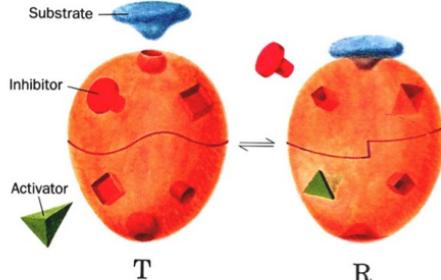


ENZYME REGULATION 1

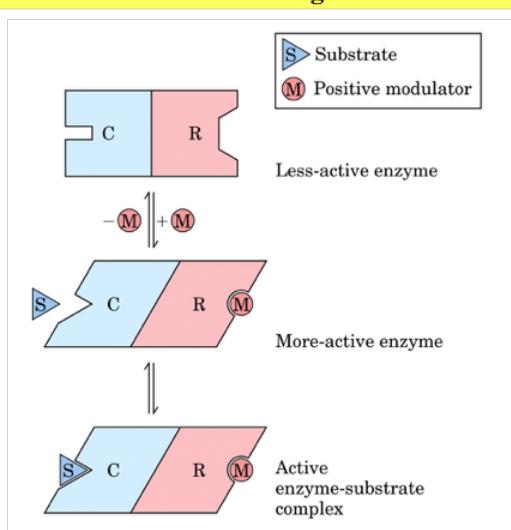
ALLOSTERY: binding of one ligand to enzyme/protein is affected by the binding of another (effector or modulator) at different site



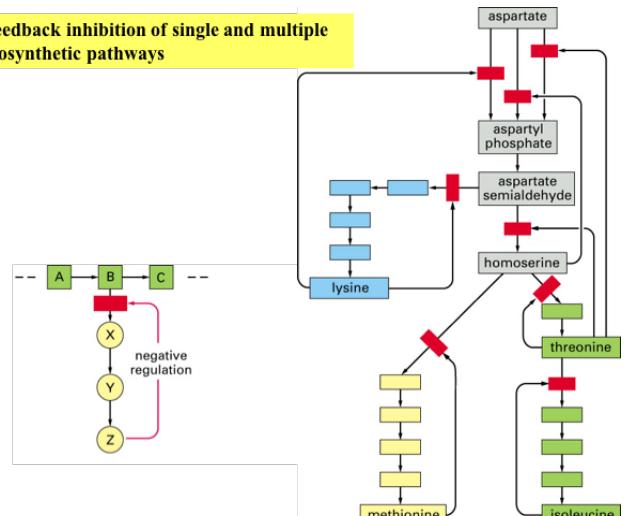
HOMOTROPIC EFFECT: ligands identical (substrate)
HETEROTROPIC EFFECT: ligands different

Allosteric Control. Allosteric proteins contain distinct regulatory sites and multiple functional sites. The binding of small signal molecules at regulatory sites is a significant means of controlling the activity of these proteins. Moreover, allosteric proteins show the property of cooperativity: activity at one functional site affects the activity at others. Proteins displaying allosteric control are thus information transducers: their activity can be modified in response to signal molecules or to information shared among active sites.

Subunit interactions in a general allosteric enzyme



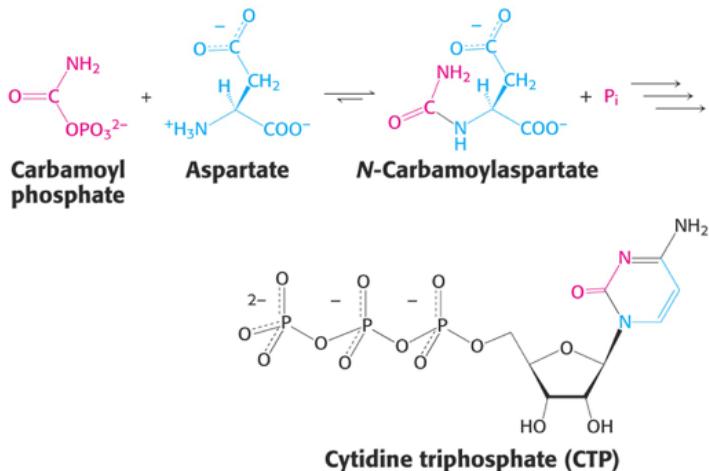
Feedback inhibition of single and multiple biosynthetic pathways



Feedback inhibition is always regulated by allosteric regulation. Aspartate is a key molecule that is also key for nucleotides. Isoleucine inhibits the first enzyme that converts threonine into isoleucine itself. It does that by shifting the decision point to switch it on.

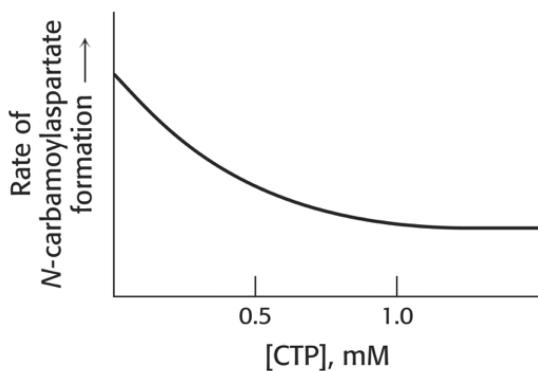
ASPARTATE TRANS-CARBAMOYLASE

Catalysis by aspartate trans-carbamoylase of the first step in pyrimidine biosynthesis is inhibited by cytidine triphosphate, the final product of that biosynthesis, in an example of feedback inhibition.

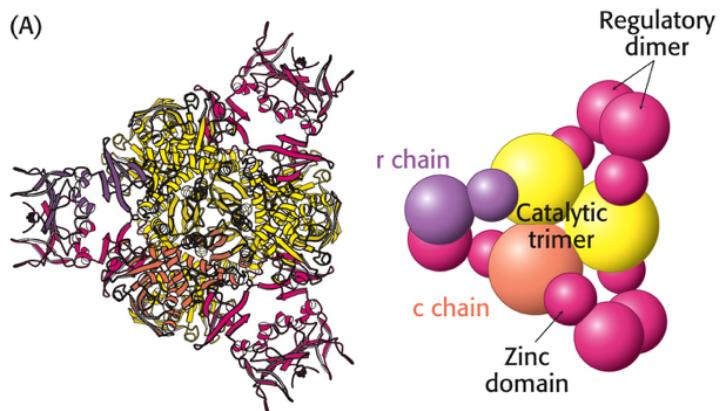


ATCase is inhibited by CTP, the final product of the ATCase-initiated pathway. The rate of the reaction catalyzed by ATCase is fast at low concentrations of CTP but slows as CTP concentration increases. Thus, the pathway continues to make new pyrimidines until sufficient quantities of CTP have accumulated. The inhibition of ATCase by CTP is an example of feedback inhibition, the inhibition of an enzyme by the end product of the pathway. Feedback inhibition by CTP ensures that *N*-carbamoylaspartate and subsequent intermediates in the pathway are not needlessly formed when pyrimidines are abundant.

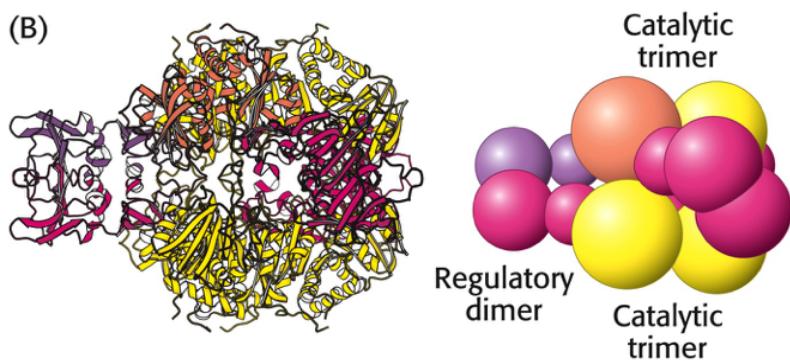
The inhibitory ability of CTP is remarkable because CTP is structurally quite different from the substrates of the reaction. Thus CTP must bind to a site distinct from the active site at which substrate binds. Such sites are called allosteric or regulatory sites. CTP is an example of an allosteric inhibitor. In ATCase (but not all allosterically regulated enzymes), the catalytic sites and the regulatory sites are on separate polypeptide chains.



