**Lecture 13: Allosteric Effects and Cooperative Binding**

**Key Terms:**

* Oxygen transport.
* Homotropic Allosteric effects
* Heterotropic Allosteric effects
* T and R states of cooperative systems.
* Role of proximal His residue in cooperativity
* Role of BPG in oxygen adaptation.

**Pre-Lecture Quiz:**

1. Sketch the binding curve for non-cooperative binding and mark the KD on the graph.

2. Now draw a binding curve for a ligand that binds more tightly, and mark its KD on the graph.

3. The initial slope of a binding curve, at low [L] is equal to \_\_\_\_\_\_\_\_\_\_\_:

2. Myoglobin binds \_\_\_\_\_\_\_ O2, while hemoglobin binds \_\_\_\_\_\_\_ O2.

4. Sketch the binding curve for Myo (KD = 0.2 kPa).



**13A. Allosteric Effects and Cooperativity:**

**Allosteric** effects are the change in the conformation of a protein due to binding of a ligand.

**Cooperative** effects occur when the binding properties of the protein towards ligands change as a consequence of another ligand binding to the protein. There need not be a direct connection between the two ligands (i.e. they may bind to opposite sides of the protein, or even to different subunits).

Cooperative behavior requires the presence of:

i) At least two binding sites, these may be on the same polypeptide chain, or different subunits.

ii) Two forms of the macromolecule. One form, usually called the T or tense state, binds the primary ligand (e.g. oxygen) with low affinity. The other form, usually called the R or relaxed state, binds ligand with high affinity. The T and R states are in equilibrium with each other.

* In the case of positive cooperativity the fraction of T states exceeds that of the R state and the binding of ligand increases the amount of R state, thus increases the ease of ligand binding.
* In the case of negative cooperativity, the fraction of the T state is smaller than that of the R state. Thus, the initial binding affinity is high. However, the binding of ligand increases the amount of T state, thus reducing the binding affinity.

Allosteric effects are important in the regulation of enzymatic reactions.

**13B. Homotropic Allosteric Moderators:**

* If the two ligands are the same (e.g. oxygen affecting its own binding) then this is called a **homotropic** allosteric effect. Example shown below is a *positive* homotropic modulator, it increases the affinity of the system.



**Mechanism of positive homotropic cooperativity in Hb:**

* Binding of O2 to Fe+2 in heme moves the proximal His residue and its attached helix (F)
* Helix F adjusts its conformation by movement of the α and β subunits.
* Change in interaction between the α and β subunits causes a conformational change to the R-state.

**13C. Heterotropic Allosteric Effects.**

* If the two ligands are different, then this is called a **heterotropic** allosteric effect. The example shows both a heterotropic *negative* allosteric modulator (I), and a *positive* allosteric activator (A).



**Non-cooperative binding:**

* Allosteric activators decrease the KD for the other ligand
* Allosteric inhibitors increase the KD for the other ligand

**Heterotropic Allosteric Effectors in Cooperative systems** These will change the affinity and cooperatively for the second ligand.

* Activators – increase affinity, curve shifts to the left.
* Inhibitors – decrease affinity, curve shifts to the right.

**Hemoglobin:** There are many heterotropic allosteric effectors of oxygen binding in Hemoglobin, two examples are:

1. Protons: oxygen affinity is decreased at low pH, such as in active muscle that is producing lactic acid. This provides an immediate response to the metabolic state of the tissue.

2. BPG: bis-phosphoglycerate binds to the deoxy form of hemoglobin. Therefore it reduces oxygen affinity. This is an adaptive response, requiring several days at high altitude. The production of excess BPG, although it reduces the oxygen affinity, it makes the protein more efficient at delivering oxygen to the tissues.

In the above examples, the tense state of hemoglobin becomes more prevalent than the relaxed state. The allosteric effector stabilizes the tense state, or lowers its energy relative to that of the relaxed state. Consequently, the oxygen affinity is reduced and the binding curve is shifted to the right. Note that the system, with respect to oxygen binding is still cooperative, and eventually high levels of O2 will shift the equilibrium to the R state.



In the case of BPG, this seemingly contradictory behavior actually enhances O2 delivery because of a change in the shape of the binding curve. Note that oxygen still acts as a homotropic allosteric activator, producing cooperative oxygen binding curves, but higher levels of oxygen are required to saturate, and the shape of the binding curve changes.

**Molecular nature of the action of BPG:**

* In deoxy hemoglobin, a positively charged binding pocket exists between two of the four subunits. Thus BPG can easily bind, and when it does so, it stabilizes the deoxy , or tense, form of the protein.
* In oxy-hemoglobin, the relative movement of the chains that occurs during the allosteric transition to the R state closes this pocket, so BPG can no longer fit.





**Effect of BPG on O2 Delivery:** This graph shows the effect of BPG (bisphosphoglycerate) on the oxygen affinity of normal hemoglobin. The level of BPG in the blood at sea level is ~5mM. After adaptation to high altitudes in 2-4 days the BPG level rises to about 8 mM.

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| --- | --- | --- | --- | --- | --- | --- | --- |
|  | **pO2 (kPa)** | **5mM BPG** |  | **5mM BPG** |  | **8 mM BPG** |  |
| **Sea level** | 12 | Y=0.96 |  |  |  |  |  |
| **Rockies** | 8 |  |  | Y=0.90 |  | Y=0.87 |  |
| **Muscle** | 4 | Y=0.56 |  | Y=0.56 |  | Y=0.47 |  |