

CHAPTER 2

Enzymes

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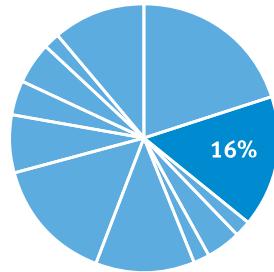
Introduction

There is an obesity epidemic in the United States that is paralleled by an increase in high blood pressure, or hypertension. This is extremely relevant to medical students because hypertension increases the risk of stroke, heart failure, and kidney failure.

Each year, physicians encourage millions of Americans to improve their diets, add exercise to their daily regimens, or even take prescription drugs to control their hypertension. Many of these anti-hypertensive medications are called ACE (*angiotensin-converting enzyme*) inhibitors. In healthy patients, ACE catalyzes a reaction that converts a peptide called angiotensin I to angiotensin II. The angiotensin II peptide then not only directly causes constriction of the blood vessels to raise blood pressure, but also stimulates the release of the hormone aldosterone, which activates the kidneys to reabsorb more water back into the bloodstream. The increase in blood volume also increases blood pressure. Physicians take advantage of this complicated pathway with a straightforward solution: stop the pathway early by inhibiting ACE and blood pressure will decrease.

Enzymes are crucial proteins that dramatically increase the rate of biological reactions. They're used to regulate homeostatic mechanisms in every organ system and are highly regulated themselves by environmental conditions, activators, and inhibitors. These regulators may be naturally occurring or may be given as a drug, such as

CHAPTER PROFILE



The content in this chapter should be relevant to about 16% of all questions about biochemistry on the MCAT.

This chapter covers material from the following AAMC content categories:

1A: Structure and function of proteins and their constituent amino acids

5E: Principles of chemical thermodynamics and kinetics



the ACE inhibitors used to treat hypertension. Some enzymes are kept in an inactivated form called a zymogen and are only activated as needed. In this chapter, we'll learn about how enzymes work and how different conditions influence their activity. We'll also see how enzymes are regulated, which will help us tie together concepts about every organ system and metabolic process we learn about for the MCAT.

2.1 Enzymes as Biological Catalysts

High-Yield

LEARNING OBJECTIVES

After Chapter 2.1, you will be able to:

- Explain the major features of enzyme function, including specificity and catalysis
- Describe all six classes of enzymes: oxidoreductases, transferases, hydrolases, lyases, isomerases, and ligases
- Recognize how enzymes affect thermodynamics and kinetics of a reaction

Enzymes are incredibly important as biological catalysts. **Catalysts** do not impact the thermodynamics of a biological reaction; that is, the ΔH_{rxn} and equilibrium position do not change. Instead, they help the reaction proceed at a much faster rate. As a catalyst, the enzyme is not changed during the course of the reaction. Enzymes increase the reaction rate of a process by a factor of 100, 1000, or even 1,000,000,000,000 (10^{12}) times when compared to the uncatalyzed reaction. Without this increase, we wouldn't be alive. Table 2.1 summarizes the key points to remember about enzymes.

Lower the activation energy

Increase the rate of the reaction

Do not alter the equilibrium constant

Are not changed or consumed in the reaction (which means that they will appear in both the reactants and products)

Are pH- and temperature-sensitive, with optimal activity at specific pH ranges and temperatures

Do not affect the overall ΔG of the reaction

Are specific for a particular reaction or class of reactions

Table 2.1 Key Features of Enzymes

Enzyme Classifications

Enzymes are picky. The molecules upon which an enzyme acts are called substrates; a given enzyme will only catalyze a single reaction or class of reactions with these substrates, a property known as **enzyme specificity**. For example, *urease* only catalyzes the breakdown of urea. *Chymotrypsin*, on the other hand, can cleave peptide bonds





around the amino acids phenylalanine, tryptophan, and tyrosine in a variety of polypeptides. Although those amino acids aren't identical, they all contain an aromatic ring, which makes chymotrypsin specific for a class of molecules.

Enzymes can be classified into six categories, based on their function or mechanism. We'll review each type of enzyme and give examples of those that you are most likely to see on Test Day. If you encounter an unfamiliar enzyme on the MCAT, keep in mind that most enzymes have descriptive names ending in the suffix **-ase**: *lactase*, for example, breaks down lactose.