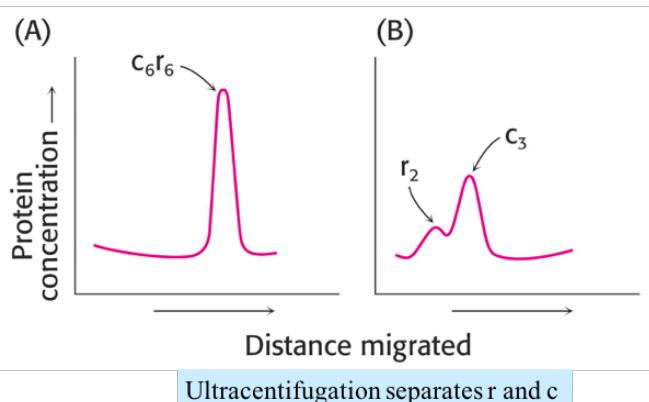
ATCase can be literally separated into regulatory (r) and catalytic (c) subunits by treatment with a mercurial compound such as p-hydroxymercuribenzoate, which reacts with sulfhydryl groups. The larger subunit is the catalytic subunit. This subunit displays catalytic activity but is unresponsive to CTP and does not display sigmoidal kinetics. The isolated smaller subunit can bind CTP, but has no catalytic activity. Hence, that subunit is the regulatory subunit. The catalytic subunit (c3) consists of three chains (34 kd each), and the regulatory subunit (r2) consists of two chains (17 kd each). The catalytic and regulatory subunits combine rapidly when they are mixed. The resulting complex has the same structure, c6r6, as the native enzyme: two catalytic trimers and three regulatory dimers.



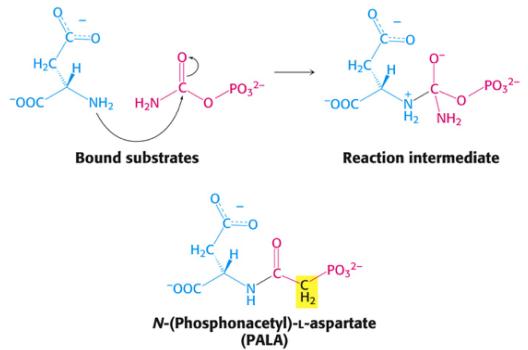
Most strikingly, the reconstituted enzyme has the same allosteric and kinetic properties as those of the native enzyme. Thus, ATCase is composed of discrete catalytic and regulatory subunits, and the interaction of the subunits in the native enzyme produces its regulatory and catalytic properties.



ATCase can be dissociated into catalytic (c) and regulatory (r) subunits

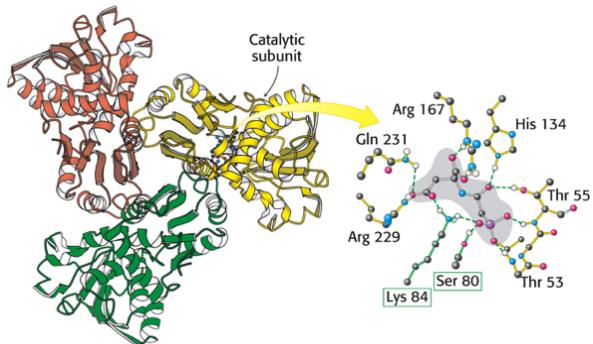


Two catalytic trimers are stacked one on top of the other, linked by three dimers of the regulatory chains. There are significant contacts between the catalytic and the regulatory subunits: each r chain within a regulatory dimer interacts with a c chain within a catalytic trimer. The c chain makes contact with a structural domain in the r chain that is stabilized by a zinc ion bound to four cysteine residues. The mercurial compound p-hydroxymercuribenzoate is able to dissociate the catalytic and regulatory subunits because mercury binds strongly to the cysteine residues, displacing the zinc and destabilizing this r-subunit domain.

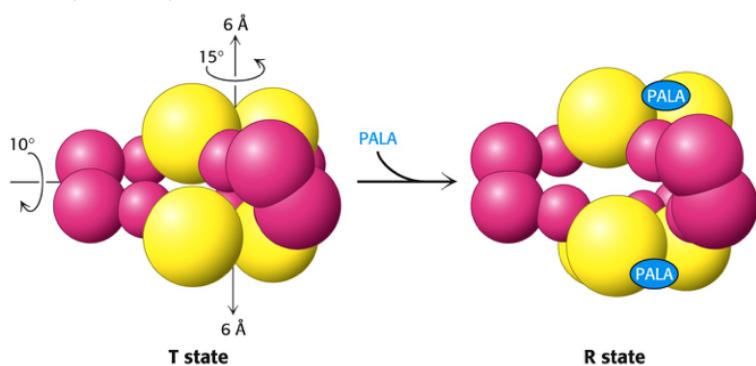


To locate the active sites, the enzyme was crystallized in the presence of *N*-(phosphonacetyl)-L-aspartate (PALA), a bisubstrate analog (an analog of the two substrates) that resembles an intermediate along the pathway of catalysis. PALA is a potent competitive inhibitor of ATCase; it binds to the active sites and blocks them. The structure of the ATCase–PALA complex reveals that PALA binds at sites lying at the boundaries between pairs of c chains within a catalytic trimer. Each catalytic trimer contributes three active sites to the complete enzyme. Further examination of the ATCase–PALA complex reveals a remarkable change in quaternary structure on binding of PALA. The two catalytic trimers move 12 Å farther apart and rotate approximately 10 degrees about their common threefold axis of symmetry.

The active site of ATCase contains residues from more than one subunit

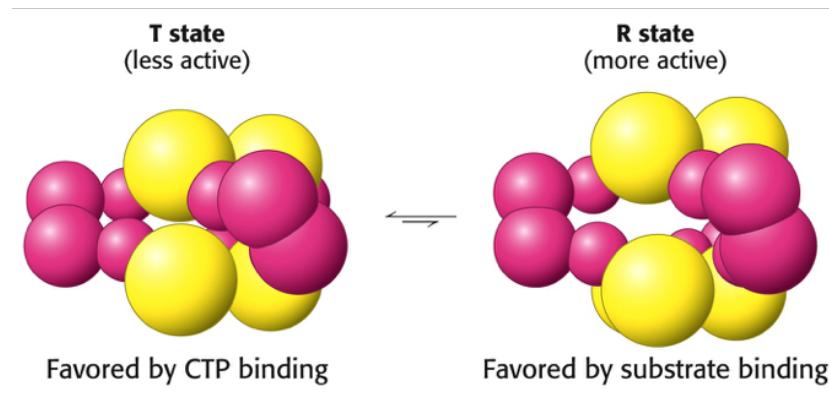


Moreover, the regulatory dimers rotate approximately 15 degrees to accommodate this motion . The enzyme literally expands on PALA binding. In essence, ATCase has two distinct quaternary forms: one that predominates in the absence of substrate or sub- strate analogs and another that predominates when substrates or analogs are bound. We call these forms the T (for tense) state and the R (for relaxed) state, respectively



The enzyme exists in an equilibrium between the T state and the R state. In the absence of substrate, almost all the enzyme molecules are in the T state. The T state has a low affinity for substrate and hence shows a low catalytic activity. The occasional binding of a substrate molecule to one active site in an enzyme increases the likelihood that the entire enzyme shifts to the R state with its higher binding affinity. The addition of more substrate has two effects. First, it increases the probability that each enzyme molecule will bind at least one substrate molecule. Second, it increases the average number of substrate molecules bound to each enzyme. The presence of additional substrate will increase the fraction of enzyme molecules in the more active R state because the position of the equilibrium depends on the number of active sites that are occupied by substrate.

