9 Regulation of Enzymes

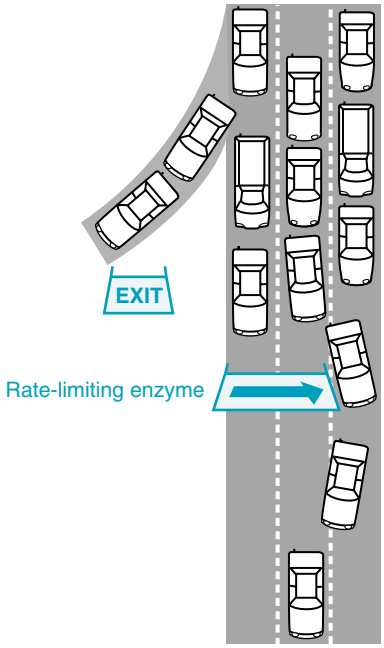


Fig. 9.1. The flux of substrates down a metabolic pathway is analogous to cars traveling down a highway. The rate-limiting enzyme is the portion of the highway that is narrowed to one lane by a highway barrier. This single portion of the highway limits the rate at which cars can arrive at their final destination miles later. Cars will back up before the barrier (similar to the increase in concentration of a precursor when a rate-limiting enzyme is inhibited). Some cars may exit and take an alternate route (similar to precursors entering another metabolic pathway.) Moving the barrier just a little to open an additional lane is like activating a rate-limiting enzyme; it increases flow through the entire length of the pathway.

In the body, thousands of diverse enzymes are regulated to fulfil their individual functions without waste of dietary components. Thus, with changes in our physiologic state, time of eating, environment, diet, or age, the rates of some enzymes must increase and others decrease. In this chapter, we describe the mechanisms for regulation of enzyme activity and the strategies employed to regulate the metabolic pathways in which they reside.

***Regulation matches function**. Changes in the rate of a metabolic pathway occur because at least one enzyme in that pathway, the **regulatory enzyme**, has been **activated** or **inhibited,** or the amount of enzyme has increased or decreased. Regulatory enzymes usually catalyze the **rate-limiting**, or slowest, step in the pathway, so that increasing or decreasing their rate changes the rate of the entire pathway (Fig. 9.1). The mechanisms used to regulate the rate-limiting enzyme in a pathway reflect the function of the pathway.*

***Substrate concentration**. The rate of all enzymes is dependent on substrate concentration. Enzymes exhibit **saturation kinetics**; their rate increases with increasing substrate concentration [S], but reaches a maximum velocity ($\boldsymbol{V_{max}}$) when the enzyme is saturated with substrate. For many enzymes, the **Michaelis-Menten equation** describes the relationship between $\boldsymbol{v_i}$ (the initial velocity of a reaction), [S], V_{max}, and the $\boldsymbol{K_m}$ (the substrate concentration at which $v_i = ½\ V_{max}$).*

***Reversible inhibition**. Enzymes are reversibly inhibited by **structural analogs** and **products**. These inhibitors are classified as **competitive**, **noncompetitive**, or **uncompetitive,** depending on their effect on formation of the **enzyme–substrate complex**.*

***Allosteric enzymes**. **Allosteric activators** or **inhibitors** are compounds that bind at sites other than the active catalytic site and regulate the enzyme through **conformational changes** affecting the catalytic site.*

***Covalent modification**. Enzyme activity also may be regulated by a covalent modification, such as **phosphorylation** of a serine, threonine, or tyrosine residue by a **protein kinase**.*

***Protein–protein interactions**. Enzyme activity can be modulated through the reversible binding of a **modulator protein**, such as **Ca^{2+} calmodulin**. **Monomeric G proteins** (GTP-binding proteins) activate target proteins through reversible binding.*

***Zymogen cleavage**. Some enzymes are synthesized as inactive precursors, called zymogens, that are activated by **proteolysis** (e.g., the digestive enzyme chymotrypsin).*

***Changes in enzyme concentration**. The concentration of an enzyme can be regulated by changes in the rate of enzyme synthesis (e.g., induction of gene transcription) or the rate of degradation.*

***Regulation of metabolic pathways**. The regulatory mechanisms for the rate-limiting enzyme of a pathway always reflects the **function of the pathway in a particular tissue**. In **feedback regulation,** the end product of a pathway directly or indirectly controls its own rate of synthesis; in **feedforword regulation**, the*

substrate controls the rate of the pathway. ***Biosynthetic*** *and* ***degradative*** *pathways are controlled through different but* ***complementary regulation****. Pathways are also regulated through* ***compartmentation*** *of enzymes.*

THE WAITING ROOM

Al Martini is a 44-year-old man who has been an alcoholic for the past 5 years. He was recently admitted to the hospital for congestive heart failure (see Chapter 8). After being released from the hospital, he continued to drink. One night he arrived at a friend's house at 7:00 P.M. Between his arrival and 11:00 P.M., he drank four beers and five martinis (for a total ethanol consumption of 9.5 oz). His friends encouraged him to stay an additional hour and drink coffee to sober up. Nevertheless, he ran his car off the road on his way home. He was taken to the emergency room of the local hospital and arrested for driving under the influence of alcohol. His blood alcohol concentration at the time of his arrest was 240 mg/dL, compared with the legal limit of ethanol for driving of 80 mg/dL.

Ann O'Rexia, a 23-year old woman, 5 feet 7 inches tall, is being treated for anorexia nervosa (see Chapters 1–3). She has been gaining weight, and is now back to 99 lb from a low of 85 lb. Her blood glucose is still below normal (fasting blood glucose of 72 mg/dL, compared with a normal range of 80-100 mg/dL). She complains to her physician that she feels tired when she jogs, and she is concerned that the "extra weight" she has gained is slowing her down.

Although the regulation of metabolic pathways is an exceedingly complex subject, dealt with in most of the subsequent chapters of this text, a number of common themes are involved. Physiologic regulation of a metabolic pathway depends on the ability to alter flux through the pathway by activating the enzyme catalyzing the rate-limiting step in the pathway (see Fig. 9.1). The type of regulation employed always reflects the function of the pathway and the need for that pathway in a particular tissue or cell type. Pathways producing a necessary product are usually feedback-regulated through a mechanism directly or indirectly involving the concentration of product (e.g., allosteric inhibition or induction/repression of enzyme synthesis). The concentration of product signals when enough of the product has been synthesized. Storage and toxic disposal pathways are usually regulated directly or indirectly through a feedforward mechanism reflecting the availability of precursor. Regulatory enzymes are often tissue-specific isozymes whose properties reflect the different functions of a pathway in particular tissues. Pathways are also regulated through compartmentation, collection of enzymes with a common function within a particular organelle or at a specific site in the cell.

The mechanisms employed to regulate enzymes have been organized into three general categories: regulation by compounds that bind reversibly in the active site (including dependence of velocity on substrate concentration and product levels); regulation by changing the conformation of the active site (including allosteric regulators, covalent modification, protein–protein interactions, and zymogen cleavage); and regulation by changing the concentration of enzyme (enzyme synthesis and degradation).

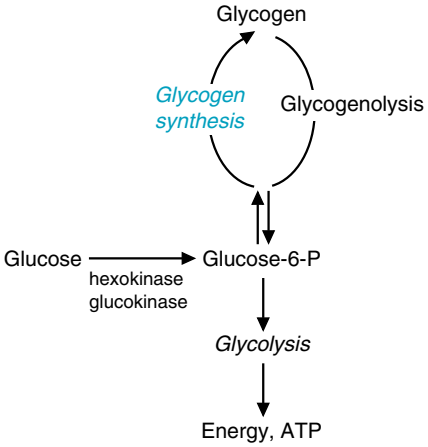
We will generally be using the pathways of fuel oxidation to illustrate the role of various mechanisms of enzyme regulation in metabolic pathways. Chapters 1 through 3 provide an overview of the names and functions of these pathways, including the TCA cycle, glycolysis, glycogen synthesis, glycogenolysis, and fatty acid oxidation.

Al Martini was not able to clear his blood ethanol rapidly enough to stay within the legal limit for driving. Ethanol is cleared from the blood at about ½ ounce/hr (15 mg/dL per hour). Liver metabolism accounts for more than 90% of ethanol clearance from the blood. The major route of ethanol metabolism in the liver is the enzyme liver alcohol dehydrogenase (ADH), which oxidizes ethanol to acetaldehyde with generation of NADH.

$$\text{Ethanol} + NAD^+ \rightarrow \text{Acetaldehyde} + NADH + H^+$$

The multienzyme complex MEOS (**m**icrosomal **e**thanol **o**xidizing **s**ystem), which is also called cytochrome P450-2E1, provides an additional route for ethanol oxidation to acetaldehyde in the liver.

One of the fuels used by **Ann O' Rexia**'s skeletal muscles for jogging is glucose, which is converted to glucose 6-phosphate (glucose 6-P) by the enzymes hexokinase (HK) and glucokinase (GK). Glucose 6-P is metabolized in the pathway of glycolysis to generate ATP. This pathway is feedback regulated, so that as her muscles use ATP, the rate of glycolysis will increase to generate more ATP.



When she is resting, her muscles and liver will convert glucose 6-phosphate to glycogen (a fuel storage pathway, shown in blue). Glycogen synthesis is feed-forward regulated by the supply of glucose and by insulin and other hormones that signal glucose availability.

Glycogenolysis (glycogen degradation) is activated during exercise to supply additional glucose 6-P for glycolysis. Unless Ann consumes sufficient calories, her glycogen stores will not be replenished after exercise, and she will tire easily.