Oxidoreductases

Oxidoreductases catalyze oxidation–reduction reactions; that is, the transfer of electrons between biological molecules. They often have a cofactor that acts as an electron carrier, such as NAD^+ or NADP^+ . In reactions catalyzed by oxidoreductases, the electron donor is known as the **reductant**, and the electron acceptor is known as the **oxidant**. Enzymes with *dehydrogenase* or *reductase* in their names are usually oxidoreductases. Enzymes in which oxygen is the final electron acceptor often include *oxidase* in their names.

Transferases

Transferases catalyze the movement of a functional group from one molecule to another. For example, in protein metabolism, an *aminotransferase* can convert aspartate and α -ketoglutarate, as a pair, to glutamate and oxaloacetate by moving the amino group from aspartate to α -ketoglutarate. Most transferases will be straightforwardly named, but remember that *kinases* are also a member of this class. **Kinases** catalyze the transfer of a phosphate group, generally from ATP, to another molecule.

Hydrolases

Hydrolases catalyze the breaking of a compound into two molecules using the addition of water. In common usage, many hydrolases are named only for their substrate. For example, one of the most common hydrolases you will encounter on the MCAT is a *phosphatase*, which cleaves a phosphate group from another molecule. Other hydrolases include *peptidases*, *nucleases*, and *lipases*, which break down proteins, nucleic acids, and lipids, respectively.

Lyases

Lyases catalyze the cleavage of a single molecule into two products. They do not require water as a substrate and do not act as oxidoreductases. Because most enzymes can also catalyze the reverse of their specific reactions, the synthesis of two molecules into a single molecule may also be catalyzed by a lyase. When fulfilling this function, it is common for them to be referred to as *synthases*.

BRIDGE

The convention for naming reductants and oxidants of oxidoreductases is the same as the convention for naming reducing agents and oxidizing agents in general and organic chemistry. This is a good time to brush up on oxidation–reduction reactions if you haven't seen them in a while—they're covered in Chapter 11 of *MCAT General Chemistry Review* and Chapter 4 of *MCAT Organic Chemistry Review*.

MNEMONIC

Major Enzyme Classifications: **LIP'L HOT**

- Ligase
- Isomerase
- Lyase
- Hydrolase
- Oxidoreductase
- Transferase



Isomerases

Isomerases catalyze the rearrangement of bonds within a molecule. Some isomerases can also be classified as oxidoreductases, transferases, or lyases, depending on the mechanism of the enzyme. Keep in mind that isomerases catalyze reactions between stereoisomers as well as constitutional isomers.

Ligases

Ligases catalyze addition or synthesis reactions, generally between large similar molecules, and often require ATP. Synthesis reactions with smaller molecules are generally accomplished by lyases. Ligases are most likely to be encountered in nucleic acid synthesis and repair on Test Day.

Impact on Activation Energy

BRIDGE

Gibbs free energy and endergonic/exergonic reactions are covered in Chapter 7 of *MCAT General Chemistry Review*.

Recall that thermodynamics relates the relative energy states of a reaction in terms of its products and reactants. An **endergonic** reaction is one that requires energy input ($\Delta G > 0$), whereas an **exergonic** reaction is one in which energy is given off ($\Delta G < 0$). Remember that *endo-* means “in” and *exo-* means “out,” so endergonic reactions take in energy as they proceed, whereas exergonic reactions release energy as they proceed. We can look at the reaction diagram in Figure 2.1 to see this demonstrated more clearly.

