteric Kinetic Models, con't.

- For example, the random substrate addition model can, under the right condition of relative rate constants, lead to an equation for a sigmoid curve.



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Monod, Wyman, Changeux Allosteric Model

- Most widely accepted model, also called the **symmetry model** or the **MWC** model
  - Two conformational states of the enzyme
    - **R** (relaxed) and **T** (taut)
  - All subunits in same conformation
  - Equilibrium in absence of ligand favors the **T** form.
    - i.e. for $L = [T_o]/[R_o]$, L is very large

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MWC Symmetry Model, con't.

- Binding of Substrate or Effectors can shift the equilibrium to the **R** conformation.
- In the extreme case, S binds **only** to the **R** conformation.
- The model allows for substrate binding to **T**, but the affinity for **T** must be much lower than for **R**.

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MWC Symmetry Model, con't.

- A cartoon illustrating the essential features of the model is shown in Figure 15.9.
- The kinetic rate law plots for the model are shown in Figure 15.10.
  - In 15.10a, the effect of **L** is shown.
  - In 15.10b, the effect of the relative binding affinities of S to **R** and **T** is shown.

In 15.10 b, c is the ratio of the dissociation constants of S from R and T, i.e. K_R/K_T. Note as c gets smaller, the curve gets more sigmoid.

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MWC Symmetry Model, con't.

- S binds **cooperatively**, because as it shifts the equilibrium to the **R** state, it opens up more S binding sites.
  - S is called a **positive homotropic effector.**
- Effectors which bind to other sites are called **heterotropic effectors**.
  - Positive heterotropic effectors are **activators**.
    - They bind preferably to the **R** form.
  - Negative heterotropic effectors are **inhibitors**
    - They bind preferably to the **T** form.
  - (See Figure 15.11)

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The Koshland Sequential Allosteric Model

- Similar in many respects to the MWC model.
- Major difference is that it does not require all subunits to be in either the **R** or **T** state.
- There can be **sequential** conformational changes as substrate or effectors bind.

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K Systems and V Systems

- MWC model is a K system.
  - It affects the $K_{0.5}$ of the system, not the maximum velocity that can be achieved. (Effectors shift the sigmoid curve to the right or left.)
- Some systems are called V systems because the V_{max} is affected by effectors, while the $K_{0.5}$ (or K_m) is not changed. (See Figure 15.12)

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Glycogen Phosphorylase

- Catalyzes breakdown of glycogen
- Two levels of regulation
  - Covalent modification, under hormonal control
  - Allosteric effectors, responding to energy situation in the cell
- We will skip this section of the chapter and come back to it when we discuss glycogen metabolism.

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Oxygen Binding Proteins

- Two very similar proteins, but different functions:
  - Myoglobin binds oxygen to store it temporarily in tissues
  - Hemoglobin transports oxygen from lungs to tissues, so its binding affinity must change in the two locations
- Both have heme as a prosthetic group which serves as the binding site of oxygen.

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Myoglobin

- MW 16,700, 153 AA residues
- Heme fits in crevice; Iron in Fe^{+2} state
  - (Figure 15.24)
- His F_8 occupies one coordination position, O_2 occupies the other (Figure 15.25)
- His E_7 rests against the tilted O_2, and prevents CO from binding perpendicular.
  - (Figure 15.26)

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Myoglobin, con't.

- Oxygen binding causes Fe to move into plane of porphyrin ring, causing a small movement of His F_8 and a small shift in the polypeptide backbone. (Figure 15.27)
- The protein prevents the oxygen from oxidizing the Fe^{+2} to Fe^{+3}—a reaction which would require two heme groups interacting with the oxygen.
- (Fe^{+3} containing heme is called hematin, and myoglobin containing Fe^{+3} is called metmyoglobin.)

Myoglobin gives meat its red color. The brownish color of red meat is from metmyoglobin.

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Hemoglobin

- MW 64,500, tetramer with $\alpha_2\beta_2$ structure
  - Actually a dimer of two $\alpha\beta$ units (Figure 15.30)
- Each subunit is very similar to myoglobin
  - See Figure 15.28
    - (Large sequence homologies between myoglobin and the chains of hemoglobin, and between the alpha and beta chain, indicate an evolutionary relationship.)