I. REGULATION BY SUBSTRATE AND PRODUCT CONCENTRATION

A. Velocity and Substrate Concentration

The velocity of all enzymes is dependent on the concentration of substrate. This dependence is reflected in conditions such as starvation, in which a number of pathways are deprived of substrate. In contrast, storage pathways (e.g., glucose conversion to glycogen in the liver) and toxic waste disposal pathways (e.g., the urea cycle, which prevents NH_4^+ toxicity by converting NH_4^+ to urea) are normally regulated to speed up when more substrate is available. In the following sections, we use the Michaelis-Menten equation to describe the response of an enzyme to changes in substrate concentration and use glucokinase to illustrate the role of substrate supply in regulation of enzyme activity.

1. MICHAELIS-MENTEN EQUATION

The equations of enzyme kinetics provide a quantitative way of describing the dependence of enzyme rate on substrate concentration. The simplest of these equations, the Michaelis-Menten equation, relates the initial velocity (v_i) to the concentration of substrate [S] and the two parameters K_m and V_{max} (Equation 9.1) The V_{max} of the enzyme is the maximal velocity that can be achieved at an infinite concentration of substrate, and the K_m of the enzyme for a substrate is the concentration of substrate required to reach ½ V_{max}. The Michaelis-Menten model of enzyme kinetics applies to a simple reaction in which the enzyme and substrate form an enzyme–substrate complex (ES) that can dissociate back to the free enzyme and substrate. The initial velocity of product formation, v_i, is proportionate to the concentration of enzyme–substrate complexes [ES]. As substrate concentration is increased, the concentration of enzyme–substrate complexes increases, and the reaction rate increases proportionately.

The graph of the Michaelis-Menten equation (v_i as a function of substrate concentration) is a rectangular hyperbola that approaches a finite limit, V_{max}, as the fraction of total enzyme present as enzyme–substrate complex increases (Fig. 9.2).

The Michaelis-Menten equation relates the initial velocity of the reaction (v_i) to the concentration of enzyme substrate complexes (ES). This equation is derived for a reaction in which a single substrate, S, is converted to a single product, P. The enzyme (E) and S associate to form ES with the rate constant of k_1. The complex dissociates with the rate constant of k_2, or is converted to P with the rate constant k_3. Under conditions in which [S] >> [E], [P] is negligible, and the rate of conversion of ES to an enzyme-product complex is very fast, $v_i = k_3$ [ES]. The concentration of ES is a fraction of E_T, the total amount of enzyme present as ES and E.

Therefore,

$$v_i = k_3[ES] = \frac{k_3[ET][S]}{K_m + [S]}$$

Where $K_m = (k_2 + k_3)/k_1$. Substitution of V_{max} for k_3 [ET] gives the Michaelis-Menten equation (see Equation 9.1)

Equation 9.1. The Michaelis-Menten equation:

For the reaction

$$E + S \underset{k_2}{\overset{k_1}{\rightleftarrows}} ES \xrightarrow{k_3} P$$

the Michaelis-Menten equation is given by

$$v_i = \frac{V_{max}[S]}{K_m + [S]}$$

where $K_m = (k_2 + k_3)/k_1$
and $V_{max} = k_3\,[ET]$

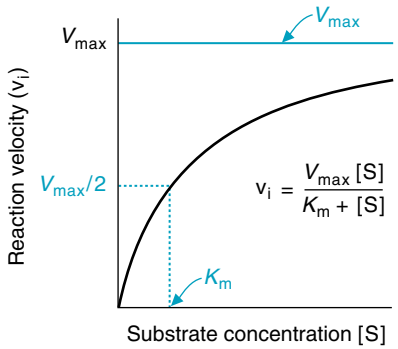


Fig. 9.2. A graph of the Michaelis-Menten equation. V_{max} (solid blue line) is the initial velocity extrapolated to infinite [S]. K_m (dashed blue line) is the concentration of S at which $v_i = V_{max}/2$.

At a hypothetical infinitely high substrate concentration, all of the enzyme molecules contain bound substrate, and the reaction rate is at $V_{max.}$ The approach to the finite limit of V_{max} is called saturation kinetics because velocity cannot increase any further once the enzyme is saturated with substrate. Saturation kinetics is a characteristic property of all rate processes dependent on the binding of a compound to a protein.

The K_m of the enzyme for a substrate is defined as the concentration of substrate at which v_i equals ½ V_{max}. The velocity of an enzyme is most sensitive to changes in substrate concentration over a concentration range below its K_m (see Fig. 9.2). At substrate concentrations less than 1/10th of the K_m, a doubling of substrate concentration nearly doubles the velocity of the reaction; at substrate concentrations 10 times the K_m, doubling the substrate concentration has little effect on the velocity.

The K_m of an enzyme for a substrate is related to the dissociation constant, K_d, which is the rate of substrate release divided by the rate of substrate binding. For example, a genetic mutation that decreases the rate of substrate binding to the enzyme decreases the affinity of the enzyme for the substrate and increases the K_d and K_m of the enzyme for that substrate. The higher the K_m, the higher is the substrate concentration required to reach ½ V_{max}.

MODY. Patients with maturity onset diabetes of the young (MODY) have a rare genetic form of diabetes mellitus in which the amount of insulin being secreted from the pancreas is too low, resulting in hyperglycemia. The disease is caused by mutations in the gene for pancreatic glucokinase (a closely related isozyme of liver glucokinase) that affect its kinetic properties (K_m or V_{max}). Glucokinase is part of the mechanism controlling release of insulin from the pancreas. A decreased activity of glucokinase results in lower insulin secretion for a given blood glucose level.

2. HEXOKINASE ISOZYMES HAVE DIFFERENT K_m VALUES FOR GLUCOSE

A comparison between the isozymes of hexokinase found in red blood cells and in the liver illustrates the significance of the K_m of an enzyme for its substrate. Hexokinase catalyses the first step in glucose metabolism in most cells, the transfer of a phosphate from ATP to glucose to form glucose 6-phosphate. Glucose 6-phosphate may then be metabolized in glycolysis, which generates energy in the form of ATP, or converted to glycogen, a storage polymer of glucose. Hexokinase I, the isozyme in red blood cells (erythrocytes), has a K_m for glucose of approximately 0.05 mM (Fig. 9.3). The isozyme of hexokinase, called glucokinase, which is found in the liver and pancreas, has a much higher K_m of approximately 5 to 6 mM. The red blood cell is totally dependent on glucose metabolism to meet its needs for ATP. At the low K_m of the erythrocyte hexokinase, blood glucose could fall drastically below its normal fasting level of approximately 5 mM, and the red blood cell could still phosphorylate glucose at rates near V_{max}. The liver, however, stores large amounts of "excess" glucose as glycogen or converts it to fat. Because glucokinase has a K_m of approximately 5 mM, the rate of glucose phosphorylation in the liver will tend to increase as blood glucose increases after a high-carbohydrate meal, and decrease as blood glucose levels fall. The high K_m of hepatic glucokinase thus promotes the storage of glucose as liver glycogen or as fat, but only when glucose is in excess supply.

As **Ann O'Rexia** eats a high carbohydrate meal, her blood glucose will rise to approximately 20 mM in the portal vein, and much of the glucose from her carbohydrate meal will enter the liver. How will the activity of glucokinase in the liver change as glucose is increased from 4 mM to 20 mM? (Hint: Calculate v_i as a fraction of V_{max} for both conditions, using a K_m for glucose of 5 mM and the Michaelis-Menten equation).

A: Glucokinase, which has a high K_m for glucose, phosphorylates glucose to glucose 6-phosphate about twice as fast after a carbohydrate meal than during fasting. Substitute the values for S and K_m into the Michaelis-Menten equation. The initial velocity will be 0.44 times V_{max} when blood glucose is at 4 mM and about 0.80 times V_{max} when blood glucose is at 20 mM. In the liver, glucose 6-phosphate is a precursor for both glycogen and fat synthesis. Thus, these storage pathways are partially regulated through a direct effect of substrate supply. They are also partially regulated through an increase of insulin and a decrease of glucagon, two hormones that signal the supply of dietary fuel.

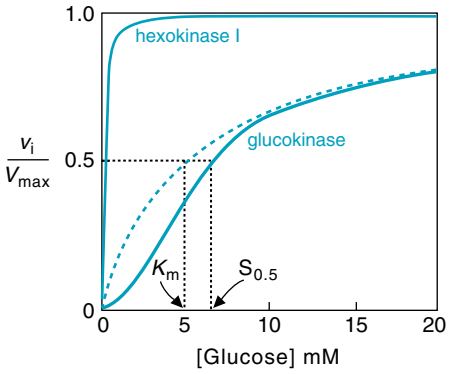


Fig. 9.3. A comparison between hexokinase I and glucokinase. The initial velocity (v_i) as a fraction of V_{max} is graphed as a function of glucose concentration. The plot for glucokinase (heavy blue line) is slightly sigmoidal (S-shaped), possibly because the rate of an intermediate step in the reaction is so slow that the enzyme does not follow Michaelis-Menten kinetics. The dashed blue line has been derived from the Michaelis-Menten equation fitted to the data for concentrations of glucose above 5 mM. For S-shaped curves, the concentration of substrate required to reach half V_{max}, or half-saturation, is sometimes called the $S_{0.5}$ or $K_{0.5}$, rather than K_m. At $v_i/V_{max} = 0.5$, the K_m is 5 mM, and the $S_{0.5}$ is 6.7 mM.