The R and T states are in equilibrium

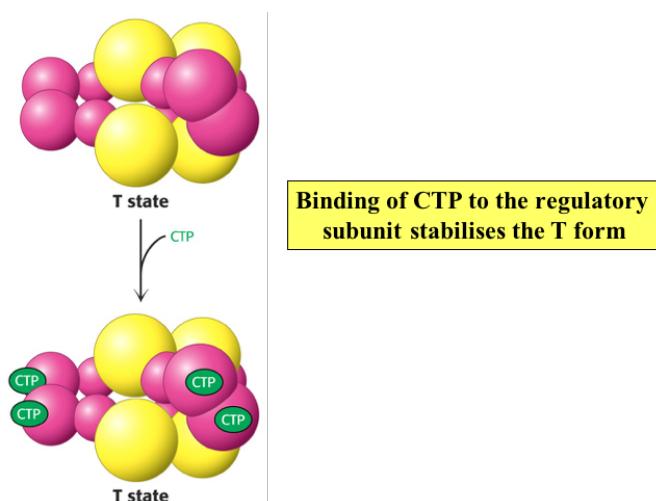


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This mechanism for allosteric regulation is referred to as the concerted mechanism because the change in the enzyme is “all or none”; the entire enzyme is converted from T into R, affecting all of the catalytic sites equally. In contrast, the sequential model assumes that the binding of ligand to one site on the complex can affect neighboring sites without causing all subunits to undergo the T-to-R transition. Although the concerted mechanism explains the behavior of ATCase well, most other allosteric enzymes have features of both models.

The sigmoidal curve for ATCase can be pictured as a composite of two Michaelis–Menten curves, one corresponding to the T state and the other to the R state. An increase in substrate concentration favors a transition from the T-state curve to the R-state curve. Note that such sigmoidal behavior has an additional consequence: in the concentration range at which the T-to-R transition is taking place, the curve depends quite steeply on the substrate concentration.

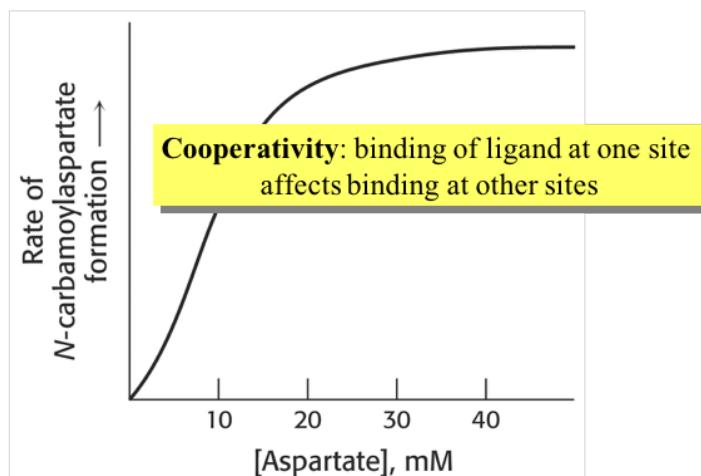


CTP inhibits the action of ATCase. X-ray studies of ATCase in the presence of CTP revealed (1) that the enzyme is in the T state when bound to CTP and (2) that a binding site for this nucleotide exists in each regulatory chain in a domain that does not interact with the catalytic subunit. Each active site is more than 50 Å from the nearest CTP-binding site.

The quaternary structural changes observed on substrate-analog binding suggest a mechanism for inhibition by CTP.

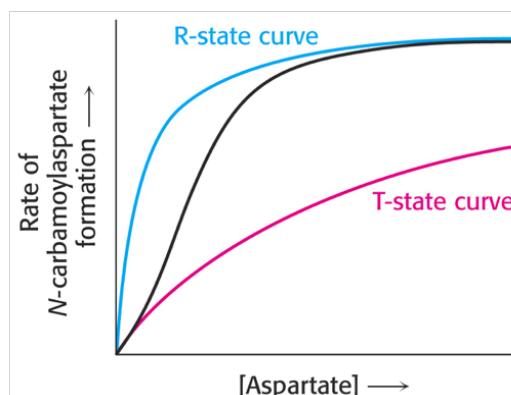
The binding of the inhibitor CTP shifts the equilibrium toward the T state, decreasing net enzyme activity. The binding of CTP makes it more difficult for substrate binding to convert the enzyme into the R state. Consequently, CTP increases the initial phase of the sigmoidal curve. More substrate is required to attain a given reaction rate.

ATCase shows sigmoidal kinetics - the active sites cooperate



The sigmoidal curve for ATCase can be pictured as a composite of two Michaelis–Menten curves, one corresponding to the T state and the other to the R state. An increase in substrate concentration favors a transition from the T-state curve to the R-state curve. Note that such sigmoidal behavior has an additional consequence: in the concentration range at which the T-to-R transition is taking place, the curve depends quite steeply on the substrate concentration. The enzyme is switched from a less active state to a more active state within a narrow range of substrate concentration. This behavior is beneficial when a response to small changes in substrate concentration is physiologically important. In studies of the isolated catalytic trimer, the catalytic subunit shows the hyperbolic curve characteristic of Michaelis–Menten kinetics, which is indistinguishable from the curve deduced for the R state. Thus, the term *tense* is apt: in the T state, the regulatory dimers hold the two catalytic trimers sufficiently close to each other that key loops on their surfaces collide and interfere with conformational adjustments necessary for high-affinity substrate binding and catalysis.

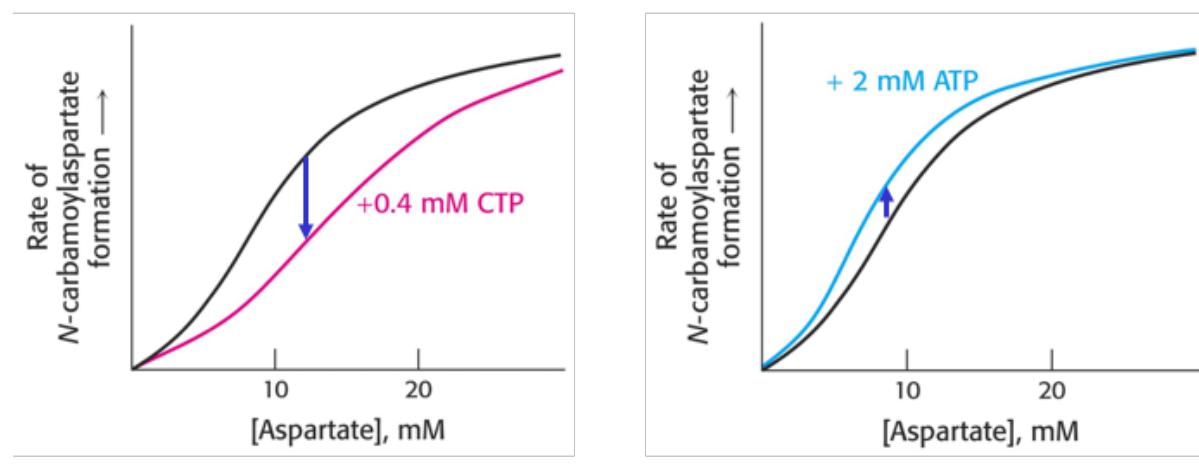
Model for cooperativity: two Michaelis-Menten enzymes with different K_m values



ATP, too, is an allosteric effector of ATCase. However, the effect of ATP is to increase the reaction rate at a given aspartate concentration. At high concentrations of ATP, the kinetic profile shows a less-pronounced sigmoidal behavior. ATP competes with CTP for binding to regulatory sites. Consequently, high levels of ATP prevent CTP from inhibiting the enzyme. The effects of nonsubstrate molecules on allosteric enzymes (such as those of CTP and ATP on ATCase) are referred to as heterotropic effects. Substrates generate the sigmoidal curve (homotropic effects), whereas regulators shift the KM (heterotropic effects). Note, however, that both types of effect are generated by altering the T/R ratio.

The increase in ATCase activity in response to increased ATP concentration has two potential physiological explanations. First, high ATP concentration signals a high concentration of purine nucleotides in the cell; the increase in ATCase activity will tend to balance the purine and pyrimidine pools. Second, a high concentration of ATP indicates that energy is available for mRNA synthesis and DNA replication and leads to the synthesis of pyrimidines needed for these processes.

