

Regulation of Enzymes

Control of any metabolic process depends on control of the enzymes responsible for mediating the reactions involved in the pathway. Because regulating metabolic pathways is critically important for living organisms, the ability to regulate enzymatic activities is required for survival. A number of methods are used to regulate enzymes and the rates of the reactions they catalyze.

Control of the amount of enzyme

Two methods can be used to change the amount of an enzyme present in a cell:

- 1) change the rate of enzyme **synthesis**
- 2) change the rate of enzyme **degradation**

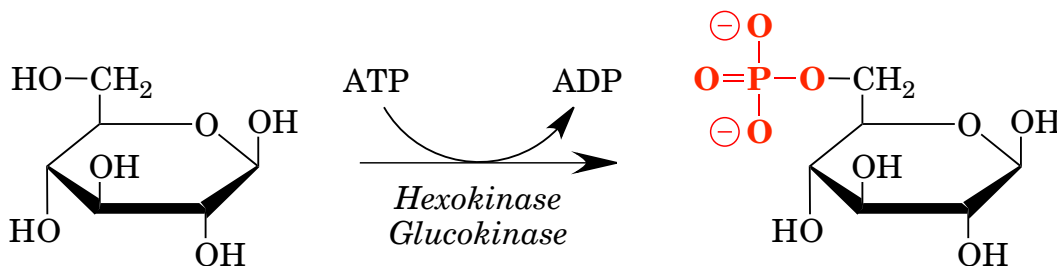
The effect of both of these processes is to change the net amount of enzyme. Because V_{max} is directly proportional to the enzyme concentration, and because the velocity of a reaction is directly proportional to the V_{max} , changing the amount of enzyme changes the rate of a reaction. A number of hormones induce changes in cellular functioning by altering the enzyme concentration.

Changing the amount of an enzyme is conceptually simple method for changing the amount of enzyme activity. However, altering enzyme concentration is a relatively slow process (the minimum time required is about 15 minutes to allow increased or decreased protein synthesis to have an effect). As a result, other methods are frequently used in addition to effects related to altered gene expression.

Control of the type of enzyme

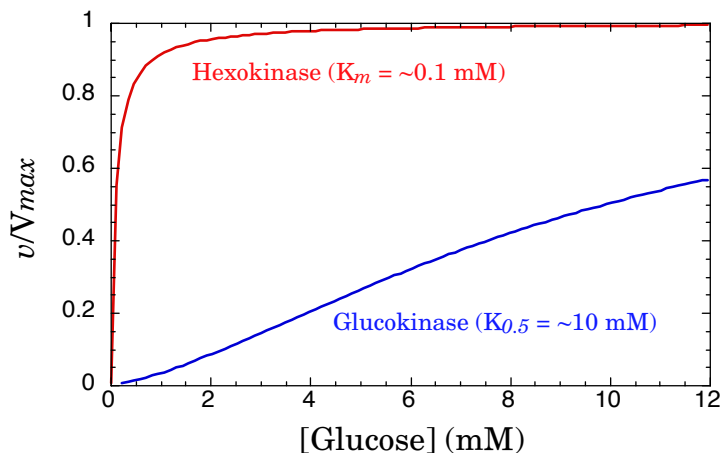
In many cases, more than one form of an enzyme will catalyze a particular reaction. Different **isoenzymes** or **isozymes** are products of different genes. Some multimeric proteins can be synthesized from more than one isozyme. The resulting multimers are different **isoforms**.

In most cases, the different isozymes have somewhat different properties, and therefore can be used to regulate the reaction, or the rate of the reaction, that occurs. Different **isozymes may have markedly different affinities for the same substrate**. An example of this is provided by hexokinase and glucokinase, which both catalyze the phosphorylation of glucose.



Although they catalyze the same reaction, these enzymes differ dramatically in

their affinity for glucose.



The normal concentration for glucose in the bloodstream varies from 4 to 6.5 mM depending on conditions (glucose concentration rarely varies outside this range in normal individuals, although abnormal states such as diabetes mellitus may result in much larger variation in glucose concentration). The graph indicates that hexokinase will be nearly saturated with substrate over the entire normal range. However, glucokinase activity will increase significantly as glucose concentration rises from 4 to 6.5 mM. These enzymes are present in different tissues. Hexokinase is expressed in tissues where glucose utilization is regulated by other processes, and it is necessary for hexokinase to operate at maximal rate regardless of the glucose concentration. In contrast, glucokinase is found largely in the liver and pancreas, where the ability of glucokinase to increase its velocity with increasing glucose concentration has an important regulatory role for glucose metabolism.

We will see these enzymes again during the section on carbohydrate metabolism.

Control of preformed enzyme

In addition to modulating the **amount** of an enzyme, it is possible to modulate the **activity** of an enzyme. These alterations in activity can involve changes in K_m or k_{cat} or both. Possible mechanisms include:

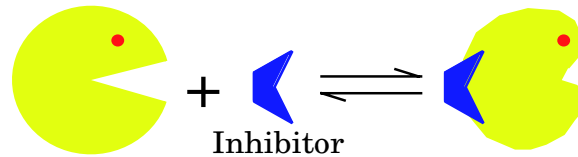
- 1) Covalent modification of the enzyme (most commonly by phosphorylation).
- 2) Protein-protein interaction. For example, calmodulin binds calcium; when bound to calcium calmodulin binds to a number of other proteins, and regulates their activity.
- 3) Competitive inhibition by substrate analogs
- 4) Non-competitive inhibition by small molecules
- 5) Allosteric effectors (see below)

With the exception of alterations in activity mediated by competitive inhibitors, these mechanisms for control of preformed enzyme all involve changes in the conformation of the protein, induced either by the covalent or non-covalent binding of the modulator molecule.