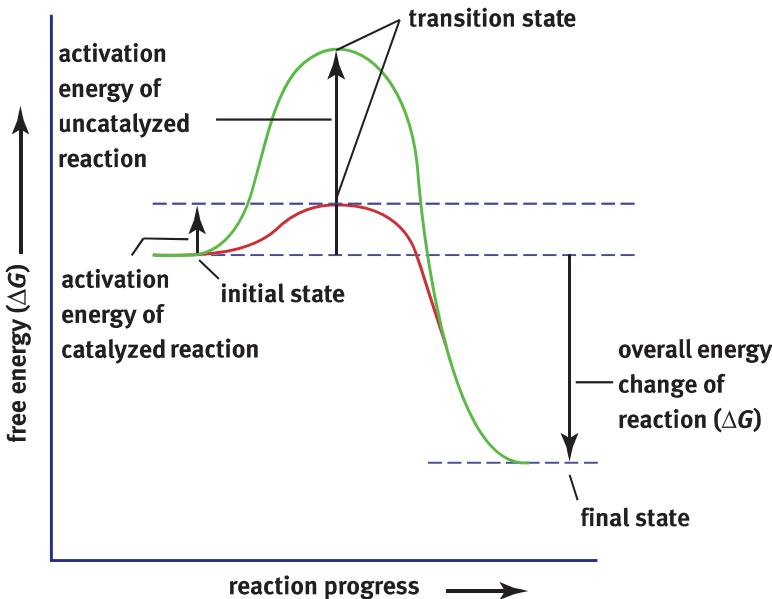


Figure 2.1 Exergonic Reaction Diagram

The activation energy required for a catalyzed reaction is lower than that of an uncatalyzed reaction while the ΔG (and ΔH) remains the same.

The reaction shown in Figure 2.1 is spontaneous. Note that the ΔG for this reaction is negative. A very important characteristic of enzymes is that they do not alter the overall free energy change for a reaction, nor do they change the equilibrium of a



reaction. Rather, they affect the rate (kinetics) at which a reaction occurs; thus, they can affect how quickly a reaction gets to equilibrium but not the actual equilibrium state itself. For example, a reaction could take years to approach equilibrium without an enzyme. In comparison, with the enzyme, equilibrium might be attained within seconds. Enzymes ensure that many important reactions can occur in a reasonable amount of time in biological systems. Recall that enzymes, as catalysts, are unchanged by the reaction. What is the functional consequence of this? Far fewer copies of the enzyme are required relative to the overall amount of substrate because one enzyme can act on many, many molecules of substrate over time.

Catalysts exert their effect by lowering the **activation energy** of a reaction; in other words, they make it easier for the substrate to reach the transition state. Imagine having to walk to the other side of a tall hill. The only way to get there is to climb to the top of the hill and then walk down the other side—but wouldn’t it be easier if the top of the hill was cut off so one wouldn’t have to climb so high? That’s exactly what catalysts do for chemical reactions when they make it easier for substrates to achieve their transition state. Most reactions catalyzed by enzymes are technically reversible, although that reversal may be extremely energetically unfavorable and therefore essentially nonexistent.

MCAT CONCEPT CHECK 2.1

Before you move on, assess your understanding of the material with these questions.

1. How do enzymes function as biological catalysts?

2. What is enzyme specificity?

3. What are the names and main functions of the six different classes of enzymes?

Name	Function

4. In what ways do enzymes affect the thermodynamics *vs.* the kinetics of a reaction?



2.2 Mechanisms of Enzyme Activity

LEARNING OBJECTIVES

After Chapter 2.2, you will be able to:

- Differentiate between coenzymes and cofactors
- Compare the induced fit and lock and key models of enzyme function

While enzyme mechanisms will vary depending on the reaction that is being catalyzed, they tend to share some common features. Enzymes may act to provide a favorable microenvironment in terms of charge or pH, stabilize the transition state, or bring reactive groups nearer to one another in the active site. The formation of the enzyme–substrate complex in the active site of an enzyme is the key catalytic activity of the enzyme, which reduces the activation energy of the reaction as described above. This interaction between a substrate and the active site of an enzyme also accounts for the selectivity and some regulatory mechanisms of enzymes.

Enzyme–Substrate Binding

The molecule upon which an enzyme acts is known as its **substrate**. The physical interaction between these two is referred to as the **enzyme–substrate complex**. The **active site** is the location within the enzyme where the substrate is held during the chemical reaction, as shown in Figure 2.2.

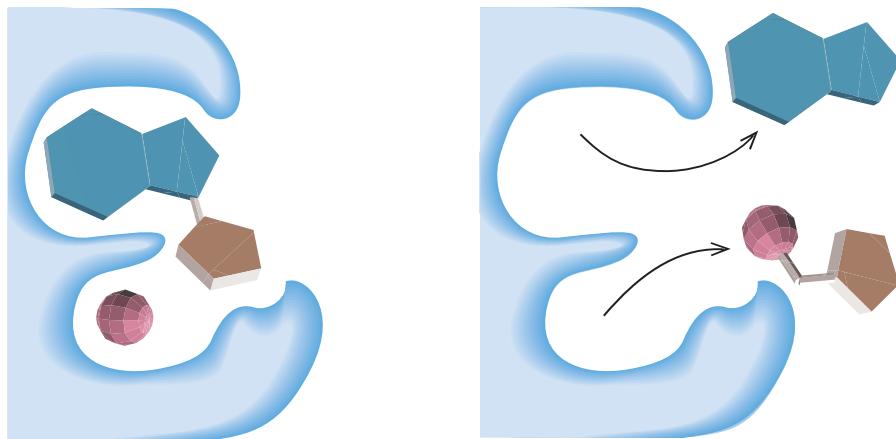


Figure 2.2 Reaction Catalysis in the Active Site of an Enzyme
This transferase has catalyzed the formation of a bond between two substrate molecules.