3. VELOCITY AND ENZYME CONCENTRATION

The rate of a reaction is directly proportional to the concentration of enzyme; if you double the amount of enzyme, you will double the amount of product produced per minute, whether you are at low or at saturating concentrations of substrate. This important relationship between velocity and enzyme concentration is not immediately apparent in the Michaelis-Menten equation because the concentration of total enzyme present (E_t) has been incorporated into the term V_{max} (that is, V_{max} is equal to the rate constant k_3 times E_t.) However, V_{max} is most often expressed as product produced per minute per milligram of enzyme and is meant to reflect a property of the enzyme that is not dependent on its concentration.

The use of V_{max} in the medical literature to describe the maximal rate at which a certain amount of tissue converts substrate to product can be confusing. The best way to describe an increase in enzyme activity in a tissue is to say that the maximal capacity of the tissue has increased. In contrast, the term k_{cat} has been developed to clearly describe the speed at which an enzyme can catalyse a reaction under conditions of saturating substrate concentration. The rate constant k_{cat}, the turnover number of the enzyme, has the units of min^{-1} (micromoles of product formed per minute divided by the micromoles of active site).

4. MULTISUBSTRATE REACTIONS

Most enzymes have more than one substrate, and the substrate binding sites overlap in the catalytic (active) site. When an enzyme has more than one substrate, the sequence of substrate binding and product release affect the rate equation. As a

The liver alcohol dehydrogenase most active in oxidizing ethanol has a very low K_m for ethanol of approximately 0.04 mM, and is at over 99% of its V_{max} at the legal limit of blood alcohol concentration for driving (80 mg/dL or about 17 mM). In contrast, the MEOS isozyme most active toward ethanol has a K_m of approximately 11 mM. Thus, MEOS makes a greater contribution to ethanol oxidation and clearance from the blood at higher ethanol levels than lower ones. Liver damage, such as cirrhosis, results partly from toxic byproducts of ethanol oxidation generated by MEOS. **Al Martini**, who has blood alcohol levels of 240 mg/dL (approximately 52 mM), is drinking enough to potentially cause liver damage, as well as his car accident and arrest for driving under the influence of alcohol. The various isozymes and polymorphisms of alcohol dehydrogenase and MEOS are discussed in more detail in Chapter 25.

consequence, an apparent value of K_m ($K_{m,app}$) depends on the concentration of cosubstrate or product present.

5. RATES OF ENZYME-CATALYZED REACTIONS IN THE CELL

Equations for the initial velocity of an enzyme-catalyzed reaction, such as the Michaelis-Menten equation, can provide useful parameters for describing or comparing enzymes. However, many multisubstrate enzymes, such as glucokinase, have kinetic patterns that do not fit the Michaelis-Menten model (or do so under nonphysiologic conditions). The Michaelis-Menten model is also inapplicable to enzymes present in a higher concentration than their substrates. Nonetheless, the term "K_m" is still used for these enzymes to describe the approximate concentration of substrate at which velocity equals $\frac{1}{2}\ V_{max}$.

B. Reversible Inhibition within the Active Site

One of the ways of altering enzyme activity is through compounds binding in the active site. If these compounds are not part of the normal reaction, they inhibit the enzyme. An inhibitor of an enzyme is defined as a compound that decreases the velocity of the reaction by binding to the enzyme. It is a reversible inhibitor if it is not covalently bound to the enzyme and can dissociate at a significant rate. Reversible inhibitors are generally classified as competitive, noncompetitive, or uncompetitive with respect to their relationship to a substrate of the enzyme. In most reactions, the products of the reaction are reversible inhibitors of the enzyme producing them.

1. COMPETITIVE INHIBITION

A competitive inhibitor "competes" with a substrate for binding at the enzyme's substrate recognition site and therefore is usually a close structural analog of the substrate (Fig. 9.4). An increase of substrate concentration can overcome competitive inhibition; when the substrate concentration is increased to a sufficiently high level, the substrate binding sites are occupied by substrate, and inhibitor molecules cannot bind. Competitive inhibitors, therefore, increase the apparent K_m

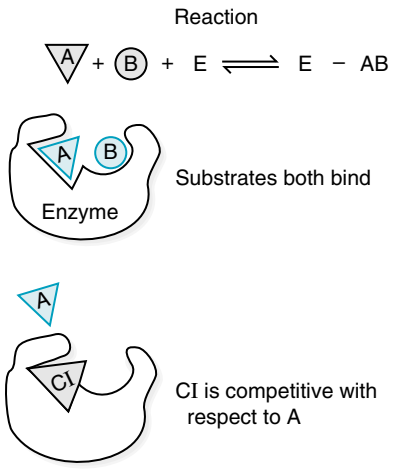


Fig. 9.4. Competitive inhibition with respect to substrate A. A and B are substrates for the reaction forming the enzyme substrate complex (E-AB). The enzyme has separate binding sites for each substrate, which overlap in the active site. The competitive inhibitor (CI) competes for the binding site of A, the substrate it most closely resembles.

Some of **Al Martini**'s problems have arisen from product inhibition of liver alcohol dehydrogenase by NADH. As ethanol is oxidized in liver cells, NAD^+ is reduced to NADH and the NADH/NAD^+ ratio rises. NADH is an inhibitor of alcohol dehydrogenase, competitive with respect to NAD^+, so the increased NADH/NAD^+ ratio slows the rate of ethanol oxidation and ethanol clearance from the blood.

NADH is also a product inhibitor of enzymes in the pathway that oxidizes fatty acids. Consequently, these fatty acids accumulate in the liver, eventually contributing to the alcoholic fatty liver.

of the enzyme ($K_{m,app}$) because they raise the concentration of substrate necessary to saturate the enzyme. They have no effect on V_{max}.

2. NONCOMPETITIVE AND UNCOMPETITIVE INHIBITION

If an inhibitor does not compete with a substrate for its binding site, the inhibitor is either a noncompetitive or uncompetitive inhibitor with respect to that particular substrate (Fig. 9.5). To illustrate noncompetitive inhibition, consider a multisubstrate reaction in which substrates A and B react to form a product. An inhibitor (NI) that is a structural analog of substrate B would fit into substrate B's binding site, but the inhibitor would be a noncompetitive inhibitor with regard to the other substrate, substrate A. An increase of A will not prevent the inhibitor from binding to substrate B's binding site. The inhibitor, in effect, lowers the concentration of the active enzyme and therefore changes the V_{max} of the enzyme. If the inhibitor has absolutely no effect on the binding of substrate A, it will not change the K_m for A (a pure noncompetitive inhibitor).

Some inhibitors, such as metals, might not bind at either substrate recognition site. In this case, the inhibitor would be noncompetitive with respect to both substrates.

An inhibitor that is uncompetitive with respect to a substrate will bind only to enzyme containing that substrate. Suppose, for example, that in Figure 9.5 an inhibitor that is a structural analog of B and binds to the B site could only bind to an enzyme that contains A. That inhibitor would be called uncompetitive with respect to A. It would decrease both the V_{max} of the enzyme and its apparent K_m for A.

3. SIMPLE PRODUCT INHIBITION IN METABOLIC PATHWAYS

All products are reversible inhibitors of the enzymes that produce them and may be competitive, noncompetitive, or uncompetitive relative to a particular substrate. Simple product inhibition, a decrease in the rate of an enzyme caused by the accumulation of its own product, plays an important role in metabolic pathways; it prevents one enzyme in a sequence of reactions from generating a product faster than it can be used by the next enzyme in that sequence.

Product inhibition of hexokinase by glucose 6-phosphate conserves blood glucose for tissues needing it. Tissues take up glucose from the blood and phosphorylate it to glucose 6-phosphate, which can then enter a number of different pathways (including glycolysis and glycogen synthesis). As these pathways become more active, glucose 6-phosphate concentration decreases, and the rate of hexokinase increases. When these pathways are less active, glucose 6-phosphate concentration increases, hexokinase is inhibited, and glucose remains in the blood for other tissues.

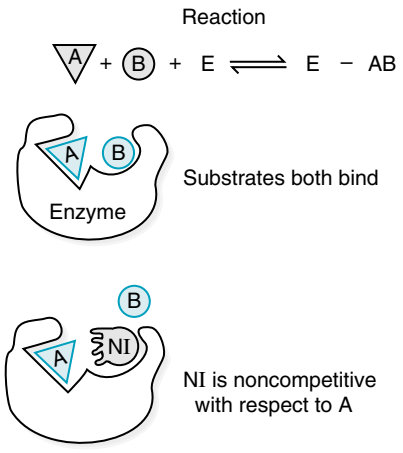


Fig. 9.5. NI is a noncompetitive inhibitor with respect to substrate A. A can still bind to its binding site in the presence of NI. However, NI is competitive with respect to B because it binds to the B binding site. In contrast, an inhibitor that is uncompetitive with respect to A might also resemble B, but it could only bind to the B site after A is bound.

III. REGULATION THROUGH CONFORMATIONAL CHANGES

In substrate response and product inhibition, the rate of the enzyme is affected principally by the binding of a substrate or a product within the catalytic site. Most rate-limiting enzymes are also controlled through regulatory mechanisms that change the conformation of the enzyme in a way that affects the catalytic site. These regulatory mechanisms include: (1) allosteric activation and inhibition; (2) phosphorylation or other covalent modification; (3) protein–protein interactions between regulatory and catalytic subunits, or between two proteins; and (4) proteolytic cleavage. These types of regulation can rapidly change an enzyme from an inactive form to a fully active conformation.

In the sections below, we describe the general characteristics of these regulatory mechanisms and illustrate the first three with glycogen phosphorylase, glycogen phosphorylase kinase, and protein kinase A.

A. Conformational Changes in Allosteric Enzymes

Allosteric activators and inhibitors (allosteric effectors) are compounds that bind to the allosteric site (a site separate from the catalytic site) and cause a conformational change that affects the affinity of the enzyme for the substrate. Usually an allosteric enzyme has multiple interacting subunits that can exist in active and inactive conformations, and the allosteric effector promotes or hinders conversion from one conformation to another.