Allostery and Cooperativity

Allostery

Allosteric means “other shape” or “other space”. The term refers to phenomena where ligand binding at one site affects a different, distant site, and to the fact that this binding alters the shape of the protein.



Allosteric effects can occur within one subunit and hence, do not necessarily require an oligomeric protein structure or an enzyme that displays cooperativity relative to the substrate.

Allosteric effectors that **decrease** activity are termed **negative effectors**, whereas those that **increase** enzyme activity are called **positive effectors**.

When the **substrate itself** serves as an effector, the effect is said to be **homotropic**. In such a case, the presence of a substrate molecule at one site on the enzyme alters the catalytic properties of the other substrate-binding site. Homotropic effects can occur in proteins with more than one subunit: if each subunit contains a catalytic site for the same enzymatic process, the binding of substrate at one site may alter the affinity or catalytic rate at other active sites in the complex. Homotropic effects can also involve interactions between regulatory sites (with no catalytic function), and active sites (with no regulatory function).

Alternatively, the effector may be **different** from the substrate, in which case the effect is said to be **heterotropic**. Heterotropic effectors tend to bind to allosteric sites; binding of heterotropic effectors to the active site is unusual.

Effector binding can alter either the K_m or the V_{max} of the enzyme. Effectors that alter the V_{max} are termed **V-type** effectors. Effectors that alter the K_m are called **K-type** effectors. Most (although not all) K-type effectors modulate the activity as a result of affecting the cooperative behavior of the protein.

Cooperativity

In multisubunit proteins, the binding of a molecule to one subunit may alter the binding at the others. The binding events therefore “cooperate” because they act together. The type of effect can vary.

Positive cooperativity occurs when the binding of one molecule **increases** the affinity for binding the others.

Negative cooperativity occurs when the binding of one molecule **reduces** the affinity for binding the others.

Systems exhibiting positive cooperativity have the advantage that they can be very

responsive to small changes in concentration. In order to fully understand this concept, we will look at a well-studied protein: hemoglobin.

Hemoglobin

Hemoglobin is an oxygen-transport protein; it is the major protein present within red blood cells, and accounts for the characteristic color of both this cell type and of blood itself. Because the hemoglobin content of the red blood cell is about 150 mg/ml, this protein is readily purified, and its properties have been studied for more than 150 years.

Hemoglobin is critical to the survival of essentially all vertebrate animals. (Some invertebrates also use hemoglobin, although others use other oxygen transport molecules.) Hemoglobin appears to be an ancient molecule, since it is also found in plants (its role in plants is not understood).

In part because of its ready availability, in part because of its importance, and in part because of its complex functional features, hemoglobin has been extensively studied. Techniques originally developed by Max Perutz for the determination of the hemoglobin structure are still in wide use for X-ray crystallographic structure determination of proteins. The solution of the hemoglobin structure, and the slightly earlier solution of the related, but smaller, myoglobin structure, were milestone events in our understanding of protein structure and its effect on function. Much of the information discussed below is the result of these studies on the structure of these proteins.

Hemoglobin and myoglobin are not enzymes. Instead, they are proteins that bind to ligands, but do not catalyze reactions involving these ligands. However, many mathematical aspects of the interaction of ligands with proteins are similar to the mathematical treatment of enzyme kinetics discussed earlier.

Consider the interaction of myoglobin with oxygen. Each molecule of myoglobin binds a single oxygen molecule. Recall that K_d is the equilibrium dissociation constant, and is defined as shown below.



We can define “Y” as the fraction of myoglobin that is bound to oxygen.

$$Y = \frac{[\text{Bound}]}{[\text{Total Binding Sites}]} = \frac{[\text{Mb} \cdot \text{O}_2]}{[\text{Mb}] + [\text{Mb} \cdot \text{O}_2]}$$

Rearranging the equation for K_d gives:

$$[\text{Mb} \cdot \text{O}_2] = \frac{[\text{Mb}][\text{O}_2]}{K_d}$$

Substituting the equation for bound myoglobin into the equation for Y gives:

$$Y = \frac{\frac{[Mb][O_2]}{K_d}}{[Mb] + \frac{[Mb][O_2]}{K_d}} \text{ which reduces to: } Y = \frac{[O_2]}{K_d + [O_2]}$$

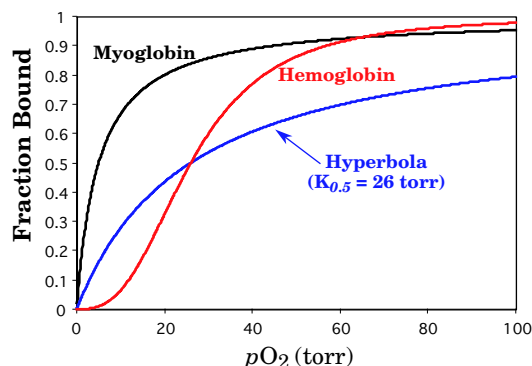
Recall that “ Y ” is the fraction of bound myoglobin. Making Y explicit and rearranging slightly gives:

$$[Mb \bullet O_2] = \frac{([Mb] + [Mb \bullet O_2])[O_2]}{K_d + [O_2]}$$

The sum of free myoglobin and bound myoglobin is the number of binding sites present in the solution and is usually termed the B_{max} (the maximum binding possible at that concentration of the protein). The term $[Mb \bullet O_2]$ is the amount of bound oxygen present. The term $[O_2]$ is the amount of free oxygen present. By substituting the term $[B]$ to indicate the concentration of bound ligand, and $[F]$ to indicate the concentration of free ligand, we obtain a general equation to describe ligand binding:

$$[B] = \frac{B_{max}[F]}{K_d + [F]}$$

Note that this equation is identical in form to the Michaelis-Menten equation. This means that binding data should fit a rectangular hyperbolic curve. Examination of actual binding data reveals that oxygen interaction with myoglobin does, in fact, follow a hyperbola. However, binding of oxygen to hemoglobin follows a **sigmoidal** curve, rather than a hyperbola.