The active site assumes a defined spatial arrangement in the enzyme–substrate complex, which dictates the specificity of that enzyme for a molecule or group of molecules. Hydrogen bonding, ionic interactions, and transient covalent bonds within the active site all stabilize this spatial arrangement and contribute to the efficiency of the enzyme. Two competing theories explain how enzymes and substrates interact, but one of the two is better supported than the other.



Lock and Key Theory

The **lock and key theory** is aptly named. It suggests that the enzyme's active site (lock) is already in the appropriate conformation for the substrate (key) to bind. As shown in Figure 2.3, the substrate can then easily fit into the active site, like a key into a lock or a hand into a glove. No alteration of the tertiary or quaternary structure is necessary upon binding of the substrate.

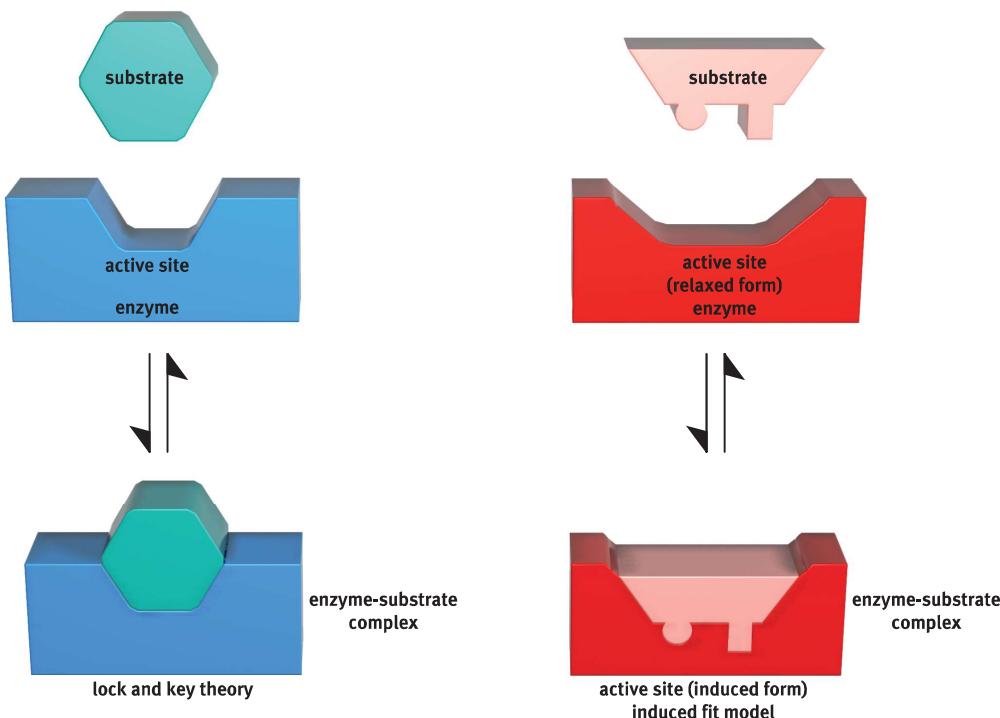


Figure 2.3 Lock and Key Theory vs. Induced Fit Model for Enzyme Catalysis

Induced Fit Model

The more scientifically accepted theory is the induced fit model; this is the one you are more likely to see referenced on Test Day. Imagine that the enzyme is a foam stress ball and the substrate is a frustrated MCAT student's hand. What's the desired interaction? The student wants to release some stress and relax. As his hand squeezes the ball, both change conformation. The ball is no longer spherical and his hand is no longer flat because they adjust to fit each other well. In this case, the substrate (the student) has induced a change in the shape of the enzyme (the stress ball). This interaction requires energy, and therefore, this part of the reaction is endergonic. Once the student lets go of the stress ball, we have our desired product: a relaxed, more confident test taker. Letting go of the stress ball is pretty easy and doesn't require extra energy; so, this part of the reaction is exergonic. Just like enzymes, foam stress balls return to their original shape once their crushers (substrates) let go of them. On a molecular level, demonstrated in Figure 2.3, the induced fit model starts with a substrate and an enzyme active site that don't seem to fit together. However, once the substrate is present and ready to interact with the active site, the molecules find that