1. COOPERATIVITY IN SUBSTRATE BINDING TO ALLOSTERIC ENZYMES

Allosteric enzymes usually contain two or more subunits and exhibit positive cooperativity; the binding of substrate to one subunit facilitates the binding of substrate to another subunit (Fig. 9.6). The first substrate molecule has difficulty in binding to the enzyme because all of the subunits are in the conformation with a low affinity for substrate (the taut "T" conformation) (see Chapter 7, section VII.B.). The first substrate molecule to bind changes its own subunit and at least one adjacent subunit to the high-affinity conformation (the relaxed "R" state.) In the example of the tetramer hemoglobin, discussed in Chapter 7, the change in one subunit facilitated changes in all four subunits, and the molecule generally changed to the new conformation in a concerted fashion. However, most allosteric enzymes follow a more stepwise (sequential) progression through intermediate stages (see Fig. 9.6)

2. ALLOSTERIC ACTIVATORS AND INHIBITORS

Allosteric enzymes bind activators at the allosteric site, a site physically separate from the catalytic site. The binding of an allosteric activator changes the conformation of the catalytic site in a way that increases the affinity of the enzyme for the substrate.

In general, activators of allosteric enzymes bind more tightly to the high-affinity R state of the enzyme than the T state (i.e., the allosteric site is open only in the R enzyme) (Fig. 9.7). Thus, the activators increase the amount of enzyme in the active state, thereby facilitating substrate binding in their own and other subunits. In contrast, allosteric inhibitors bind more tightly to the T state, so either substrate concentration or activator concentration must be increased to overcome the effects of the allosteric inhibitor. Allosteric inhibitors might have their own binding site on the enzyme, or they might compete with the substrate at the active site and prevent cooperativity. Thus, the term "allosteric inhibitor" is more generally applied to any inhibitor of an allosteric enzyme.

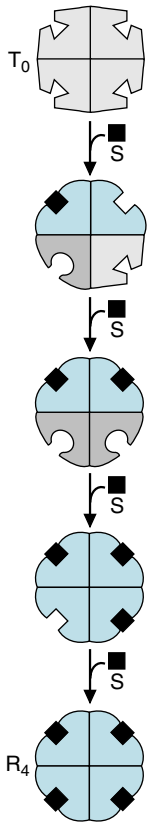


Fig. 9.6. A sequential model for an allosteric enzyme. The sequential model is actually the preferred path from the T_0 (taut, with 0 substrate bound) low-affinity conformation to the R_4 (relaxed, with four substrate molecules bound) conformation, taken from an array of all possible equilibrium conformations that differ by the conformation of only one subunit. The final result is a stepwise path in which intermediate conformations exist, and subunits may change conformations independently, depending on their geometric relationship to the subunits already containing bound substrate.

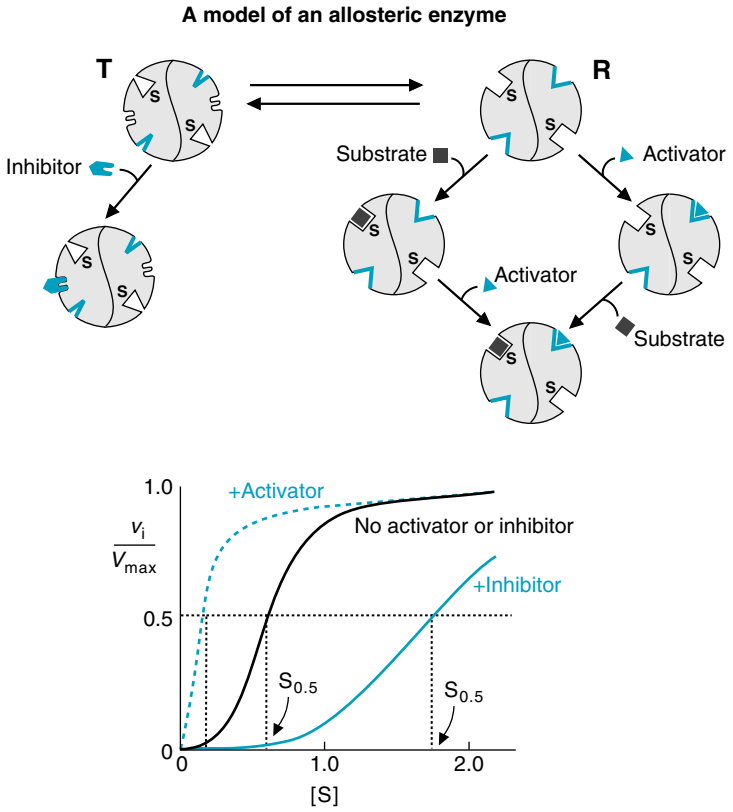


Fig. 9.7. Activators and inhibitors of an allosteric enzyme (simplified model). This enzyme has two identical subunits, each containing three binding sites: one for the substrate (s), one for the allosteric activator (blue triangle), and one for the allosteric inhibitor (two-pronged shape). The enzyme has two conformations, a relaxed active conformation (R) and an inactive conformation (T). The activator binds only to its activator site when the enzyme is in the R configuration. The inhibitor binding site is open only when the enzyme is in the T state.

A plot of velocity (v_i/V_{max}) versus substrate concentration reveals that binding of the substrate at its binding site stabilizes the active conformation so that the second substrate binds more readily, resulting in an S (sigmoidal)-shaped curve. The graph of v_i/V_{max} becomes hyperbolic in the presence of activator (which stabilizes the high-affinity R form), and more sigmoidal with a higher $S_{0.5}$ in the presence of inhibitor (which stabilizes the low-affinity form).

Some of the rate-limiting enzymes in the pathways of fuel oxidation (e.g., muscle glycogen phosphorylase in glycogenolysis, phosphofructokinase-1 in glycolysis and isocitrate dehydrogenase in the TCA cycle) are allosteric enzymes regulated by changes in the concentration of ADP or AMP, which are allosteric activators. The function of fuel oxidation pathways is the generation of ATP. When the concentration of ATP in a muscle cell begins to decrease, ADP and AMP increase; ADP activates isocitrate dehydrogenase, and AMP activates glycogen phosphorylase and phosphofructokinase-1. The response is very fast, and small changes in the concentration of activator can cause large changes in the rate of the reaction.

In the absence of activator, a plot of velocity versus substrate concentration for an allosteric enzyme usually results in a sigmoid or S-shaped curve (rather than the rectangular hyperbola of Michaelis-Menten enzymes) as the successive binding of substrate molecules activates additional subunits (see Fig. 9.7). In plots of velocity versus substrate concentration, the effect of an allosteric activator generally makes the sigmoidal S-shaped curve more like the rectangular hyperbola, with a substantial decrease in the $S_{0.5}$ (K_m) of the enzyme, because the activator changes all of the subunits to the high-affinity state. Such allosteric effectors are "K effectors"; they change the K_m but not the V_{max} of the enzyme. An allosteric inhibitor makes it more difficult for substrate or activators to convert the subunits to the most active conformation, and therefore inhibitors generally shift the curve to the right, either increasing the $S_{0.5}$ alone, or increasing it together with a decrease in the V_{max}.

3. ALLOSTERIC ENZYMES IN METABOLIC PATHWAYS

Regulation of enzymes by allosteric effectors provides several advantages over other methods of regulation. Allosteric inhibitors usually have a much stronger

effect on enzyme velocity than competitive, noncompetitive, and uncompetitive inhibitors in the active catalytic site. Because allosteric effectors do not occupy the catalytic site, they may function as activators. Thus, allosteric enzymes are not limited to regulation through inhibition. Furthermore, the allosteric effector need not bear any resemblance to substrate or product of the enzyme. Finally, the effect of an allosteric effector is rapid, occurring as soon as its concentration changes in the cell. These features of allosteric enzymes are often essential for feedback regulation of metabolic pathways by endproducts of the pathway or by signal molecules that coordinate multiple pathways.

B. Conformational Changes from Covalent Modification

1. PHOSPHORYLATION

The activity of many enzymes is regulated through phosphorylation by a protein kinase or dephosphorylation by a protein phosphatase (Fig. 9.8). Serine/threonine protein kinases transfer a phosphate from ATP to the hydroxyl group of a specific serine (and sometimes threonine) on the target enzyme; tyrosine kinases transfer a phosphate to the hydroxyl group of a specific tyrosine residue. Phosphate is a bulky, negatively charged residue that interacts with other nearby amino acid residues of the protein to create a conformational change at the catalytic site. The conformational change makes certain enzymes more active and other enzymes less active. The effect is reversed by a specific protein phosphatase that removes the phosphate by hydrolysis.

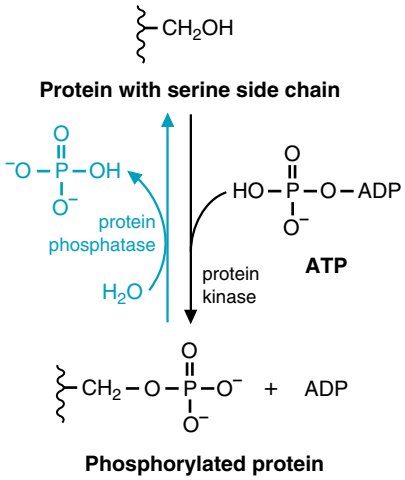


Fig. 9.8. Protein kinases and protein phosphatases.