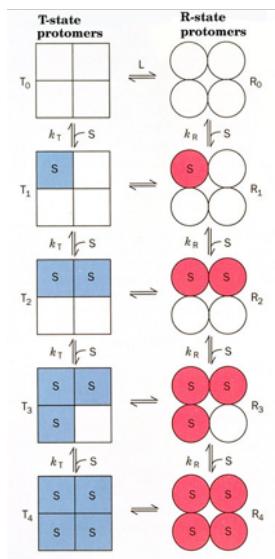
Allosteric regulators modulate the T-to-R equilibrium



**Negative regulation
stabilises T state**

**Positive regulation
stabilises R state**

Symmetry or concerted model of Monod, Wyman and Changeux (MWC)

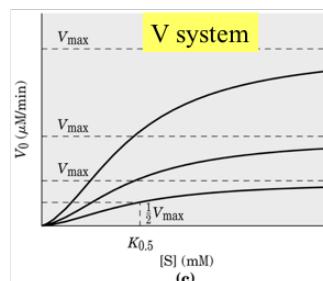
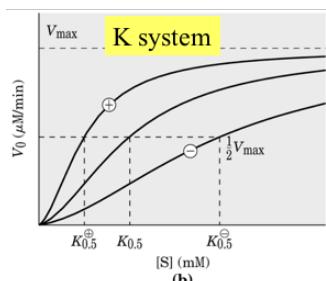
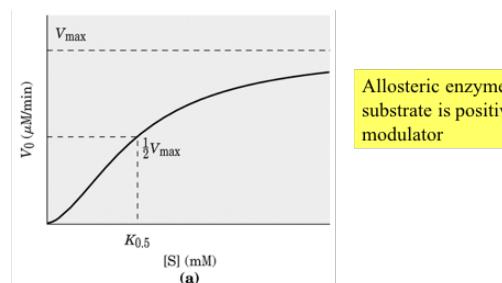


Rules:

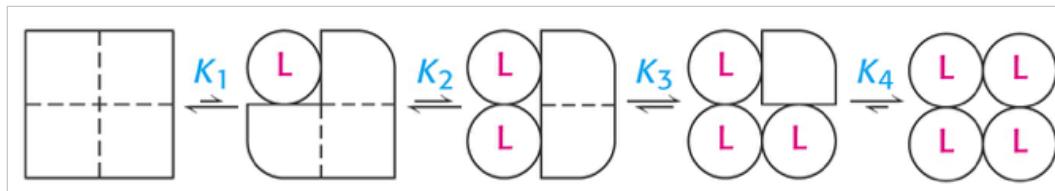
- Oligomeric
- Two forms (R and T) in equilibrium

R	Relaxed
T	Tense
- T is dominant form in absence of ligand (L, allosteric constant = [T]/[R])
- T state has lower affinity for ligand
- Molecular symmetry in the OLIGOMER is conserved (either all T or all R)

For enzymes:
either K_m affected (K system)
or V_{max} (V system)



Sequential model of Koshland, Némethy and Filmer



Rules:

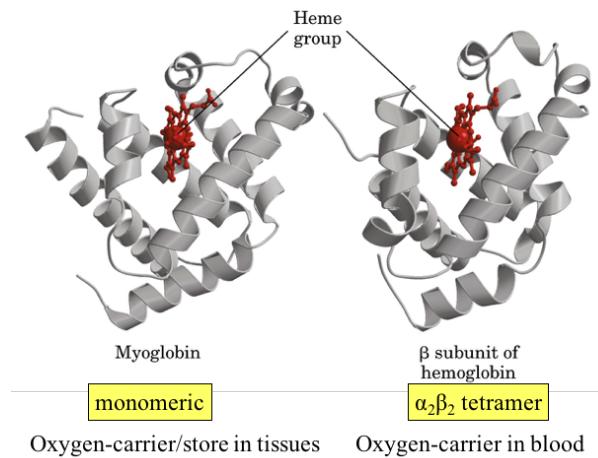
- Only one conformational state in absence of ligand
- Conformational change is sequential upon binding of ligands
- Interactions between subunits can be positive or negative
- Different dissociation constants (K₁, K₂...K_n)

Positive cooperativity
Negative cooperativity

binding affinity improves
binding affinity declines

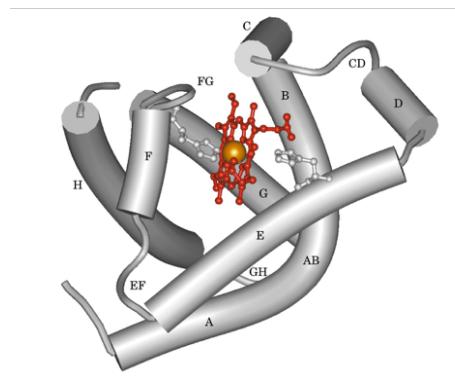
ENZYME REGULATION 2

Haemoglobin and myoglobin: an oxygen transport system



A comparison of myoglobin and haemoglobin illuminates some key aspects of protein structure and function. These two evolutionarily related proteins employ nearly identical structures for oxygen binding. However, haemoglobin is a remarkably efficient oxygen carrier, able to use as much as 90% of its potential oxygen-carrying capacity effectively. Under similar conditions, myoglobin would be able to use only 7% of its potential capacity. What accounts for this dramatic difference? Myoglobin exists as a single polypeptide, whereas hemoglobin comprises four polypeptide chains. The four chains in haemoglobin bind oxygen cooperatively, meaning that the binding of oxygen to a site in one chain increases the likelihood that the remaining chains will bind oxygen. Furthermore, the oxygen-binding properties of hemoglobin are modulated by the binding of hydrogen ions and carbon dioxide in a manner that enhances oxygen-carrying capacity. Both cooperativity and the response to modulators are made possible by variations in the quaternary structure of hemoglobin when different combinations of molecules are bound.

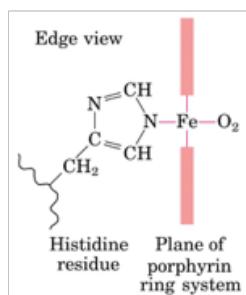
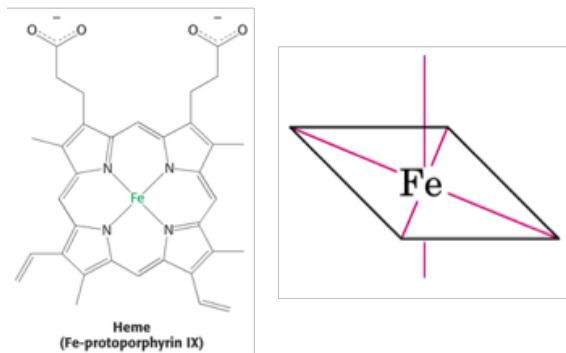
Myoglobin has a single binding site for oxygen



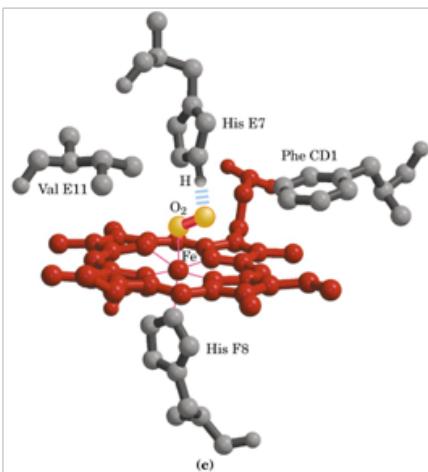
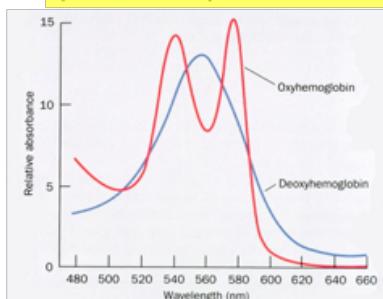
Myoglobin can exist in an oxygen-free form called deoxymyoglobin or in a form with an oxygen molecule bound called oxymyoglobin. The ability of myoglobin and hemoglobin to bind oxygen depends on the presence of a bound prosthetic group called heme.

The heme group gives muscle and blood their distinctive red color. It consists of an organic component and a central iron atom. The organic component, called protoporphyrin, is made up of four pyrrole rings linked by methene bridges to form a tetrapyrrole ring. Four methyl groups, two vinyl groups, and two propionate side chains are attached.