the induced form, or transition state, is more comfortable for both of them. Thus, the shape of the active site becomes truly complementary only after the substrate begins binding to the enzyme. Similarly, a substrate of the wrong type will not cause the appropriate conformational shift in the enzyme. Thus, the active site will not be adequately exposed, the transition state is not preferred, and no reaction occurs.

Cofactors and Coenzymes

REAL WORLD

Deficiencies in vitamin cofactors can result in devastating disease. Thiamine is an essential cofactor for several enzymes involved in cellular metabolism and nerve conduction. Thiamine deficiency, often a result of excess alcohol consumption and poor diet, results in diseases including Wernicke–Korsakoff syndrome. In this disorder, patients suffer from a variety of neurologic deficits, including delirium, balance problems, and, in severe cases, the inability to form new memories.

BRIDGE

Vitamins come in two major classes: fat- and water-soluble. This is important to consider in digestive diseases, where different parts of the gastrointestinal tract may be affected by different disease processes. Because different parts of the gastrointestinal tract specialize in the absorption of different types of biomolecules, loss of different parts of the gastrointestinal tract or its accessory organs may result in different vitamin deficiencies. The digestive system is discussed in Chapter 9 of *MCAT Biology Review*.

Many enzymes require nonprotein molecules called **cofactors** or **coenzymes** to be effective. These cofactors and coenzymes tend to be small in size so they can bind to the active site of the enzyme and participate in the catalysis of the reaction, usually by carrying charge through ionization, protonation, or deprotonation. Cofactors and coenzymes are usually kept at low concentrations in cells, so they can be recruited only when needed. Enzymes without their cofactors are called **apoenzymes**, whereas those containing them are **holoenzymes**. Cofactors are attached in a variety of ways, ranging from weak noncovalent interactions to strong covalent ones. Tightly bound cofactors or coenzymes that are necessary for enzyme function are known as **prosthetic groups**.

Cofactors and coenzymes are topics that we are likely to see on Test Day, so they are important to know. Cofactors are generally inorganic molecules or metal ions, and are often ingested as dietary minerals. Coenzymes are small organic groups, the vast majority of which are vitamins or derivatives of vitamins such as NAD^+ , FAD, and coenzyme A. The water-soluble vitamins include the B complex vitamins and ascorbic acid (vitamin C), and are important coenzymes that must be replenished regularly because they are easily excreted. The fat-soluble vitamins—A, D, E, and K—are better regulated by partition coefficients, which quantify the ability of a molecule to dissolve in a polar vs. nonpolar environment. Enzymatic reactions are not restricted to a single cofactor or coenzyme. For example, metabolic reactions often require magnesium, NAD^+ (derived from vitamin B_3), and biotin (vitamin B_7) simultaneously.

The MCAT is unlikely to expect memorization of the B vitamins; however, familiarity with their names may make biochemistry passages easier on Test Day:

- B_1 : thiamine
- B_2 : riboflavin
- B_3 : niacin
- B_5 : pantothenic acid
- B_6 : pyridoxal phosphate
- B_7 : biotin
- B_9 : folic acid
- B_{12} : cyanocobalamin



**MCAT CONCEPT CHECK 2.2**

Before you move on, assess your understanding of the material with these questions.

- How do the lock and key theory and induced fit model differ?

Lock and Key	Induced Fit

- What do cofactors and coenzymes do? How do they differ?

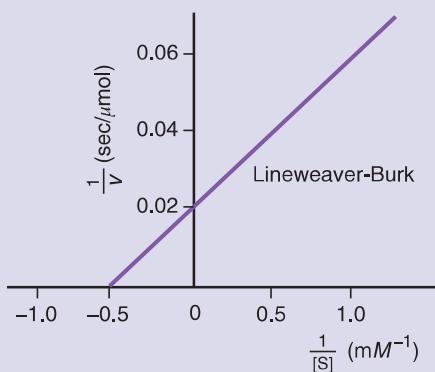
2.3 Enzyme Kinetics

High-Yield

LEARNING OBJECTIVES

After Chapter 2.3, you will be able to:

- Predict how changes in enzyme and solute concentration will affect enzyme kinetics
- Define enzyme cooperativity
- Compare Lineweaver–Burk and Michaelis–Menten plots
- Explain key points on a Lineweaver–Burk or Michaelis–Menten plot:



Kinetics of Monomeric Enzymes

Enzyme kinetics is a high-yield topic that can score us several points on Test Day. Just as the relief our student derives from squeezing a stress ball depends on a number of factors, such as size and shape of the ball and his or her baseline level of stress, enzyme kinetics are dependent on factors like environmental conditions and concentrations of substrate and enzyme.