The plot shows “Fraction Bound” on the y -axis; this is the amount of oxygen bound as a fraction of the total amount that could be bound to the protein. “Fraction Bound” is the same as “ Y ” (see above).

Myoglobin has a high affinity for oxygen ($K_d = 2.8$ torr). The hemoglobin curves were generated using a value of 26 torr⁵ for the oxygen concentration required for 50% of maximal binding (the $K_{0.5}$, a parameter analogous to the K_d).

Careful analysis of the binding curve for hemoglobin indicates that the apparent affinity for oxygen changes with oxygen concentration (the affinity for oxygen is low at low concentrations of oxygen, and then increases when oxygen concentration increases). This behavior is typical of positive cooperativity.

Analyzing cooperativity

The standard Michaelis-Menten equation does not correspond to a sigmoidal curve. In addition, the term K_m does not apply to cooperative systems; instead, we need a new term, $K_{0.5}$. $K_{0.5}$ is similar to the K_m , in that $K_{0.5}$ is the concentration for 50% of maximum binding, although its derivation is considerably different from that of K_m .

Before looking at cooperativity, it is useful to first consider a different analysis of the Michaelis-Menten equation. The equation can be rearranged as shown:

$$v = \frac{V_{max}[S]}{K_m + [S]} \quad Y = \frac{v}{V_{max}} = \frac{[S]}{K_m + [S]}$$

The term “Y” is merely used to simplify the later steps (it is the essentially the same Y that we used earlier to look at binding).

Simple algebra then results in a modified equation:

$$\frac{Y}{1 - Y} = \frac{[S]}{K_m}$$

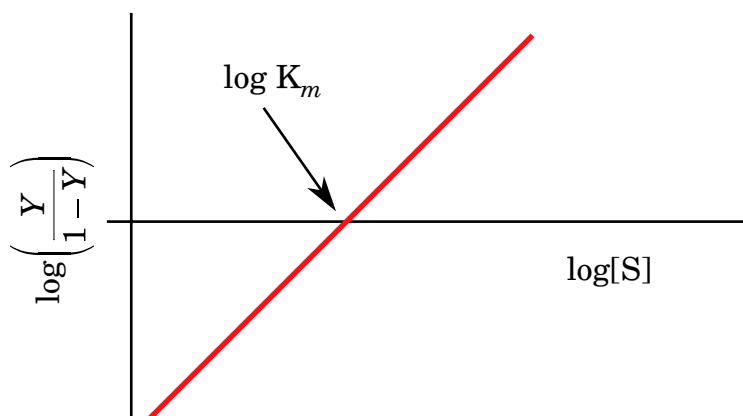
Taking the log of both sides results in this equation:

$$\log\left(\frac{Y}{1 - Y}\right) = \log[S] - \log(K_m)$$

Archibald Hill did this first in 1913. The Hill treatment allows determination of the K_m from the x -intercept of the straight line. Because the Hill plot requires that V_{max} be known (since “Y” depends on V_{max}), ***the Hill plot tends to yield inaccurate results for many data sets***, but it is otherwise a fairly useful way of

⁵ Note: pO₂ is the partial pressure of oxygen; torr are units of pressure equivalent to millimeters of mercury. In weather reports, the barometric pressure readings given are always about 30 inches; 30 inches = 762 mm. The standard earth sea-level atmospheric pressure is 760 mm of mercury = 760 torr. On earth, oxygen comprises about 21% of the atmospheric gas, and therefore has a partial pressure of about 160 mm of mercury (21% of 760 mm). In blood passing through the lungs, the pO₂ is ~100 torr; in the peripheral tissues, the pO₂ drops to 20-40 torr.)

determining K_m . Note that a Hill plot for a normal Michaelis-Menten enzyme has a slope = 1.



Cooperative enzymes (and cooperative binding proteins such as hemoglobin) require a somewhat modified equation called the **Hill equation** to describe their sigmoidal curves. (It is the usefulness of Hill's approach in simple modeling of cooperative proteins that is the reason for much of the above discussion.)

$$\text{For enzymes: } v = \frac{V_{max} [S]^n}{K_{0.5}^n + [S]^n}$$

$$\text{For binding proteins: } [B] = \frac{B_{max} [F]^n}{K_{0.5}^n + [F]^n}$$

The additional term " n " in the Hill equations above is often called the **Hill coefficient**, and is a measure of the deviation from hyperbolic behavior. A **non-cooperative protein has an $n = 1$** and exhibits a hyperbolic curve on a plot of v versus $[S]$. If $n > 1$ then the protein exhibits **positive cooperativity**, while if $n < 1$, then the protein exhibits **negative cooperativity**. Note that n will always be greater than zero.

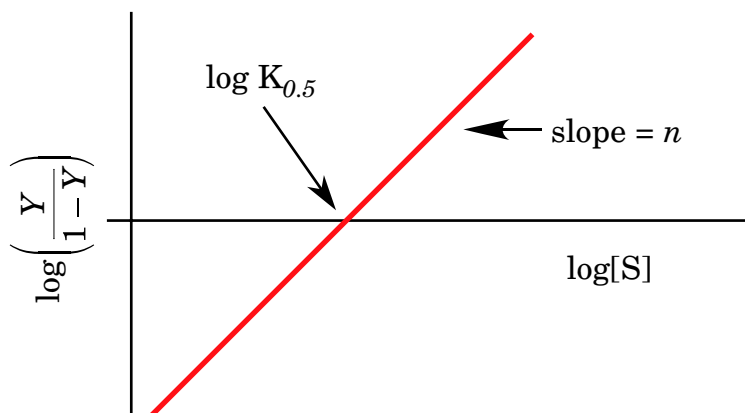
Determining the Hill coefficient requires either a non-linear regression fit to the Hill equation (the preferred method, but frequently more challenging than performing a non-linear regression fit to the Michaelis-Menten equation), or the use of a modified form of the Hill plot. Performing the same rearrangement as before results in this equation:

$$\log\left(\frac{Y}{1-Y}\right) = n \log[S] - n \log(K_{0.5})$$

$$y = mx + b$$

A **Hill plot** of this equation therefore has a **slope of n** , and an x -intercept of

$\log(K_{0.5})$:



The Hill plot is useful for illustrative purposes. However, a ***much better method*** for actually determining the parameters $K_{0.5}$, V_{max} (or B_{max}), and n is to use a **non-linear regression fit** to the Hill equation.