2. MUSCLE GLYCOGEN PHOSPHORYLASE

Muscle glycogen phosphorylase, the rate-limiting enzyme in the pathway of glycogen degradation, degrades glycogen to glucose 1-phosphate. It is regulated by the allosteric activator AMP, which increases in the cell as ATP is used for muscular contraction (Fig. 9.9). Thus, a rapid increase in the rate of glycogen degradation to glucose 1-phosphate is achieved when an increase of AMP signals that more fuel is needed for ATP generation in the glycolytic pathway.

Glycogen phosphorylase also can be activated through phosphorylation by glycogen phosphorylase kinase. Either phosphorylation or AMP binding can change the enzyme to the same fully active conformation. The phosphate is removed by protein

When **Ann O'Rexia** begins to jog, AMP activates her muscle glycogen phosphorylase, which degrades glycogen to glucose 1-phosphate. This compound is converted to glucose 6-phosphate, which feeds into the glycolytic pathway to generate ATP for muscle contraction. As she continues to jog, her adrenaline (epinephrine) levels rise, producing the signal that activates glycogen phosphorylase kinase. This enzyme phosphorylates glycogen phosphorylase, causing it to become even more active than with AMP alone (see Fig. 9.9).

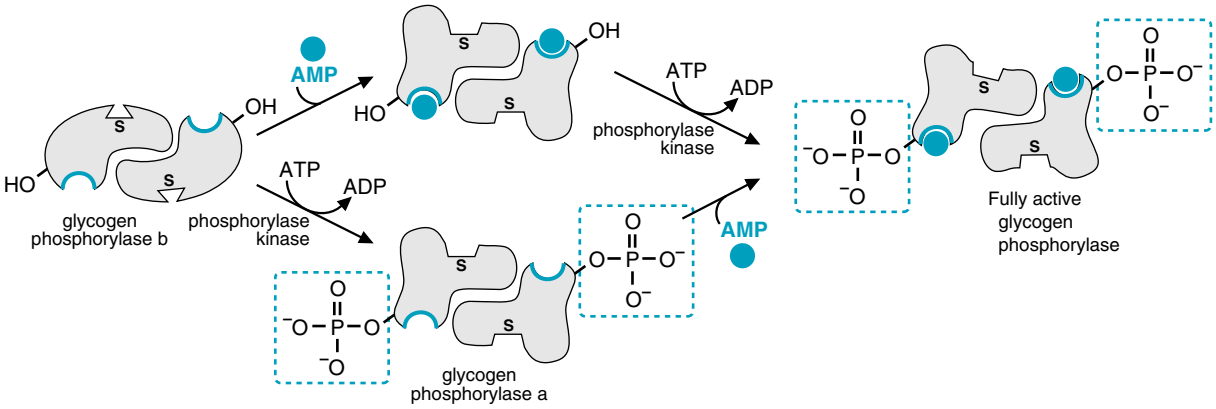


Fig. 9.9. Activation of muscle glycogen phosphorylase by AMP and by phosphorylation. Muscle glycogen phosphorylase is composed of two identical subunits. The substrate binding sites in the active catalytic site are denoted by S. AMP binds to the allosteric site, a site separate from the active catalytic site. Glycogen phosphorylase kinase can transfer a phosphate from ATP to one serine residue in each subunit. Either phosphorylation or binding of AMP causes a change in the active site that increases the activity of the enzyme. The first event at one subunit facilitates the subsequent events that convert the enzyme to the fully active form.

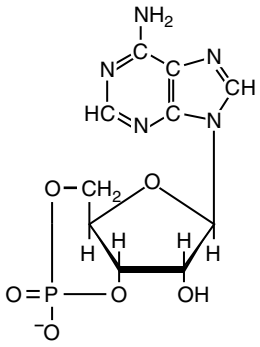


Fig. 9.10. Structure of cAMP (3′,5′ -cyclic AMP.) The phosphate group is attached to hydroxyl groups on both the 3rd (3′) and 5th (5′) carbons of ribose, forming a cycle.

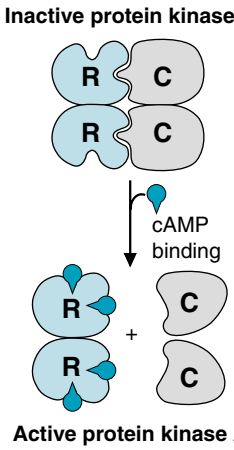


Fig. 9.11. Protein kinase A. When the regulatory subunits (R) of protein kinase A bind the allosteric activator, cAMP, they dissociate from the enzyme, thereby releasing active catalytic subunits (C).

phosphatase-1. Glycogen phosphorylase kinase links the activation of muscle glycogen phosphorylase to changes in the level of the hormone adrenaline in the blood. It is regulated through phosphorylation by protein kinase A and by activation of Ca^{2+}-calmodulin (a modulator protein) during contraction.

3. PROTEIN KINASE A

Some protein kinases, called dedicated protein kinases, are tightly bound to a single protein and regulate only the protein to which they are tightly bound. However, other protein kinases and protein phosphatases will simultaneously regulate a number of rate-limiting enzymes in a cell to achieve a coordinated response. For example, protein kinase A, a serine/threonine protein kinase, phosphorylates a number of enzymes that regulate different metabolic pathways. One of these enzymes is glycogen phosphorylase kinase (see Fig. 9.9).

Protein kinase A provides a means for hormones to control metabolic pathways. Adrenaline and many other hormones increase the intracellular concentration of the allosteric regulator 3′, 5′-cyclic AMP (cAMP), which is referred to as a hormonal second messenger (Fig. 9.10). cAMP binds to regulatory subunits of protein kinase A, which dissociate and release the activated catalytic subunits (Fig. 9.11). Dissociation of inhibitory regulatory subunits is a common theme in enzyme regulation. The active catalytic subunits phosphorylate glycogen phosphorylase and other enzymes at serine residues.

In the example shown in Figure 9.9, adrenaline indirectly increases cAMP, which activates protein kinase A, which phosphorylates phosphorylase kinase, which phosphorylates glycogen phosphorylase. The sequence of events in which one kinase phosphorylates another kinase is called a phosphorylation cascade. Because each stage of the phosphorylation cascade is associated with one enzyme molecule activating many enzyme molecules, the initial activating event is greatly amplified.

4. OTHER COVALENT MODIFICATIONS

A number of proteins are covalently modified by the addition of groups such as acetyl, ADP-ribose, or lipid moieties (see Chapter 6). These modifications may directly activate or inhibit the enzyme. However, they also may modify the ability of the enzyme to interact with other proteins or to reach its correct location in the cell.

C. Conformational Changes from Protein–Protein Interactions

Changes in the conformation of the active site also can be regulated by direct protein–protein interaction. This type of regulation is illustrated by Ca^{2+}-calmodulin and small (monomeric) G proteins.

1. THE CALCIUM-CALMODULIN FAMILY OF MODULATOR PROTEINS

Modulator proteins bind to other proteins and regulate their activity by causing a conformational change at the catalytic site or by blocking the catalytic site (steric hindrance). They are protein allosteric effectors that can either activate or inhibit the enzyme or protein to which they bind.

Ca^{2+}-calmodulin is an example of a dissociable modulator protein that binds to a number of different proteins and regulates their function. It also exists in the cytosol and functions as a Ca^{2+} binding protein (Fig. 9.12). The center of the symmetric molecule is a hinge region that bends as Ca^{2+}-calmodulin folds over the protein it is regulating.

One of the enzymes activated by Ca^{2+}-calmodulin is muscle glycogen phosphorylase kinase, which is also activated by protein kinase A (see Fig. 9.9). When a

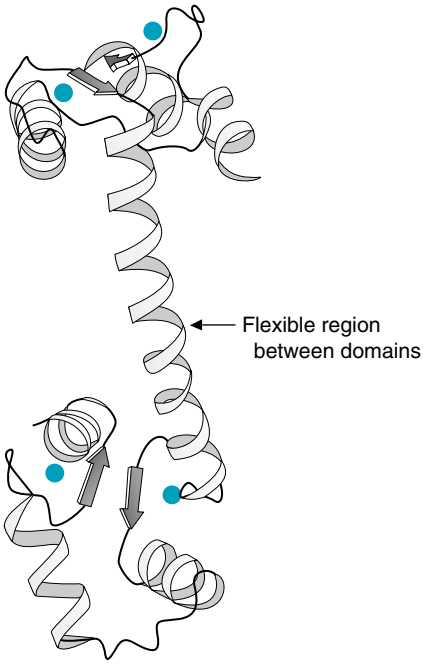


Fig. 9.12. Calcium-calmodulin has four binding sites for calcium (shown in blue). Each calcium forms a multiligand coordination sphere by simultaneously binding several amino acid residues on calmodulin. Thus, it can create large conformational changes in proteins when it binds. Calmodulin has a flexible region in the middle connecting the two domains.

neural impulse triggers Ca^{2+} release from the sarcoplasmic reticulum, Ca^{2+}binds to the calmodulin subunit of muscle glycogen phosphorylase kinase, which undergoes a conformational change. This activated kinase then phosphorylates glycogen phosphorylase, ultimately increasing the generation of ATP to supply energy for muscle contraction. Simultaneously, Ca^{2+} binds to troponin-C, a member of the Ca^{2+}-calmodulin superfamily that serves as a nondissociable regulatory subunit of troponin, a regulator of muscle contraction. Calcium binding to troponin prepares the muscle for contraction. Thus, the supply of energy for contraction is activated simultaneously with the contraction machinery.