The concentrations of the substrate, $[S]$, and enzyme, $[E]$, greatly affect how quickly a reaction will occur. Let's say that we have 100 stress balls (enzymes) and only 10 frustrated students (substrates) to derive stress relief from them. This represents a high enzyme concentration relative to substrate. Because there are many active sites available, we will quickly form products (students letting go and feeling relaxed); in a chemical sense, we would reach equilibrium quickly. As we slowly add more substrate (students), the rate of the reaction will increase; that is, more people will relax in the same amount of time because we have plenty of available stress balls for them to squeeze. However, as we add more and more people (and start approaching 100 students), we begin to level off and reach a maximal rate of relaxation. There are fewer and fewer available stress balls until finally all active sites are occupied. Unlike before, inviting more students into the room will not change the rate of the reaction. It cannot go any faster once it has reached **saturation**. At this rate, the enzyme is working at maximum velocity, denoted by v_{\max} . The only way to increase v_{\max} is by increasing the enzyme concentration. In the cell, this can be accomplished by inducing the expression of the gene encoding the enzyme. These concepts are represented graphically in Figure 2.4.

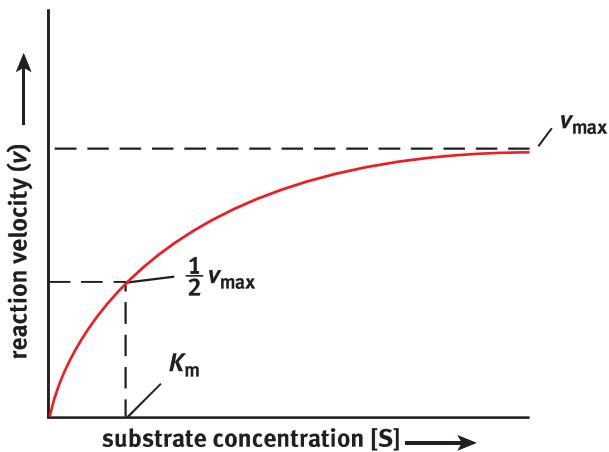
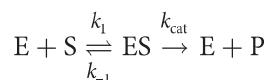


Figure 2.4 Michaelis–Menten Plot of Enzyme Kinetics
As the amount of substrate increases, the enzyme is able to increase its rate of reaction until it reaches a maximum enzymatic reaction rate (v_{\max}); once v_{\max} is reached, adding more substrate will not increase the rate of reaction.

Michaelis–Menten Equation

For most enzymes, the Michaelis–Menten equation describes how the rate of the reaction, v , depends on the concentration of both the enzyme, $[E]$, and the substrate, $[S]$, which forms product, $[P]$. Enzyme–substrate complexes form at a rate k_1 . The ES complex can either dissociate at a rate k_{-1} or turn into E + P at a rate k_{cat} :



Equation 2.1



Note that in either case, the enzyme is again available. On Test Day, the concentration of enzyme will be kept constant. Under these conditions, we can relate the velocity of the enzyme to substrate concentration using the Michaelis–Menten equation:

$$v = \frac{v_{\max} [S]}{K_m + [S]}$$

Equation 2.2

Some important and Test Day–relevant math can be derived from this equation.

When the reaction rate is equal to half of v_{\max} , $K_m = [S]$:

$$\begin{aligned}\frac{v_{\max}}{2} &= \frac{v_{\max}[S]}{K_m + [S]} \\ v_{\max}(K_m + [S]) &= 2(v_{\max}[S]) \\ K_m + [S] &= 2[S] \\ K_m &= [S]\end{aligned}$$

K_m can, therefore, be understood to be the substrate concentration at which half of the enzyme's active sites are full (half the stress balls are in use). K_m is the **Michaelis constant** and is often used to compare enzymes. Under certain conditions, K_m is a measure of the affinity of the enzyme for its substrate. When comparing two enzymes, the one with the higher K_m has the lower affinity for its substrate because it requires a higher substrate concentration to be half-saturated. The K_m value is an intrinsic property of the enzyme–substrate system and cannot be altered by changing the concentration of substrate or enzyme.