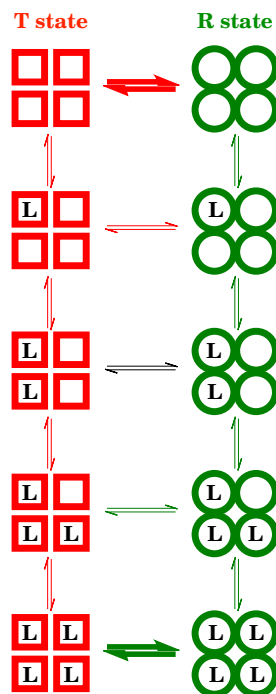
For positive cooperativity, n is less than or equal to the number of interacting sites. Because most systems are not perfectly cooperative, the Hill coefficient is usually significantly less than the number of sites. For hemoglobin, a protein with four binding sites, the Hill coefficient is about 2.8 (it actually ranges from ~2.6 to 3.1 depending on the conditions used for the measurement).

Theoretical aspects of cooperativity

Several models have been proposed to describe the molecular basis of cooperativity. Most of the current models are refinements of the 1965 MWC model. Jacques Monod, Jeffries Wyman, and Jean-Pierre Changeux proposed that cooperative proteins exist in equilibrium between two states, ***Tense (T)*** and ***Relaxed (R)***, where the ***R state has a higher affinity for the ligand***. Binding of ligands or effector molecules alters the equilibrium between the *T* and *R* states. If binding of ligand increases the probability that the protein is in the *R* state, the effect will be an observed increase in the affinity of the protein for the ligand as ligands bind.

The implication of the MWC model is that although the protein can adopt the high affinity *R* state even in the absence of ligand, relatively little of the protein is in the *R* state without ligand.

The MWC model is probably an over-simplification of the true mechanism, but the MWC is closer to reality than the model described by the Hill equation; the Hill equation yields a sigmoidal curve, but makes no real predictions about the



mechanism for the cooperative behavior of the protein. The Hill equation is, however, still useful, since most of the more complex models are too difficult to fit to real data to obtain additional insight into the system.

In the MWC model diagram (above), the two states, T and R , are always in equilibrium. However the binding of ligand alters the equilibrium. For positive cooperativity, ligand binding makes the R state more favorable. In the absence of ligand (or in the presence of negative effectors), the T state is more favored, although some protein may still be in the R state.

The diagram shows a tetrameric protein (such as hemoglobin); however, multimeric complexes varying from dimers to very large multimers have been observed to exhibit cooperativity.

Side Note: Cooperativity in the real world

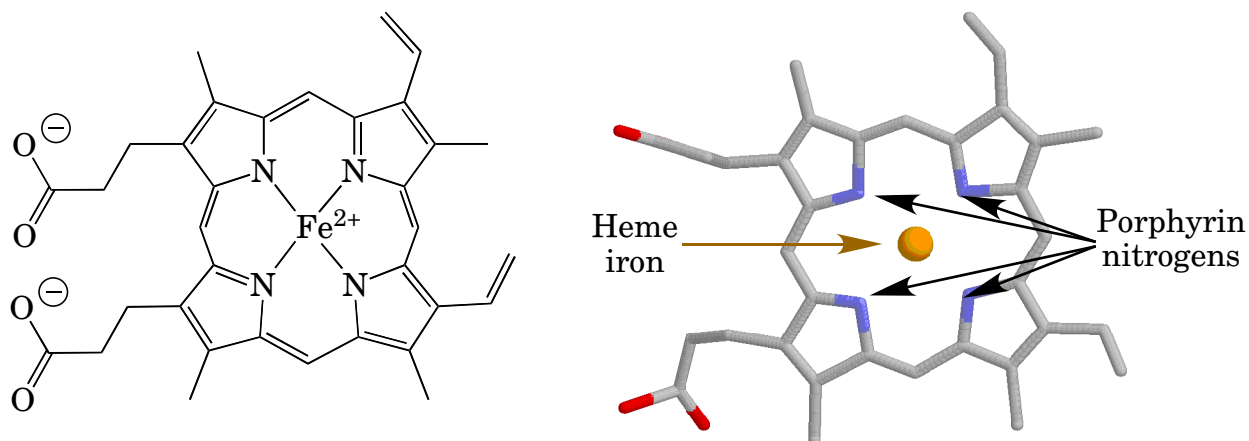
For real proteins, the relatively simple Hill equation is often a simplification of much more complex processes. This is true for a number of reasons. One reason is that at low protein concentrations, the multimeric complex may dissociate into monomers, which generally do not exhibit cooperativity. Another reason is that many cooperative systems (such as hemoglobin, for which some additional complexities are briefly discussed below) are subject to perturbations by other molecules that alter the binding parameters, which means that the binding parameters depend upon the exact conditions under which the measurement is carried out. In addition, at very low concentrations and very high concentrations of ligand, the system is frequently non-cooperative, because at the extremes of the ligand concentration range, the binding protein tends to be entirely in either the T state (at very low concentrations of ligand) or the R state (at very high concentrations of ligand). Finally, sigmoidal data sets are more challenging to analyze than hyperbolic data sets; determining reproducible values of the Hill coefficient for actual proteins using real data is frequently quite difficult, because the Hill coefficient tends to be much more dependent on the quality of the data than are the $K_{0.5}$ and V_{max} values.