2. SMALL (MONOMERIC) G PROTEINS REGULATE THROUGH CONFORMATIONAL CHANGES

The masters of regulation through reversible protein association in the cell are the monomeric G proteins, small single-subunit proteins that bind and hydrolyze GTP. GTP (guanosine triphosphate) is a purine nucleotide that, like ATP, contains high-energy phosphoanhydride bonds that release energy when hydrolyzed. When G proteins bind GTP, their conformation changes so that they can bind to a target protein, which is then either activated or inhibited in carrying out its function (Fig. 9.13, step 1).

G proteins are said to possess an internal clock because they are GTPases that slowly hydrolyze their own bound GTP to GDP and phosphate. As they hydrolyze GTP, their conformation changes and the complex they have formed with the target protein disassembles (see Fig. 9.13, step 2). The bound GDP on the inactive G protein is eventually replaced by GTP, and the process can begin again (see Fig. 9.13, step 3).

The activity of many G proteins is regulated by accessory proteins (GAPs, GEFs, and GDIs), which may, in turn, be regulated by allosteric effectors. GAPs (**G**TPase **a**ctivating **p**roteins) increase the rate of GTP hydrolysis by the G protein, and therefore the rate of dissociation of the G protein-target protein complex (see Fig. 9.13,

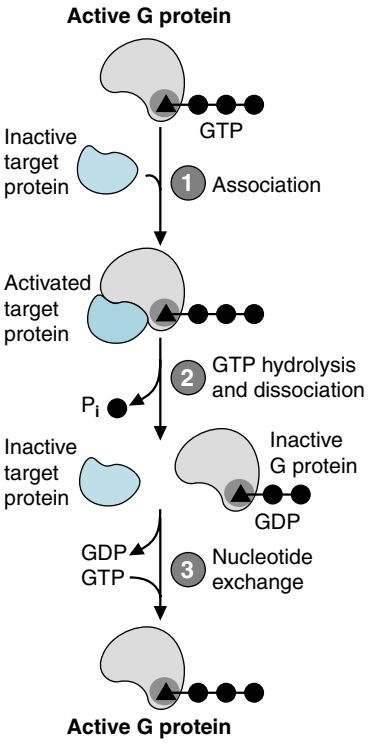


Fig. 9.13. Monomeric G proteins. (1) When GTP is bound, the conformation of the G protein allows it to bind target proteins, which are then activated. (2) The G protein hydrolyzes a phosphate from GTP to form GDP, which changes the G-protein conformation and causes it to dissociate from the target protein. (3) GDP is exchanged for GTP, which reactivates the G protein.

step 2). When a GEF protein (**g**uanine nucleotide **e**xchange **f**actor) binds to a G-protein, it increases the rate of GTP exchange for a bound GDP, and therefore activates the G-protein (see Fig. 9.13, step 3). GDI proteins (**G**DP **d**issociation **i**nhibitor) bind to the GDP-G protein complex and inhibit dissociation of GDP, thereby keeping the G protein inactive.

The Ras superfamily of small G proteins is divided into five families: Ras, Rho, Arf, Rab, and Ran. These monomeric G proteins play major roles in the regulation of growth, morphogenesis, cell motility, axonal guidance, cytokinesis, and trafficking through the Golgi, nucleus, and endosomes. They are generally bound to a lipid membrane through a lipid anchor, such as a myristoyl group or farnesyl group, and regulate the assembly and activity of protein complexes at these sites. The small G protein Ras, for example, is involved in regulation of cellular proliferation by a number of hormones called growth factors (Fig. 9.14). It is attached to the plasma membrane by a farnesyl group (see Chapter 6, section IV.B.). The activity of Ras is regulated by a guanine nucleotide exchange protein called SOS (**s**on **o**f **s**evenless). When SOS is in its active conformation, it binds to Ras, thereby activating dissociation of GDP and binding of GTP. When Ras binds GTP, it is activated, allowing it to bind and activate a protein kinase called Raf. The net effect will be the activation of transcription of certain genes. (Rho, Arf, Rab, and Ran are illustrated in Chapter 10, and the function of Ras is discussed in greater detail in Chapter 11).

D. Proteolytic Cleavage

Although many enzymes undergo some cleavage during synthesis, others enter lysosomes, secretory vesicles or are secreted as proenzymes, which are precursor proteins that must undergo proteolytic cleavage to become fully functional. Unlike most other forms of regulation, proteolytic cleavage is irreversible.

Most of the proteases involved in blood clotting are zymogens, such as fibrinogen and prothrombin, which circulate in blood in the inactive form. They are cleaved to the active form (fibrin and thrombin, respectively) by other proteases, which have been activated by their attachment to the site of injury in a blood vessel wall. Thus, clots form at the site of injury and not randomly in circulation.

The precursor proteins of proteases (enzymes that cleave specific peptide bonds) are called zymogens. To denote the inactive zymogen form of an enzyme, the name is modified by addition of the suffix "ogen" or the prefix "pro." The synthesis of zymogens as inactive precursors prevents them from cleaving proteins prematurely at their sites of synthesis or secretion. Chymotrypsinogen, for example, is stored in vesicles within pancreatic cells until secreted into ducts leading to the intestinal lumen. In the digestive tract, chymotrypsinogen is converted to chymotrypsin by the proteolytic enzyme trypsin, which cleaves off a small peptide from the N-terminal region (and two internal peptides). This cleavage activates chymotrypsin by causing

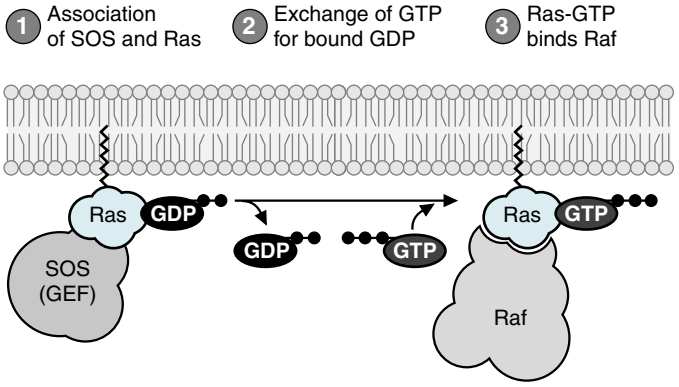


Fig. 9.14. The monomeric G protein Ras. When SOS is activated, it binds to Ras, a monomeric G protein anchored to the plasma membrane. SOS is a guanine nucleotide exchange protein that activates the exchange of GTP for bound GDP on Ras. Activated Ras containing GTP binds the target enzyme Raf, thereby activating it.

a conformational change in the spacing of amino acid residues around the binding site for the denatured protein substrate and around the catalytic site.

IV. REGULATION THROUGH CHANGES IN AMOUNT OF ENZYME

Tissues continuously adjust the rate at which different proteins are synthesized to vary the amount of different enzymes present. The expression for V_{max} in the Michaelis-Menten equation incorporates the concept that the rate of a reaction is proportional to the amount of enzyme present. Thus, the maximal capacity of a tissue can change with increased protein synthesis, or with increased protein degradation.

A. Regulated Enzyme Synthesis

Protein synthesis begins with the process of gene transcription, transcribing the genetic code for that protein from DNA into messenger RNA. The code in messenger RNA is then translated into the primary amino acid sequence of the protein. Generally the rate of enzyme synthesis is regulated by increasing or decreasing the rate of gene transcription, processes generally referred to as induction (increase) and repression (decrease). However, the rate of enzyme synthesis is sometimes regulated through stabilization of the messenger RNA. (These processes are covered in Section Three). Compared with the more immediate types of regulation discussed above, regulation by means of induction/repression of enzyme synthesis is usually slow in the human, occurring over hours to days.

The maximal capacity of MEOS (cytochrome P450-2E1) is increased in the liver with continued ingestion of ethanol through a mechanism involving induction of gene transcription. Thus, **Al Martini** has a higher capacity to oxidize ethanol to acetaldehyde than a naive drinker (a person not previously subjected to alcohol). Nevertheless, the persistance of his elevated blood alcohol level shows he has saturated his capacity for ethanol oxidation (V-maxed out). Once his enzymes are operating near V_{max}, any additional ethanol he drinks will not appreciably increase the rate of ethanol clearance from his blood.

B. Regulated Protein Degradation

The content of an enzyme in the cell can be altered through selective regulated degradation as well as through regulated synthesis. Although all proteins in the cell can be degraded with a characteristic half-life within lysosomes, protein degradation via two specialized systems, proteosomes and caspases, is highly selective and regulated. Protein degradation is dealt with in more detail in Chapter 37.

During fasting or infective stress, protein degradation in skeletal muscle is activated to increase the supply of amino acids in the blood for gluconeogenesis, or for the synthesis of antibodies and other component of the immune response. Under these conditions, synthesis of ubiquitin, a protein that targets proteins for degradation in proteosomes, is increased by the steroid hormone cortisol.

V. REGULATION OF METABOLIC PATHWAYS

The different means of regulating enzyme activity described above are used to control metabolic pathways, cellular events, and physiologic processes to match the body's requirements. Although many metabolic pathways are present in the body, a few common themes or principles are involved in their regulation. Of course, the overriding principle is: ***Regulation of a pathway matches its function***.

A. Principles of Pathway Regulation

Metabolic pathways are a series of sequential reactions in which the product of one reaction is the substrate of the next reaction (Fig. 9.15). Each step or reaction is usually catalyzed by a separate enzyme. The enzymes of a pathway have a common function—conversion of substrate to the final endproducts of the pathway. A pathway also may have a branchpoint at which an intermediate becomes the precursor for another pathway.