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Hemoglobin, con't.

- Oxygen binding causes a conformational change
  - His F_8 and its peptide shift (Figure 15.32)
  - The dimers rotate slightly (Figure 15.31)
  - Salt bridge interactions between the dimers are broken (Figure 15.33)
  - pK of His β146 is lowered as salt bridge to Asp β94 is broken.
  - pK of N-terminus of α chain also probably drops.

Lowering the pK's of the two His β146 residues and the two N-termini of the α chain means hemoglobin must dissociate protons when it binds oxygen, if the pH is to remain constant. Conversely, it must bind protons when it dissociates oxygen in order to keep the pH constant.

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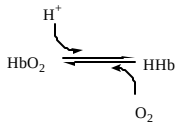
The Bohr Effect

- O_2 binding causes H^+ to dissociate.
- Likewise, an increase in $[H^+]$ will cause O_2 to dissociate. (See Figure 15.34)
- Each affects the affinity of the other:



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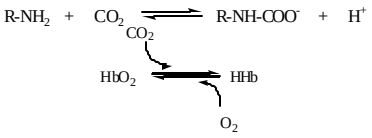
Effect of CO_2 on Oxygen Binding

- As the salt bridges are broken, and the amino groups dissociate a proton, they become free to react with CO_2 to form a **carbamate** structure
- Thus CO_2 also affects the binding of O_2, and vice versa.



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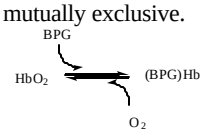
Effect of 2,3-Bisphosphoglycerate on Oxygen Binding

- 2,3-Bisphosphoglycerate (**BPG**) is a highly negatively charged small molecule. (Fig. 15.36)
- It binds to a cavity that is surrounded by positively charged groups. (Fig. 15.37)
- This cavity is only present in deoxyhemoglobin.
- Hence BPG and O_2 are mutually exclusive.
- See Figure 15.35



The cavity is lined by two lysine residues, four histidine residues, and 2 amino terminal residues.

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Binding Curves for Myoglobin and Hemoglobin

- Myoglobin shows a normal hyperbolic binding curve.
- The effect of BPG, H^+, and CO_2 act as **negative heterotropic effectors,** and O_2 becomes a **positive homotropic effector** with a sigmoid binding curve.
  - Deoxyhemoglobin is equivalent to the **T** conformation in the allosteric model.
  - Oxyhemoglobin is equivalent to the **R** conformation in the allosteric model.

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Myoglobin Binding Equation

For:

$MbO_2 \rightleftharpoons Mb + O_2$ $\quad K = \frac{[Mb][O_2]}{[MbO_2]}$

$Y =$ fractional saturation $= \frac{[MbO_2]}{[MbO_2]+[Mb]}$

substituting:

$$Y = \frac{\left(\frac{[Mb][O_2]}{K}\right)}{\left(\frac{[Mb][O_2]}{K}+[Mb]\right)} = \frac{\left(\frac{[O_2]}{K}\right)}{\left(\frac{[O_2]}{K}+1\right)} = \frac{[O_2]}{[O_2]+K}$$

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Myoglobin Binding Equation, con't.

- You should recognize this equation as a rectangular hyperbola, just like the Michaelis-Menten equation.
  - Y = 1 for maximum saturation
  - $K = pO_2$ when Y = 0.5, sometimes called P_{50}
- The ratio of **bound** Mb (Y) to **free** Mb (1-Y):

$$\frac{Y}{1-Y} = \frac{pO_2}{K} \quad \text{and} \quad \text{(reminiscent of the Henderson-Hasselbalch equation)}$$

$$\log\left(\frac{Y}{1\text{-}Y}\right) = \log pO_2 - \log K$$

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Hemoglobin Binding Equations

- The Hill Equation
  - Assumes "all or none" binding:
  - $Hb + 4\,O_2 \rightleftarrows Hb(O_2)_4$

$$K = P_{50} = \frac{[Hb][pO_2]^4}{[Hb(O_2)_4]} \quad \text{and} \quad Y = \frac{[pO_2]^4}{[pO_2]^4 + [P_{50}]^4}$$

$$\frac{Y}{1-Y} = \frac{[pO_2]^4}{[P_{50}]^4} \quad \text{or} \quad \log\left(\frac{Y}{1\text{-}Y}\right) = 4\log pO_2 - 4\log P_{50}$$

plot of $\log\left(\frac{Y}{1\text{-}Y}\right)$ vs log $[pO^2]$ gives a straight line with a slope of 4.