Hemoglobin structure and function

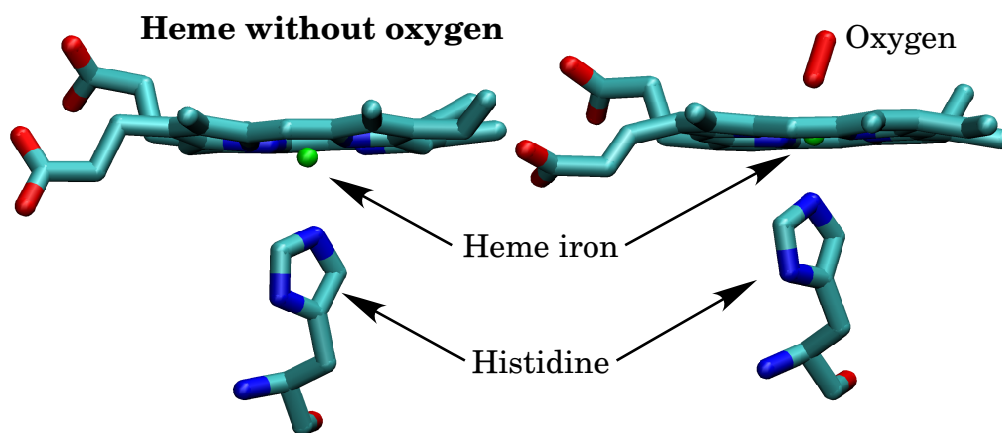
Hemoglobin is an $\alpha_2\beta_2$ tetramer. Each monomer unit contains heme, a planar iron-containing non-covalently-associated prosthetic group. A diagram of heme is shown below. Four nitrogen atoms from the porphyrin ring coordinate the heme iron. Iron normally has six coordination sites; the remaining two coordination ligands must be provided by compounds outside the porphyrin ring plane.

Heme, with the iron in the 2+ oxidation state, binds oxygen with high affinity at one of these additional sites. Heme also binds carbon monoxide and cyanide with higher affinity than oxygen. Binding of carbon monoxide and cyanide prevents oxygen binding to heme-containing proteins; interference with oxygen binding to different heme proteins accounts for the toxicity of these compounds. In hemoglobin and other heme-containing proteins, the protein structure contains features that decrease the heme affinity for carbon monoxide and cyanide; even with this

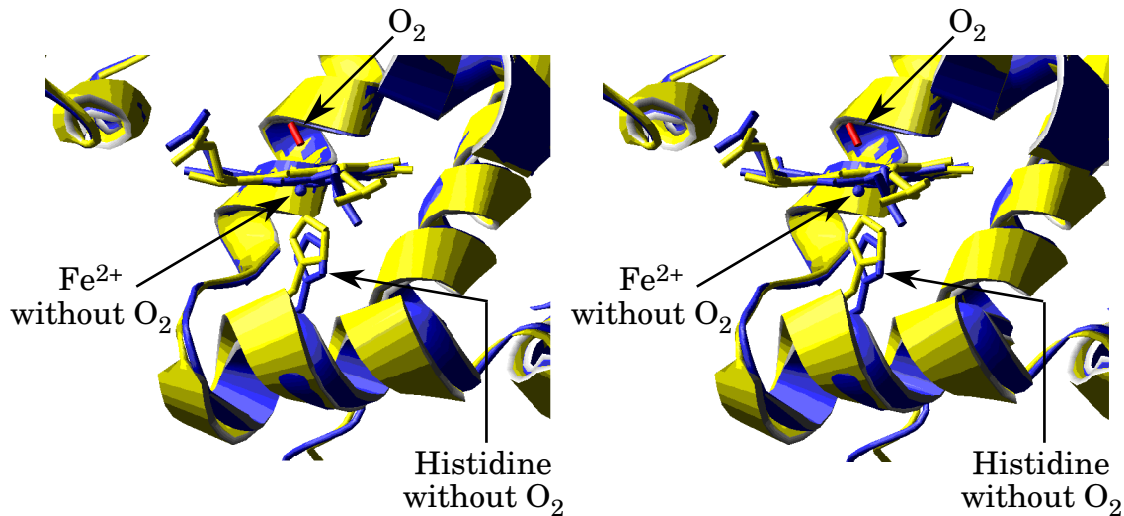
decreased affinity, however, these compounds still bind heme with higher affinity than oxygen.



In each hemoglobin monomer, a histidine is bound to the iron on the heme face opposite to the oxygen-binding site. Binding of oxygen to heme alters the position of the heme iron (see diagrams below), and therefore results in a movement of the histidine.



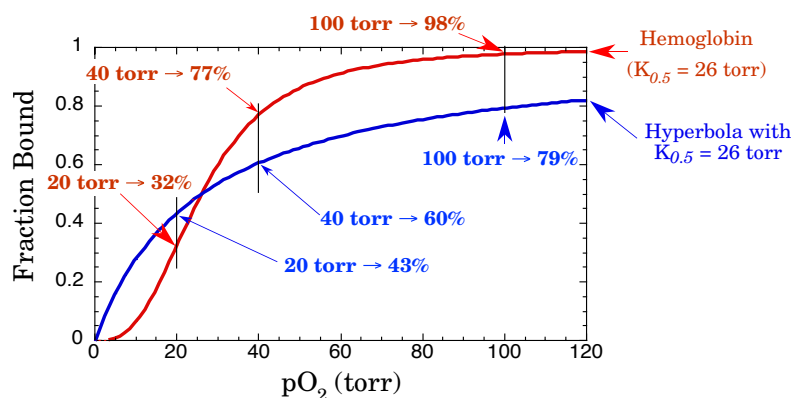
In the stereoview diagram below, the oxygen-bound hemoglobin is shown in yellow, and the non-oxygen bound hemoglobin in blue. Note the displacement of the yellow histidine, and of the helix of which this histidine is a part. The change in the structure of this subunit is fairly small, but it is enough to foster a conformational change by the other subunits of the tetrameric protein that results in an increased affinity for oxygen at each of the oxygen binding sites.