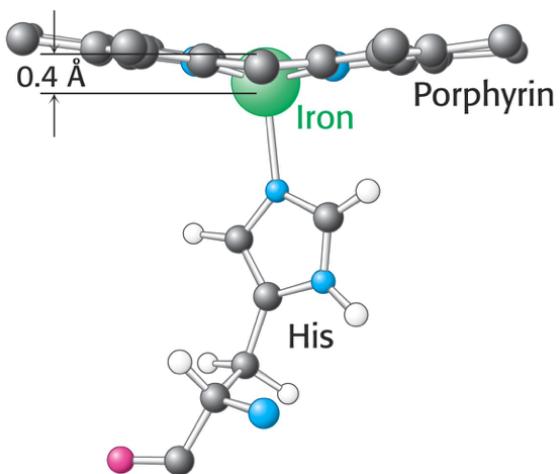
The haem Fe(II) ion has 6 potential ligand positions
(octahedrally coordinated)



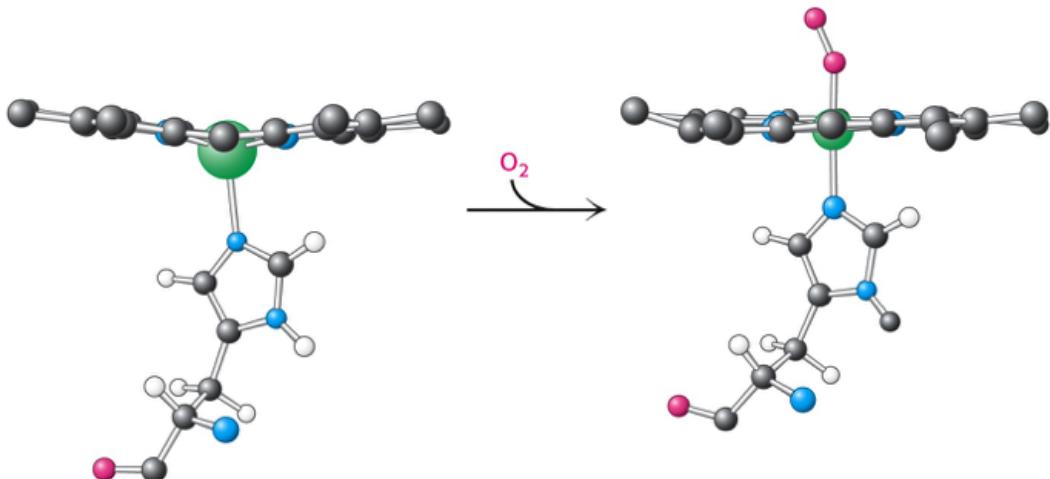
Oxygenation changes the colour (electronic state) of the haem



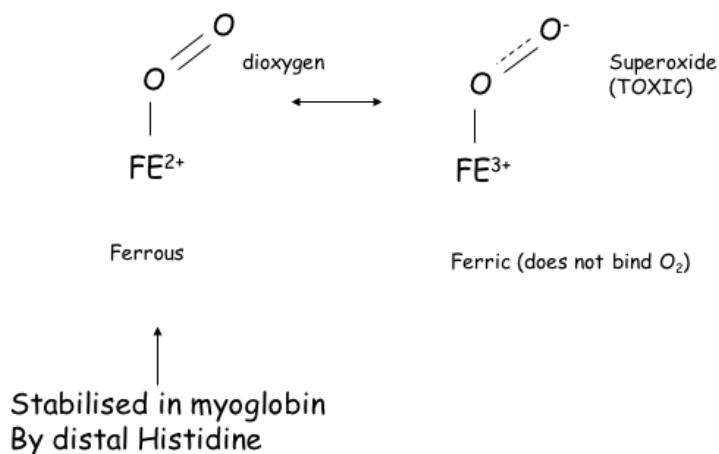
The iron atom lies in the center of the protoporphyrin, bonded to the four pyrrole nitrogen atoms. Although the heme-bound iron can be in either the ferrous (Fe^{2+}) or ferric (Fe^{3+}) oxidation state, only the Fe^{2+} state is capable of binding oxygen. The iron ion can form two additional bonds, one on each side of the heme plane. These binding sites are called the fifth and sixth coordination sites. In myoglobin, the fifth coordination site is occupied by the imidazole ring of a histidine residue from the protein. This histidine is referred to as the proximal histidine.



Oxygen binding occurs at the sixth coordination site. In deoxymyoglobin, this site remains unoccupied. The iron ion is slightly too large to fit into the well-defined hole within the porphyrin ring; it lies approximately 0.4 \AA outside the porphyrin plane. Binding of the oxygen molecule at the sixth coordination site substantially rearranges the electrons within the iron so that the ion becomes effectively smaller, allowing it to move within the plane of the porphyrin.



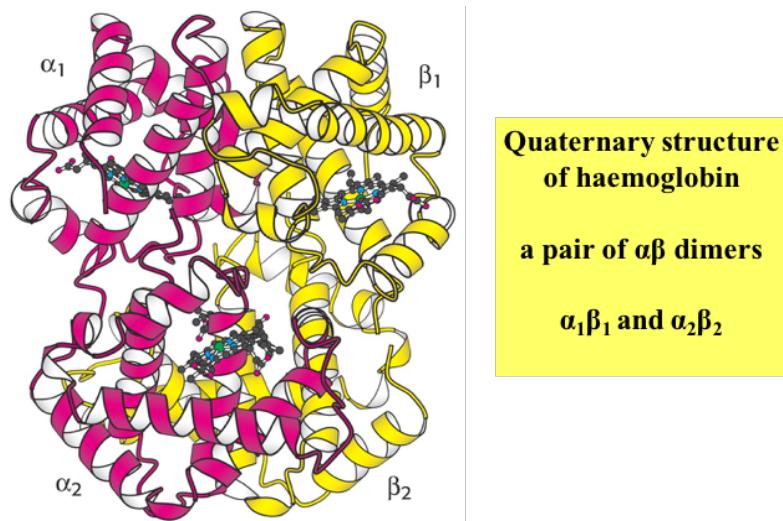
Resonance structures of bound oxygen



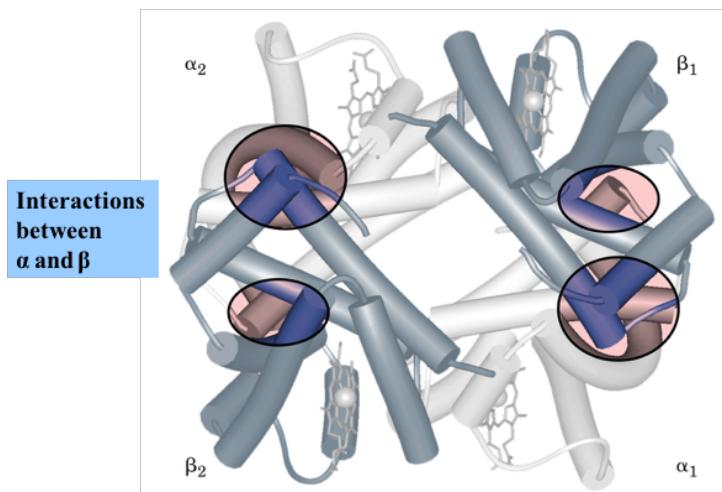
Oxygen binding to iron in heme is accompanied by the partial transfer of an electron from the ferrous ion to oxygen. In many ways, the structure is best described as a complex between ferric ion (Fe^{3+}) and superoxide anion (O_2^-). It is crucial that oxygen, when it is released, leaves as dioxygen rather than superoxide, for two important reasons. First, superoxide and other species generated from it are reactive oxygen species that can be damaging to many biological materials. Second, release of superoxide would leave the iron ion in the ferric state. This species, termed metmyoglobin, does not bind oxygen. Thus, potential oxygen- storage capacity is lost. Features of myoglobin stabilize the oxygen complex such that superoxide is less likely to be released. In particular, the binding pocket of myoglobin includes an additional histidine residue (termed the distal histidine) that donates a hydrogen bond to the bound oxygen molecule.

HAEMOGLOBIN

Hemoglobin consists of four polypeptide chains, two identical chains and two identical chains. Each of the subunits consists of a set of α helices in the same arrangement as the α helices in myoglobin. The recurring structure is called a globin fold. Consistent with this structural similarity, alignment of the amino acid sequences of the α and β chains of human hemoglobin with those of sperm whale myoglobin yields 25% and 24% identity, respectively, and good conservation of key residues such as the proximal and distal histidines. Thus, the α and β chains are related to each other and to myoglobin by divergent evolution.