1. REGULATION OCCURS AT THE RATE-LIMITING STEP

Pathways are principally regulated at one key enzyme, the regulatory enzyme, which catalyzes the rate-limiting step in the pathway. This is the slowest step and is usually not readily reversible. Thus, changes in the rate-limiting step can influence flux through the rest of the pathway (see Fig. 9.1). The rate-limiting step is usually the first committed step in a pathway, or a reaction that is related to, or influenced

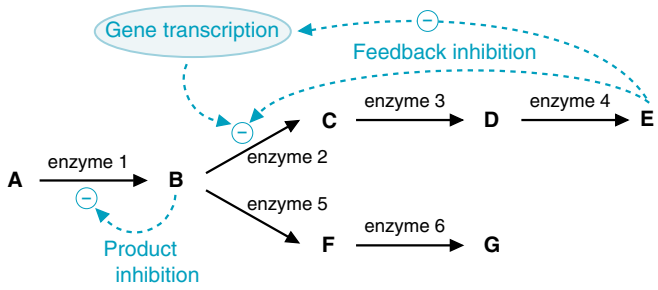


Fig. 9.15. A common pattern for feedback inhibition of metabolic pathways. The letters represent compounds formed from different enzymes in the reaction pathway. Compound B is at a metabolic branchpoint: it can go down one pathway to E or down an alternate pathway to G. The endproduct of the pathway, E, might control its own synthesis by allosterically inhibiting enzyme 2, the first committed step of the pathway, or inhibiting transcription of the gene for enzyme 2. As a result of the feedback inhibition, B accumulates and more B enters the pathway for conversion to G, which could be a storage, or disposal pathway. In this hypothetical pathway, B is a product inhibitor of enzyme 1, competitive with respect to A. Precursor A might induce the synthesis of enzyme 1, which would allow more A to go to G.

The pathways of energy production must be regulated by a mechanism that can respond rapidly to requirements for more ATP, such as the allosteric regulation of glycogen phosphorylase by AMP. However, storage pathways or biosynthetic pathways can be regulated by a mechanism that responds more slowly to changing conditions. For example, cholesterol partially feedback regulates its own rate of synthesis by decreasing transcription of the gene for the rate-limiting enzyme (HMG-CoA reductase). The enzyme concentration of a tissue may change even more slowly in response to developmental changes.

When **Ann O'Rexia** jogs, the increased use of ATP for muscle contraction results in an increase of AMP, which allosterically activates both the allosteric enzyme phosphofructokinase-1, the rate-limiting enzyme of glycolysis, and glycogen phosphorylase, the rate-limiting enzyme of glycogenolysis. This is an example of *feedback regulation* by the ATP/AMP ratio. Unfortunately, her low caloric consumption has not allowed *feed-forward activation* of the rate-limiting enzymes in her fuel storage pathways, and she has very low glycogen stores. Consequently, she has inadequate fuel stores to supply the increased energy demands of exercise.

by, the first committed step. Additional regulated enzymes occur after each metabolic branchpoint to direct flow into the branch. (e.g., in Fig. 9.15, feedback inhibition of enzyme 2 results in accumulation of B, which enzyme 5 then uses for synthesis of compound G). Inhibition of the rate-limiting enzyme in a pathway usually leads to accumulation of the pathway precursor.

2. FEEDBACK REGULATION

Feedback regulation refers to a situation in which the endproduct of a pathway controls its own rate of synthesis (see Fig. 9.15). Feedback regulation usually involves allosteric regulation of the rate-limiting enzyme by the endproduct of a pathway (or a compound that reflects changes in the concentration of the endproduct). The endproduct of a pathway may also control its own synthesis by inducing or repressing the gene for transcription of the rate-limiting enzyme in the pathway. This type of regulation is much slower to respond to changing conditions than allosteric regulation.

3. FEED-FORWARD REGULATION

Certain pathways, such as those involved in the disposal of toxic compounds, are feed-forward regulated. Feed-forward regulation may occur through an increased supply of substrate to an enzyme with a high K_m, allosteric activation of a rate-limiting enzyme through a compound related to substrate supply, substrate-related induction of gene transcription (e.g., induction of cytochrome P450-2E1 by ethanol), or increased concentration of a hormone that stimulates a storage pathway by controlling enzyme phosphorylation state.

4. TISSUE ISOZYMES OF REGULATORY PROTEINS

The human body is composed of a number of different cell types that perform specific functions unique to that cell type and synthesize only the proteins consistent with their functions. Because regulation matches function, regulatory enzymes of pathways usually exist as tissue-specific isozymes with somewhat different regulatory properties unique to their function in different cell types. For example, hexokinase and glucokinase are tissue-specific isozymes with different kinetic properties.

5. COUNTER-REGULATION OF OPPOSING PATHWAYS

A pathway for the synthesis of a compound usually has one or more enzymatic steps that differ from the pathway for degradation of that compound. A biosynthetic pathway can therefore have a different regulatory enzyme than the opposing degradative pathway, and one pathway can be activated while the other is inhibited (e.g., glycogen synthesis is activated while glycogen degradation is inhibited).

6. SUBSTRATE CHANNELING THROUGH COMPARTMENTATION

In the cell, compartmentation of enzymes into multienzyme complexes or organelles provides a means of regulation, either because the compartment provides unique conditions or because it limits or channels access of the enzymes to substrates. Enzymes or pathways with a common function are often assembled into organelles. For example, enzymes of the TCA cycle are all located within the mitochondrion. The enzymes catalyze sequential reactions, and the product of one reaction is the substrate for the next reaction. The concentration of the pathway intermediates remains much higher within the mitochondrion than in the surrounding cellular cytoplasm.

Another type of compartmentation involves the assembly of enzymes catalyzing sequential reactions into multi-enzyme complexes so that intermediates of the pathway can be directly transferred from the active site on one enzyme to the active site on another enzyme, thereby preventing loss of energy and information.

7. LEVELS OF COMPLEXITY

You may have noticed by now that regulation of metabolic pathways in the human is exceedingly complex; this might be called the second principle of metabolic regulation. As you study different pathways in the subsequent chapters of the text, it may help to develop diagrams such as Fig. 9.15 to keep track of the function and rationale behind different regulatory interactions.

The different isozymes of hexokinase (e.g., hexokinase I and glucokinase) are tissue-specific isozymes that arose through gene duplication. Glucokinase, the low-affinity enzyme found in liver, is a single polypeptide chain with a molecular weight of 55 kDa that contains one active catalytic site. The hexokinases found in erythrocytes, skeletal muscles, and most other tissues are 110 kDa and are essentially two mutated glucokinase molecules synthesized as one polypeptide chain. However, only one catalytic site is functional. All of the tissue-specific hexokinases but glucokinase have K_ms for glucose that are less than 0.2 mM.

An example of a multi-enzyme complex is provided by MEOS (microsomal ethanol oxidizing system), which is composed of two different subunits with different enzyme activities. One subunit transfers electrons from NADPH to a cytochrome Fe-heme group on the 2nd subunit, which then transfers the electrons to O_2.

The hormone epinephrine (released during stress and exercise) and glucagon (released during fasting) activate the synthesis of cAMP in a number of tissues. cAMP activates protein kinase A. Because protein kinase A is able to phosphorylate key regulatory enzymes in many pathways, these pathways can be coordinately regulated. In muscle, for example, glycogen degradation is activated while glycogen synthesis is inhibited. At the same time, fatty acid release from adipose tissue is activated to provide more fuel for muscle. The regulation of glycolysis, glycogen metabolism, and other pathways of metabolism is much more complex than we have illustrated here and is discussed in many subsequent chapters of this text.

CLINICAL COMMENTS

Al Martini. In the Emergency Room, **Al Martini** was evaluated for head injuries. From the physical examination and blood alcohol levels, it was determined that his mental state resulted from his alcohol consumption. Although his chronic ethanol consumption had increased his level of MEOS (and, therefore, rate of ethanol oxidation in his liver), his excessive drinking resulted in a blood alcohol level greater than the legal limit of 80 mg/dL. He suffered bruises and contusions but was otherwise uninjured. He left in the custody of the police officer.

Ann O'Rexia. Ann O'Rexia's physician explained that she had inadequate fuel stores for her exercise program. To jog, her muscles require an increased rate of fuel oxidation to generate the ATP for muscle contraction. The fuels used by muscles for exercise include glucose from muscle glycogen, fatty acids from adipose tissue triacylglycerols, and blood glucose supplied by liver glycogen. These fuel stores were depleted during her prolonged bout of starvation. In addition, starvation resulted in the loss of muscle mass as muscle protein was being degraded to supply amino acids for other processes, including gluconeogenesis (the synthesis of glucose from amino acids and other noncarbohydrate precursors). Therefore, Ann will need to increase her caloric consumption to rebuild her fuel stores. Her physician helped her calculate the

additional amount of calories her jogging program will need, and they discussed which foods she would eat to meet these increased caloric requirements. He also helped her visualize the increase of weight as an increase in strength.

BIOCHEMICAL COMMENTS

The Lineweaver-Burk transformation. The K_m and V_{max} for an enzyme can be visually determined from a plot of $1/v_i$ versus 1/S, called a Lineweaver-Burk or a double reciprocal plot. The reciprocal of both sides of the Michaelis-Menten equation generates an equation that has the form of a straight line, y = mx + b (Fig. 9.16). K_m and V_{max} are equal to the reciprocals of the intercepts on the abscissa and ordinate, respectively. Although double reciprocal plots are often used to illustrate certain features of enzyme reactions, they are not directly used for the determination of K_m and V_{max} values by researchers.

For the reaction in which an enzyme forms a complex with both substrates, the K_m for one substrate can vary with the concentration of cosubstrate (Fig. 9.17). At each constant concentration of cosubstrate, the plot of $1/v_i$ vs 1/[S] is a straight line. To obtain V_{max}, the graph must be extrapolated to saturating concentrations of both substrates, which is equivalent to the intersection point of these lines for different cosubstrate concentrations.