Because binding of oxygen results in (obviously) binding, *and* in an increased binding affinity, the result is the sigmoidal binding curve characteristic of positive cooperativity.

Physiological purpose of cooperativity

Why do organisms use cooperative proteins? The graph below suggests the reason. A non-cooperative oxygen carrier traveling from the lungs ($pO_2 = \sim 100$ torr) to the significantly oxygen-depleted tissues (~ 20 torr in extreme cases) would release less than half of its oxygen content as its oxygen content decreases from 79% to 43% of capacity. In contrast, real hemoglobin takes up much more oxygen in the lungs (oxygen binds to $\sim 98\%$ of the hemoglobin binding sites). When it reaches severely oxygen-depleted tissues, hemoglobin releases 66% of its maximal capacity for oxygen. This means that the same amount of hemoglobin can transport and release far more oxygen than would be the case if hemoglobin were non-cooperative.



In other words, for any system, cooperativity allows a dramatic change in binding over a relatively narrow range of ligand concentrations.

Effectors for hemoglobin

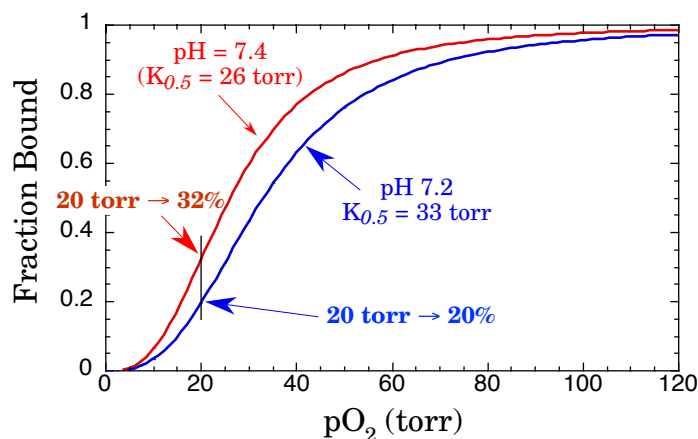
Hemoglobin changes its affinity for the binding of oxygen when it binds oxygen; this

is the definition of cooperativity. In many cases, however, even this change in affinity is not large enough to result in the release of the amount of oxygen required by the tissues. In order to result in an even more dramatic shift in oxygen binding affinity, hemoglobin is subject to regulation by allosteric effector molecules.

Protons

One major regulatory effector of oxygen binding to hemoglobin is hydrogen ion concentration. Tissues undergoing rapid metabolic processes tend to release lactic acid into circulation, and as a result have a somewhat lower local pH. These tissues need more oxygen, and organisms have evolved a mechanism to allow release of additional oxygen to these tissues.

Protons are suspected to bind to two types of sites: the N-termini of the α -subunits, and His¹⁴⁶ of the β -subunit. The effect of the proton binding is a stabilization of the low affinity (T) state of the protein, causing a shift of the binding curve to the right. As a result of this shift, the binding of oxygen at 20 torr decreases from ~32% at pH 7.4 to ~20% at pH 7.2. The release of this additional oxygen assists in supporting the metabolic requirements of the tissue.



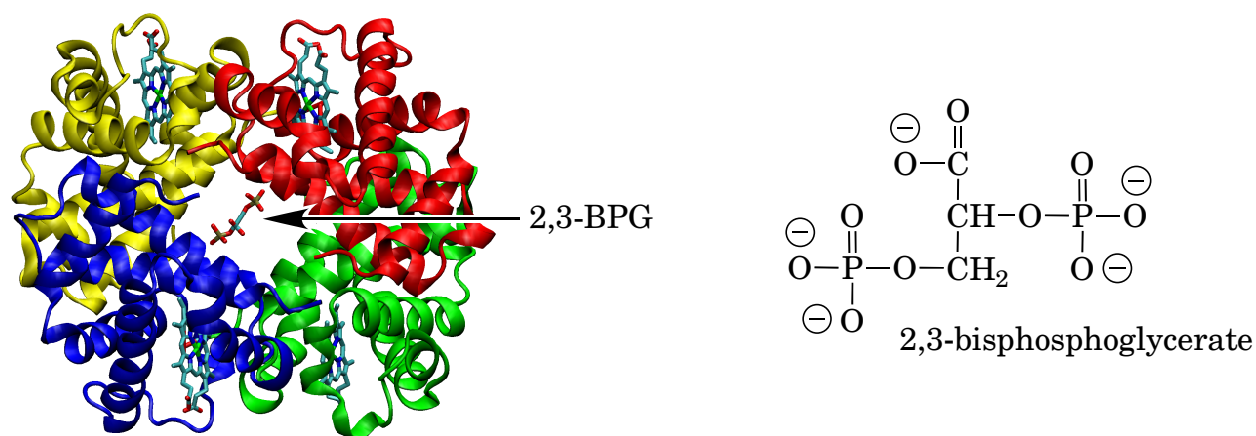
The change in binding affinity with decreasing pH is known as the **Bohr effect** in honor of its discovery by Christian Bohr (the father of the Nobel Prize winning nuclear physicist Niels Bohr).

Carbon dioxide

One simple effect of carbon dioxide is the release of protons (carbon dioxide and water combine to form carbonic acid, which results in the release of protons). In addition, carbon dioxide interacts directly with the protein to cause a decreased oxygen binding affinity. Although hemoglobin does not carry carbon dioxide very efficiently, it still accounts for about 50% of the total blood carbon dioxide transport.