Strong interactions between α₁β₁ and α₂β₂ interfaces



The hemoglobin tetramer, referred to as hemoglobin A (HbA), is best described as a pair of identical dimers (α₁β₁ and α₂β₂) that associate to form the tetramer. In deoxyhemoglobin, these ab dimers are linked by an extensive interface, which includes the carboxyl terminus of each chain. The heme groups are well separated in the tetramer by iron–iron distances ranging from 24 to 40 Å.

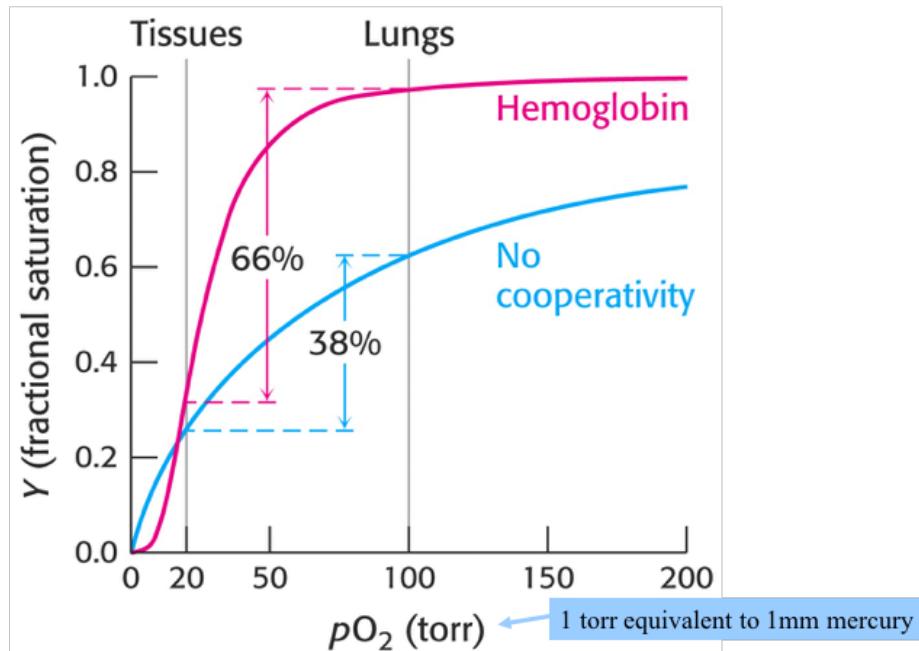
Myoglobin and haemoglobin are examples of divergent evolution

HAEMOGLOBIN BINDS OXYGEN COOPERATIVELY

We can determine the oxygen-binding properties of each of these proteins by observing its oxygen-binding curve, a plot of the fractional saturation versus the concentration of oxygen. The fractional saturation, Y , is defined as the fraction of possible binding sites that contain bound oxygen. The value of Y can range from 0 (all sites empty) to 1 (all sites filled). The concentration of oxygen is most conveniently measured by its partial pressure, pO_2 . For myoglobin, a binding curve indicating a simple chemical equilibrium is observed. Notice that the curve rises sharply as pO_2 increases and then levels off. Half-saturation of the binding sites, referred to as P_{50} (for 50% saturated), is at the relatively low value of 2 torr (mm Hg), indicating that oxygen binds with high affinity to myoglobin.

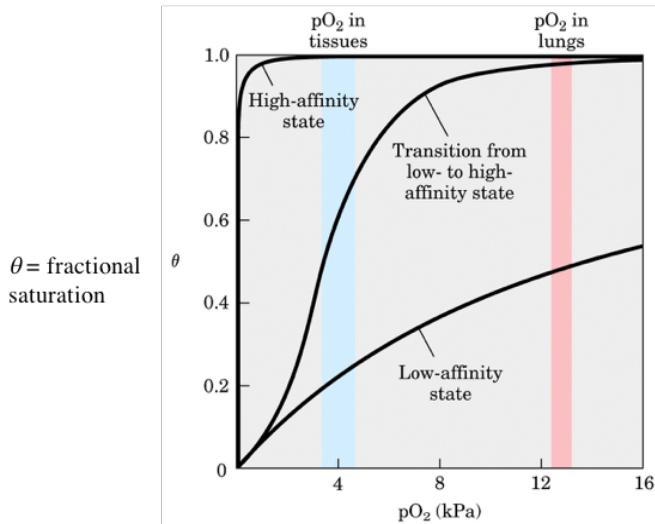
In contrast, the oxygen-binding curve for hemoglobin in red blood cells shows some remarkable features. It does not look like a simple binding curve such as that for myoglobin; instead, it resembles an "S." Such curves are referred to as sigmoid because of their S-like shape. In addition, oxygen binding for hemoglobin ($P_{50} = 26$ torr) is significantly weaker than that for myoglobin.

Binding of oxygen to haemoglobin shows cooperativity

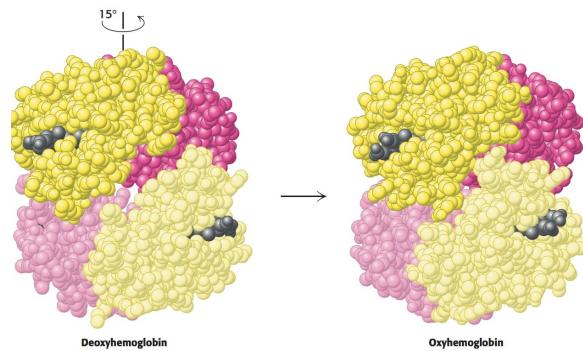


A sigmoid binding curve indicates that a protein shows a special binding behaviour. For haemoglobin, this shape suggests that the binding of oxygen at one site within the haemoglobin tetramer increases the likelihood that oxygen binds at the remaining unoccupied sites. Conversely, the unloading of oxygen at one heme facilitates the unloading of oxygen at the others. This sort of binding behaviour is referred to as cooperative, because the binding reactions at individual sites in each hemoglobin molecule are not independent of one another.

Sigmoid binding curve: a mixture of a low and high affinity states



Haemoglobin undergoes substantial changes in quaternary structure on oxygen binding: the a1b1 and a2b2 dimers rotate approximately 15 degrees with respect to one another (Figure 7.11). The dimers themselves are relatively unchanged, although there are localized conformational shifts. Thus, the interface between the a1b1 and a2b2 dimers is most affected by this structural transition. In particular, the a1b1 and a2b2 dimers are free to move with respect to one another in the oxygenated state than they are in the deoxygenated state.



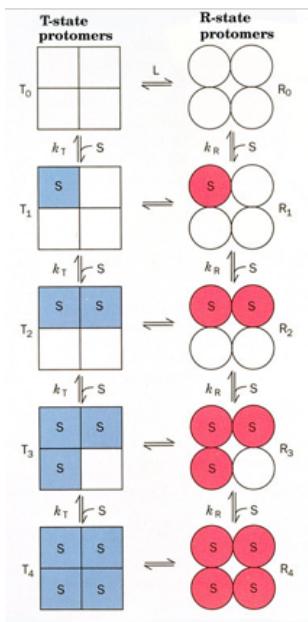
The quaternary structure observed in the deoxy form of haemoglobin, deoxyhemoglobin, is often referred to as the T (for tense) state because it is quite constrained by subunit–subunit interactions. The quaternary structure of the fully oxygenated form, oxyhemoglobin, is referred to as the R (for relaxed) state. In light of the observation that the R form of hemoglobin is less constrained, the tense and relaxed designations seem particularly apt. Importantly, in the R state, the oxygen-binding sites are free of strain and are capable of binding oxygen with higher affinity than are the sites in the T state. By triggering the shift of the hemoglobin tetramer from the T state to the R state, the binding of oxygen to one site increases the binding affinity of other sites.

MODELS TO EXPLAIN COOPERATIVITY

In the concerted model, also known as the MWC model after Jacques Monod, Jeffries Wyman, and Jean-Pierre Changeux, who first proposed it, the overall assembly can exist only in two forms: the T state and the R state. The binding of ligands simply shifts the equilibrium between these two states. Thus, as a hemoglobin tetramer binds each oxygen molecule, the probability that the tetramer is in the R state increases. Deoxyhemoglobin tetramers are almost exclusively in the T state. However, the binding of oxygen to one site in the molecule shifts the equilibrium toward the R state. If a molecule assumes the R quaternary structure, the oxygen affinity of its sites increases. Additional oxygen molecules are now more likely to bind to the three unoccupied sites. Thus, the binding curve is shallow at low oxygen concentrations when all of the molecules are in the T state, becomes steeper as the fraction of molecules in the R state increases, and flattens out again when all of the sites within the R-state molecules become filled. These events produce the sigmoid binding curve so important for efficient oxygen transport.