Since $[O_2]$ represents the **activity** of oxygen, and the **standard state** for a gas is one atmosphere pressure, one can express $[O_2]$ as a **pressure** of O_2 in atmospheres, or pO_2.

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Hemoblobin Binding Equations, con't.

Experimental Hill Equation:

$$Hb + n\ O_2 \rightleftharpoons Hb(O_2)_n$$

$$Y = \frac{[pO_2]^n}{[pO_2]^n + [P_{50}]^n} \qquad \frac{Y}{1-Y} = \frac{[pO_2]^n}{[P_{50}]^n}$$

plot of $\log\left(\frac{Y}{1\text{-}Y}\right)$ vs $\log [pO_2]$ gives a line of slope n.

Experimental hemoglobin plot gives n = 2.8.

While the experimental Hill plot doesn't fit a good mechanistic model (what does 2.8 represent in a model?), it does give a measure of the **cooperativity** of oxygen binding. That is the extent to which the binding of one oxygen molecule facilitates the binding of the next one.

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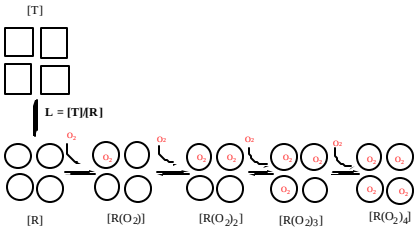
Hemoglobin Binding Equations, con't

- Monod, Wymond, Changeaux Model



The cooperativity of oxygen binding in this model is due to the fact that binding of the first oxygen creates more free binding sites as protein is converted from the T to the R state. This model is equivalent to the "all or none" model of MWC. It could be modified to allow binding to the T form with lower affinity, or can be modified to the "sequential" model of Koshland in which not every subunit has to change conformation at once.

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MWC Model for Hemoglobin, con't.

$$Y = \frac{[R(O_2)] + 2[R(O_2)_2] + 3[R(O_2)_3] + 4[R(O_2)_4]}{4\left([T] + [R] + [R(O_2)] + [R(O_2)_2] + [R(O_2)_3] + [R(O_2)_4]\right)}$$

$$Y = \frac{\frac{[pO_2]}{[P_{50}]}\left(1 + \frac{[pO_2]}{[P_{50}]}\right)^3}{L + \left(1 + \frac{[pO_2]}{[P_{50}]}\right)^4}$$

L must be very large

(See Figure 15.10)

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Fetal Hemoglobin

- In fetal hemoglobin (Hb F), the β-chains are replaced by γ- chains. They have Ser instead of His at position 143.
- The BPG binding cavity has two less positive charges. BPG does not bind as tightly.
- Therefore oxygen binds more tightly.
  - (See Figure 15.39)

By having a higher affinity for oxygen, fetal hemoglobin is able to receive oxygen from the hemoglobin of the maternal blood.

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Sickle Cell Hemoglobin

- In Sickle Cell Anemia, red blood cells at low oxygen tension will form a sickle shape, pass less freely through capillaries, and rupture more easily than normal cells.
- The sickling results from a polymerization of the Hemoglobin molecules.
- Sickle Cell Anemia is a "molecular" disease.
  - Glu_6 of the β-chain is replaced by Val, creating a **hydrophobic patch**, which allows self association of the deoxy form of hemoglobin.
    - (See Figure 15.40)

Why has the sickle-cell trait not died off by evolutionary pressure? The heterozygous condition, which gives only about 1% sickled cells and is serious only under very low oxygen pressure, seems to protect against infection by the malaria parasite. Hence this trait has survived in tropical regions where malaria is common. In some regions of Africa, the trait is found in 20% of the population.