2,3-Bisphosphoglycerate

The third major regulator of hemoglobin oxygen binding is 2,3-bisphosphoglycerate (BPG).



BPG is derived from the glycolytic intermediate 1,3-bisphosphoglycerate. The hemoglobin tetramer has a single binding site for BPG, located at the center of the molecule. This central cavity is larger in the T state than in the R state. By occupying the cavity, BPG binding stabilizes the T state, and therefore causes a decrease in oxygen binding affinity.

The concentration of BPG increases as part of the adaptation to high altitude, and therefore allows the release of additional oxygen to the tissues, a change that is necessary because of somewhat lower saturation of the hemoglobin in the lungs.

Hemoglobin isoforms

Fetal Hemoglobin

The hemoglobin produced during fetal life differs somewhat from the adult hemoglobin: it is an $\alpha_2\gamma_2$ tetramer, instead of the $\alpha_2\beta_2$ tetramer found in adults. The β and γ polypeptides are synthesized from different genes.

Fetal hemoglobin has a higher affinity for oxygen than adult hemoglobin. In addition, fetal hemoglobin has a lower affinity for BPG than does the adult hemoglobin (due to the replacement of a histidine normally forming an ionic interaction with the BPG with a non-ionizable serine). The combination of the intrinsically higher affinity and the lower binding by the allosteric inactivator allows the fetal hemoglobin to obtain oxygen from the maternal circulation.

Mutant Hemoglobins

Malaria is a serious health threat; historically, malaria has exerted sufficient selective pressure to result in several mutations that confer resistance to the disease. The majority of these mutations affect hemoglobin, because the malaria parasite must spend part of its life cycle in red blood cells. Heterozygotes for the hemoglobin mutations (~5% of the humans on earth) have resistance to malaria; homozygotes for the mutations have serious health problems.

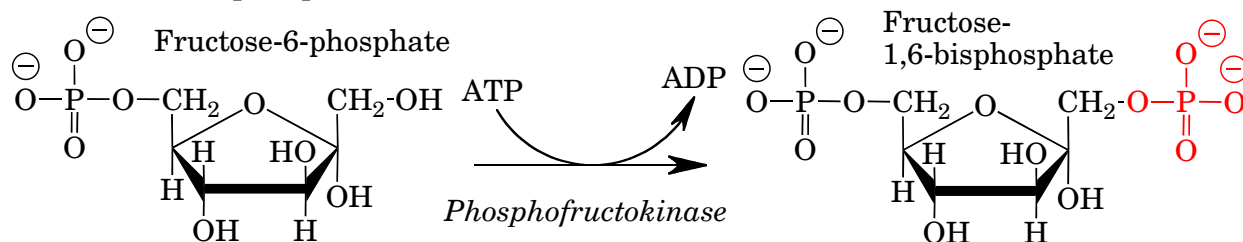
Hemoglobin mutations fall into two major categories: **thalassemias**, in which one of the adult globin genes is non-functional, and **point mutations**, in which one of the amino acids (usually in the β -subunit) is altered.

Humans have four genes for the hemoglobin α -subunit. Homozygotes for complete α -thalassemia (*i.e.* individuals missing all of the α -globin genes) invariably die shortly after birth, because the β_4 and γ_4 homotetramers they produce lack both cooperativity and the Bohr effect, and as a result do not release oxygen to the tissues. Individuals homozygous for deletions of some (but not all) of the α -globin genes are slightly anemic but are usually otherwise normal. In β -thalassemias, the disorder varies greatly in severity. In some individuals, continued production of the fetal hemoglobin ($\alpha_2\gamma_2$) completely abolishes symptoms, while others require transfusions, due both to insufficient hemoglobin production, and to tissue damage associated with α_4 homotetramers.

The most common point mutation is the sickle-cell anemia gene, in which a single base change alters the codon for Glu⁶ of the β -subunit to a valine codon. Hemoglobin formed from the mutant β -globin gene is called HbS. HbS not bound to oxygen can form polymers, because Val⁶ (unlike the normal Glu⁶) can interact with a hydrophobic pocket on the surface of another hemoglobin tetramer. HbS polymers distort the shape of the red blood cell, and tend to result in disruption of the red cell (and therefore in anemia, due to decreased numbers of red blood cells). The existence of HbS and other point mutations is a further example of the phenomenon that small changes in a protein can have dramatic effects on its function.

Phosphofructokinase

Another example of a cooperative protein is the enzyme phosphofructokinase. Phosphofructokinase catalyzes the most important of the three regulated steps in the glycolytic pathway, the ATP-dependent conversion of fructose-6-phosphate to fructose-1,6-bisphosphate.

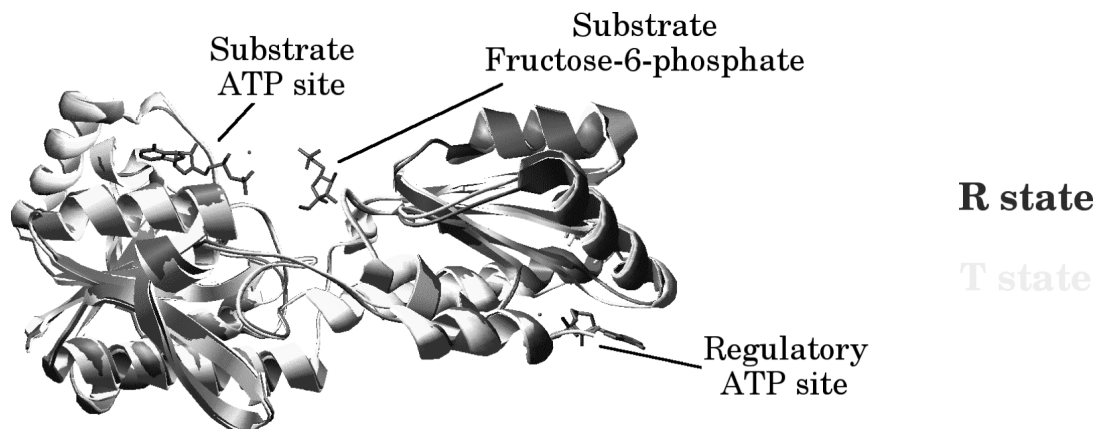


Phosphofructokinase is subject to regulation by a variety of compounds. One of the most important negative effectors is one of its substrates, ATP; in other words, ATP acts as both a substrate and an inhibitor for the enzyme. A number of other compounds increase the activity of phosphofructokinase, generally by preventing the inhibition by ATP.