In the concerted model, each tetramer can exist in only two states, the T state and the R state.

Symmetry or concerted model of Monod, Wyman and Changeux (MWC)



Rules:

- Oligomeric
- Two forms (R and T) in equilibrium

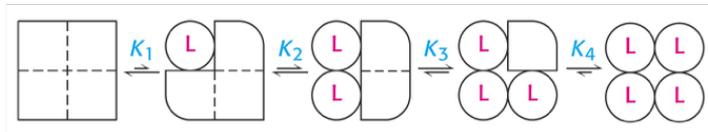
R	Relaxed
T	Tense
- T is dominant form in absence of ligand (L, allosteric constant = $[T]/[R]$)
- T state has lower affinity for ligand
- Molecular symmetry in the OLIGOMER is conserved (either all T or all R)

For enzymes:
either K_m affected (K system)
or V_{max} (V system)

In an alternative model, the sequential model, the binding of a ligand to one site in an assembly increases the binding affinity of neighboring sites without inducing a full conversion from the T into the R state .

Is the cooperative binding of oxygen by haemoglobin better described by the concerted or the sequential model? Neither model in its pure form fully accounts for the behaviour of haemoglobin. Instead, a combined model is required. Haemoglobin behaviour is concerted in that the tetramer with three sites occupied by oxygen is almost always in the quaternary structure associated with the R state. The remaining open binding site has an affinity for oxygen more than 20-fold greater than that of fully deoxygenated haemoglobin binding its first oxygen. However, the behaviour is not fully concerted, because haemoglobin with oxygen bound to only one of four sites remains primarily in the T-state quaternary structure. Yet, this molecule binds oxygen three times as strongly as does fully deoxygenated hemoglobin, an observation consistent only with a sequential model. These results highlight the fact that the concerted and sequential models represent idealized limiting cases, which real systems may approach but rarely attain.

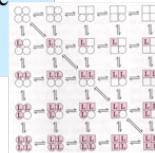
Sequential model of Koshland, Némethy and Filmer



Rules:

- Only one conformational state in absence of ligand
- Conformational change is sequential upon binding of ligands
- Interactions between subunits can be positive or negative
- Different dissociation constants ($K_1, K_2 \dots K_n$)

Positive cooperativity binding affinity improves
Negative cooperativity binding affinity declines



MEASURING THE COOPERATIVITY

The Hill coefficient: a measure of cooperativity

$$X + nL = X(L)_n$$

(Derivation)

see page 199 Stryer; Page 326 Voet & Voet

$$\Theta = [L]^n / ([L]^n + [L_{50}]^n)$$

$$(\Theta/1-\Theta) = [L]^n / [L_{50}]^n$$

$$\log(\theta/1-\theta) = n \log [L]_{eq} - \log K_d$$

HILL EQUATION

HILL COEFFICIENT

$$\log(\theta/1-\theta) \text{ vs } \log [L]_{eq}$$

HILL PLOT

L is ligand- Oxygen

K_d = dissociation constant

The Hill coefficient: a measure of cooperativity

$$\log(\theta/1-\theta) = n \log [L]_{eq} - \log K_d$$

HILL EQUATION

HILL COEFFICIENT

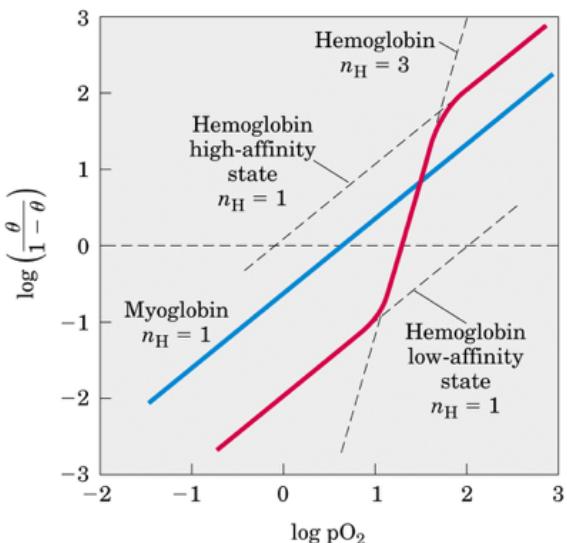
$$\log(\theta/1-\theta) \text{ vs } \log [L]_{eq}$$

HILL PLOT

L is ligand- Oxygen

K_d = dissociation constant

Hill Plots for myoglobin and haemoglobin



n_H - measure of cooperativity

<1 negative cooperativity
(binding of ligand impedes binding of others)

=1 no cooperativity

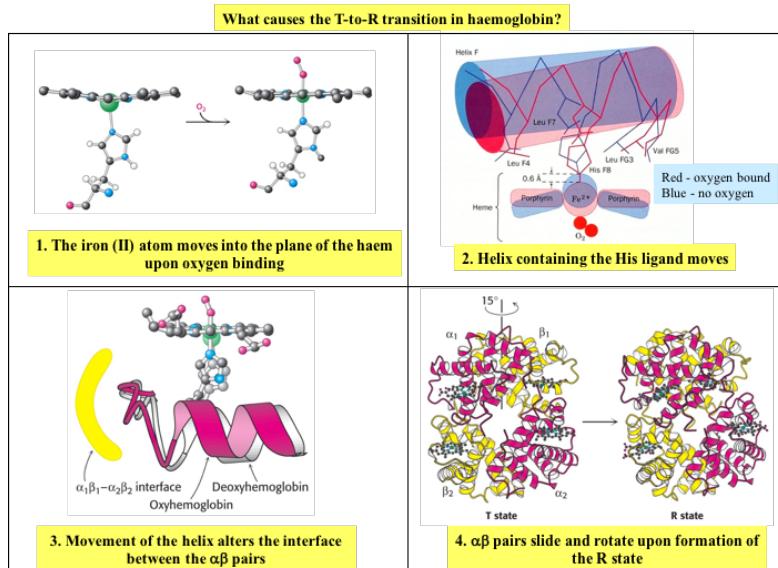
>1 positive cooperativity
(binding of ligand promotes binding of others)

$$\log(\theta/1-\theta) = n_H \log [L]_{eq} - \log K_d$$

STRUCTURAL CHANGES IN THE T-R TRANSITION

As in myoglobin, oxygen binding causes each iron atom in haemoglobin to move from outside the plane of the porphyrin into the plane. When the iron atom moves, the histidine residue bound in the fifth coordination site moves with it. This histidine residue is part of an α helix, which also moves. The carboxyl terminal end of this α helix lies in the interface between the two ab dimers. The change in position of the carboxyl terminal end of the helix favors the T-to-R transition. Consequently, the structural transition at the iron ion in one subunit is directly transmitted to the other subunits. The rearrangement of the dimer interface provides a pathway for communication between subunits, enabling the cooperative binding of oxygen.