Lineweaver-Burk plots provide a good illustration of competitive inhibition and pure noncompetitive inhibition (Fig. 9.18). In competitive inhibition, plots of 1/v vs 1/[S] at a series of inhibitor concentrations intersect on the ordinate. Thus, at infinite substrate concentration, or 1/[S] = 0, there is no effect of the inhibitor. In pure noncompetitive inhibition, the inhibitor decreases the velocity even when [S] has been extrapolated to an infinite concentration. However, if the inhibitor has no effect on the binding of the substrate, the K_m is the same for every concentration of inhibitor, and the lines intersect on the abcissa.

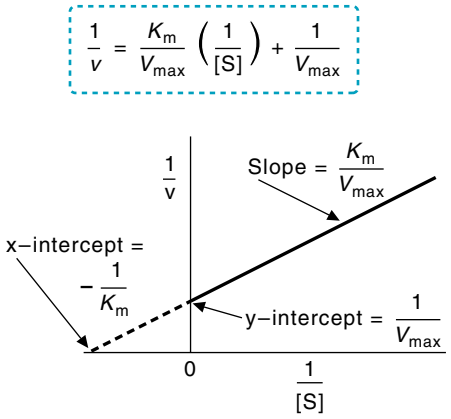


Fig. 9.16. The Lineweaver-Burk transformation (shown in blue) for the Michaelis-Menten equation converts it to a straight line of the form y = mx + b. When [S] is infinite, 1/[S] = 0, and the line crosses the ordinate (y-axis) at $1/v = 1/V_{max}$. The slope of the line is K_m/V_{max}. Where the line intersects the abscissa (x-axis), $1/[S] = -1/K_m$.

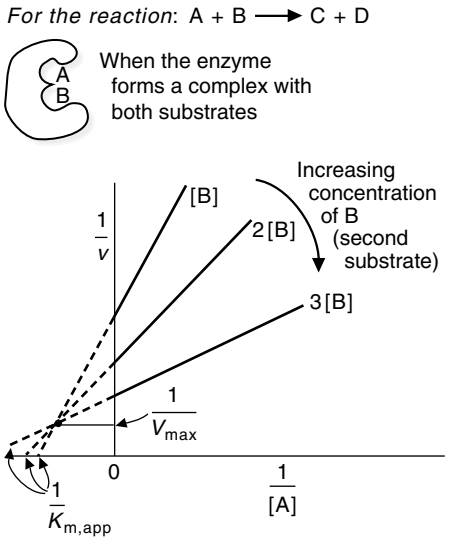


Fig. 9.17. A Lineweaver-Burk plot for a two-substrate reaction in which A and B are converted to products. In the graph, 1/[A] is plotted against 1/v for three different concentrations of the cosubstrate, [B], 2[B], and 3[B]. As the concentration of B is increased, the intersection on the abscissa, equal to $1/K_{m,app}$ is increased. The "app" represents "apparent", as the $K_{m,app}$ is the K_m at whatever concentration of cosubstrate, inhibitor, or other factor is present during the experiment.

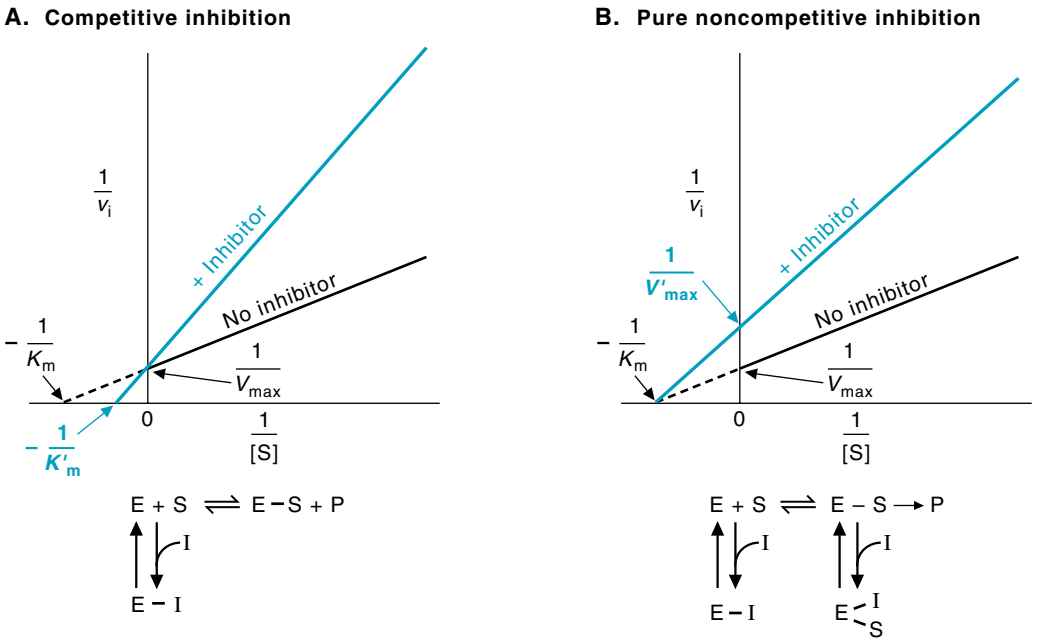


Fig. 9.18. Lineweaver-Burk plots of competitive and pure noncompetitive inhibition. A. $1/v_i$ versus 1/[S] in the presence of a competitive inhibitor. The competitive inhibitor alters the intersection on the abscissa. The new intersection is $1/K_{m,app}$ (also called $1/K'_m$). A competitive inhibitor does not affect V_{max}. B. $1/v_i$ versus 1/[S] in the presence of a pure noncompetitive inhibitor. The noncompetitive inhibitor alters the intersection on the ordinate, $1/V_{max,app}$ or $1/V'_{max}$, but does not affect $1/K_m$. A pure noncompetitive inhibitor binds to E and ES with the same affinity. If the inhibitor has different affinities for E and ES, the lines will intersect above or below the abscissa, and the noncompetitive inhibitor will change both the K'_m and the V'_m.

REVIEW QUESTIONS—CHAPTER 9

1. Which of the following describes a characteristic feature of an enzyme obeying Michaelis-Menten kinetics?

   (A) The enzyme velocity is at ½ the maximal rate when 100% of the enzyme molecules contain bound substrate.
   (B) The enzyme velocity is at ½ the maximal rate when 50% of the enzyme molecules contain bound substrate.
   (C) The enzyme velocity is at its maximal rate when 50% of the enzyme molecules contain bound substrate.
   (D) The enzyme velocity is at its maximal rate when all of the substrate molecules in solution are bound by the enzyme.
   (E) The velocity of the reaction is independent of the concentration of enzyme.

2. The pancreatic glucokinase of a patient with MODY had a mutation replacing a leucine with a proline. The result was that the K_m for glucose was decreased from a normal value of 6 mM to a value of 2.2 mM, and the V_{max} was changed from 93 units/mg protein to 0.2 units/mg protein. Which of the following best describes the patient's glucokinase compared with the normal enzyme?

   (A) The patient's enzyme requires a lower concentration of glucose to reach ½ V_{max}.
   (B) The patient's enzyme is faster than the normal enzyme at concentrations of glucose below 2.2 mM.
   (C) The patient's enzyme is faster than the normal enzyme at concentrations of glucose above 2.2 mM.
   (D) At near saturating glucose concentration, the patient would need 90 to 100 times more enzyme than normal to achieve normal rates of glucose phosphorylation.
   (E) As blood glucose levels increase after a meal from a fasting value of 5 mM to 10 mM, the rate of the patient's enzyme will increase more than the rate of the normal enzyme.

3. Methanol (CH_3OH) is converted by alcohol dehydrogenases to formaldehyde (CHO), a compound that is highly toxic in the human. Patients who have ingested toxic levels of methanol are sometimes treated with ethanol (CH_3CH_2OH) to inhibit methanol oxidation by alcohol dehydrogenase. Which of the following statements provides the best rationale for this treatment?

   (A) Ethanol is a structural analog of methanol, and might therefore be an effective noncompetitive inhibitor.
   (B) Ethanol is a structural analog of methanol that would be expected to compete with methanol for its binding site on the enzyme.
   (C) Ethanol would be expected to alter the V_{max} of alcohol dehydrogenase for the oxidation of methanol to formaldehyde.
   (D) Ethanol would be an effective inhibitor of methanol oxidation regardless of the concentration of methanol.
   (E) Ethanol would be expected to inhibit the enzyme by binding to the formaldehyde binding site on the enzyme, even though it cannot bind at the substrate binding site for methanol.

4. Which of the following describes a characteristic of most allosteric enzymes?

   (A) They are composed of single subunits.
   (B) In the absence of effectors, they generally follow Michaelis-Menten kinetics.
   (C) They show cooperativity in substrate binding.
   (D) They have allosteric activators that bind in the catalytic site.
   (E) They have irreversible allosteric inhibitors that bind at allosteric sites.

5. A rate-limiting enzyme catalyzes the first step in the conversion of a toxic metabolite to a urinary excretion product. Which of the following mechanisms for regulating this enzyme would provide the most protection to the body?

   (A) The product of the pathway should be an allosteric inhibitor of the rate-limiting enzyme.
   (B) The product of the pathway should act through gene transcription to decrease synthesis of the enzyme.
   (C) The toxin should act through gene transcription to increase synthesis of the enzyme.
   (D) The enzyme should have a high K_m for the toxin.
   (E) The product of the first enzyme should allosterically activate the subsequent enzyme in the pathway.