How can ATP act as both a substrate and an inhibitor?

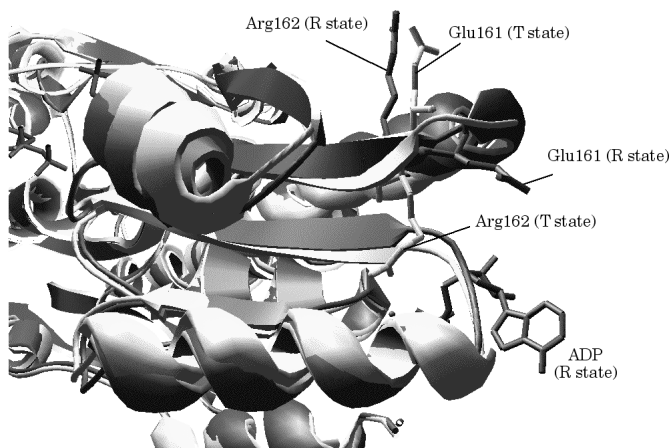
Phosphofructokinase is a tetrameric enzyme. Each monomer of the phosphofructokinase complex has two binding sites for ATP; the active site, which has a high affinity for ATP, and a lower affinity allosteric regulatory site. Binding of ATP to the regulatory site stabilizes the T state of the enzyme. The *Bacillus stearothermophilus* phosphofructokinase structure below shows the active site and

regulatory site in one monomer of the complex.



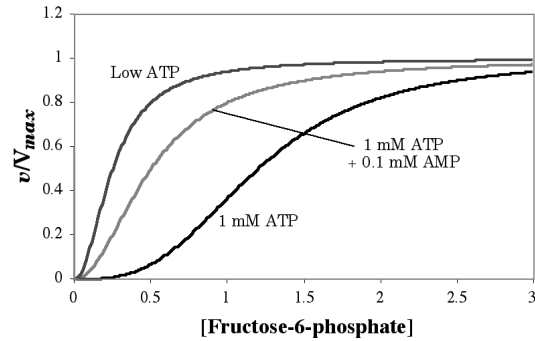
(Note: the active site in the R state crystal structure contains ADP, not ATP; crystallization of the enzyme with both substrates bound results in product formation that prevents trapping the substrate-bound state.)

The superimposition of the R and T states reveals few major structural differences. However one small difference appears to have large functional consequences. The binding of ATP is thought to cause a small conformational change near the regulatory site. This conformational change alters the shape of an α -helix. Residues Glu¹⁶¹ and Arg¹⁶² are part of this helix. In the R state, Arg¹⁶² interacts with the active site of another monomer, stabilizing the binding of the fructose-6-phosphate substrate by ionic interactions. In the T state, however, Arg¹⁶² moves, and Glu¹⁶¹ interacts with the active site of the other monomer. This results in an electrostatic repulsion between the negative charge of the substrate and the negative charge of Glu¹⁶¹. Because it alters the binding affinity for substrate, ATP is a K-type negative allosteric effector.



In contrast, the R state is stabilized by the binding of ADP, AMP, and other positive effectors. In addition, the enzyme exhibits positive cooperativity for fructose-6-phosphate. At low concentrations of ATP, the enzyme is maximally active. At higher concentrations of ATP, the phosphofructokinase is much less active. Displacement of ATP from the regulatory site by AMP (which stabilizes the R state, and has a

higher affinity for the regulatory site than does ATP) reverses the inhibition.



The plot (above) shows that the presence of AMP decreases the cooperativity of the enzyme. This is logical; in the presence of AMP, the enzyme is stabilized in the R state, and fructose-6-phosphate does not need to induce the conformational change to the higher affinity state.

Summary

Enzymes and other proteins can be regulated in a number of ways. One major mechanism is a change in the amount of enzyme present in a cell. Another major mechanism is the alteration of either V_{max} or binding affinity due to binding of substrate or other molecules.

Multimeric proteins can exhibit cooperativity, in which one substrate alters the binding of others.

Hemoglobin is a well-characterized example of **positive cooperativity** (binding of oxygen increases the affinity for other oxygen molecules). Phosphofructokinase also exhibits positive cooperativity (binding of fructose-6-phosphate to one enzyme monomer increases the affinity for binding to the other polypeptides in the complex). Proteins exhibiting positive cooperativity have Hill coefficients greater than one.

Proteins exhibiting **negative cooperativity** have Hill coefficients less than one (although greater than zero).

Both types of cooperativity allow changes in substrate concentration to exert more dramatic changes in enzymatic activity or in ligand binding.

Molecules other than the substrate can regulate cooperative proteins; an example of this is the regulation of hemoglobin by 2,3-bisphosphoglycerate and hydrogen ion concentration.

For some enzymes, increasing concentrations of substrate can decrease the enzymatic activity. This is illustrated by the effect of ATP on phosphofructokinase, where ATP acts both as a substrate and as a negative effector.

Binding of small molecules can cause conformational changes of varying degrees; even small conformational changes can have significant effects on the activity of the protein.