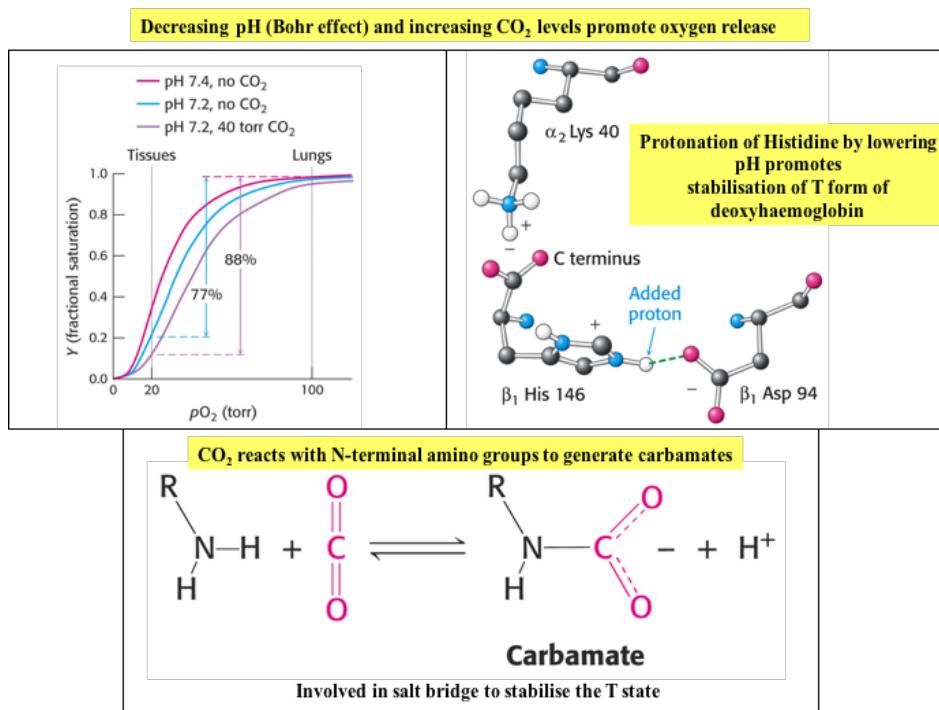
The movement of the iron ion on oxygenation brings the iron-associated histidine residue toward the porphyrin ring. The associated movement of the histidine-containing α helix alters the interface between the ab dimers, instigating other structural changes.



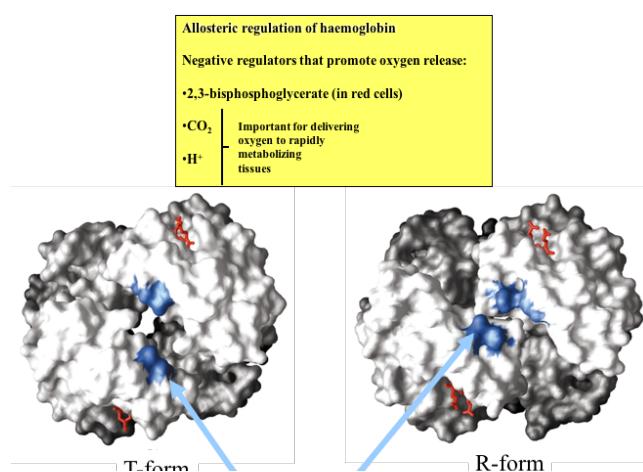
OTHER FACTORS AFFECTING OXYGEN BINDING

The binding of oxygen to haemoglobin can be dramatically altered by a small group of substances called allosteric effectors. Hydrogen ions (protons), carbon dioxide, and 2,3-bisphosphoglycerate are effectors that can promote the release of oxygen by favouring the deoxygenated form of haemoglobin. Since these allosteric effectors bind to sites that are specific to each kind of compound, their effects are cumulative.

Hydrogen ions and carbon dioxide are found in high concentrations around actively metabolizing tissues. In the capillaries, the environment favours the release of oxygen from haemoglobin and the binding of these allosteric effectors. The overall result is to facilitate oxygen release into blood plasma and subsequent uptake of oxygen by the high affinity myoglobin in the tissues. The specific reactions of the hydrogen ions and carbon dioxide with haemoglobin causing the release of additional oxygen is called the Bohr effect.



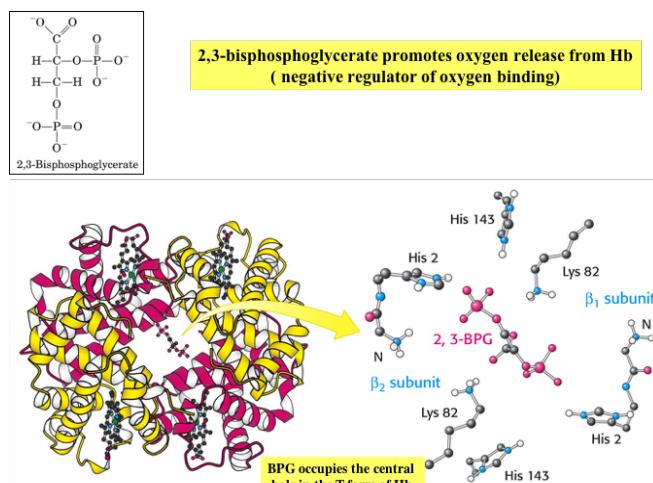
The reactions of the Bohr effect are reversible. When deoxygenated haemoglobin returns to the lungs, the concentration of the hydrogen ions and the partial pressure of carbon dioxide is low. This causes these compounds to be released from haemoglobin. The carbon dioxide is expelled out of the body through expired air. So haemoglobin not only carries oxygen to the cells, it also carries waste products from the cells to the lungs, eventually to be eliminated out of the body.



2,3-BPG

In addition to hydrogen ions and carbon dioxide, a very important allosteric effector is 2,3-bisphosphoglycerate (2,3-BPG). It is a small, organic molecule that is synthesized in red blood cells from 1,3-BPG, an intermediate in glycolysis. This "costs" the red blood cell 1 ATP that it would have gained from converting 1,3-BPG to 3-phosphoglycerate, but 2,3-BPG has a major and critical effect on the affinity of haemoglobin for oxygen.

BPG affects oxygen binding affinity by binding in a small cavity at the centre axis of deoxygenated haemoglobin. In oxygenated haemoglobin, this cavity is too small to effectively accommodate 2,3-BPG. When bound, 2,3-BPG stabilizes the deoxygenated conformation of haemoglobin, greatly diminishing the binding of oxygen and facilitating oxygen unloading to actively respiring tissues. At high altitude, when the proportion of oxygen in the atmosphere is lower and hence oxygen is harder to deliver to the tissues, the synthesis of 2,3-BPG is upregulated significantly. It takes about 24 hours for 2,3-BPG levels to rise, and over longer periods of time, the levels continue to increase. This is why athletes can train at high altitudes to temporarily increase their aerobic capacity.



The binding of 2,3-BPG to hemoglobin has other crucial physiological consequences. The globin gene expressed by human fetuses differs from that expressed by adults; fetal hemoglobin tetramers include two α chains and two γ chains. The γ chain, a result of a gene duplication, is 72% identical in amino acid sequence with the β chain. One noteworthy change is the substitution of a serine residue for His 143 in the β chain, part of the 2,3-BPG-binding site. This change removes two positive charges from the 2,3-BPG-binding site (one from each chain) and reduces the affinity of 2,3-BPG for fetal hemoglobin. Consequently, the oxygen-binding affinity of fetal hemoglobin is higher than that of maternal (adult) hemoglobin. This difference in oxygen affinity allows oxygen to be effectively transferred from maternal to fetal red blood cells. We have here an example in which gene duplication and specialization produced a ready solution to a biological challenge—in this case, the transport of oxygen from mother to fetus.

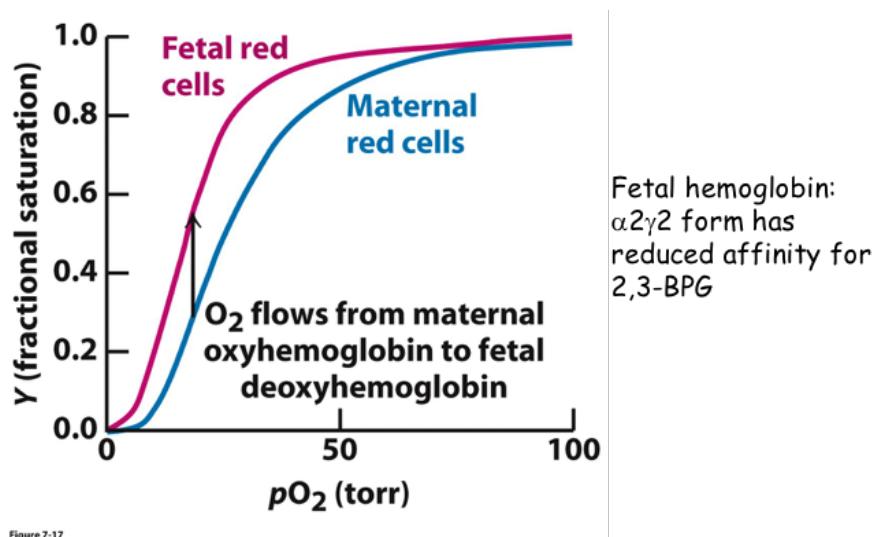


Figure 7-17
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