For a given concentration of enzyme, the Michaelis–Menten relationship generally graphs as a hyperbola, as seen in the Michaelis–Menten plot in Figure 2.4. When substrate concentration is less than K_m , changes in substrate concentration will greatly affect the reaction rate. At high substrate concentrations exceeding K_m , the reaction rate increases much more slowly as it approaches v_{\max} , where it becomes independent of substrate concentration.

The variable v_{\max} represents maximum enzyme velocity and is measured in moles of enzyme per second. Also, v_{\max} can be mathematically related to k_{cat} , which has units of s^{-1} :

$$v_{\max} = [E]k_{\text{cat}}$$

Equation 2.3

Qualitatively speaking, k_{cat} measures the number of substrate molecules “turned over,” or converted to product, per enzyme molecule per second. Most enzymes have k_{cat} values between 10¹ and 10³. The Michaelis–Menten equation above can be restated using k_{cat} :

$$v = \frac{k_{\text{cat}}[E][S]}{K_m + [S]}$$

KEY CONCEPT

We can assess an enzyme's affinity for a substrate by noting the K_m . A low K_m reflects a high affinity for the substrate (low [S] required for 50% enzyme saturation). Conversely, a high K_m reflects a low affinity of the enzyme for the substrate.



At very low substrate concentrations, where $K_m \gg [S]$, this derived equation can be further simplified as:

$$v = \frac{k_{cat}}{K_m} [E][S]$$

The ratio of k_{cat}/K_m is referred to as the **catalytic efficiency** of the enzyme. A large k_{cat} (high turnover) or a small K_m (high substrate affinity) will result in a higher catalytic efficiency, which indicates a more efficient enzyme.

Lineweaver–Burk Plots

The Lineweaver–Burk plot is a double reciprocal graph of the Michaelis–Menten equation. The same data graphed in this way yield a straight line as shown in Figure 2.5. The actual data are represented by the portion of the graph to the right of the y -axis, but the line is extrapolated into the upper left quadrant to determine its intercept with the x -axis. The intercept of the line with the x -axis gives the value of $-\frac{1}{K_m}$. The intercept of the line with the y -axis gives the value of $\frac{1}{V_{max}}$. The Lineweaver–Burk plot is especially useful when determining the type of inhibition that an enzyme is experiencing because V_{max} and K_m can be compared without estimation.

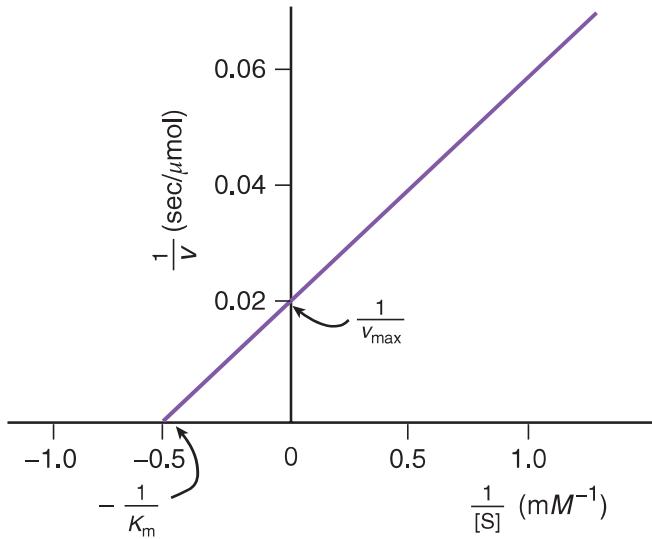


Figure 2.5 Experimentally Determined Lineweaver–Burk (Double Reciprocal) Plot Used to Calculate the Values of K_m and V_{max}

Cooperativity

MCAT EXPERTISE

The cooperative binding of hemoglobin, which acts as a transport protein rather than an enzyme, results in a characteristic sigmoidal binding curve that is an MCAT favorite.

Certain enzymes do not show the normal hyperbola when graphed on a Michaelis–Menten plot (v vs. $[S]$), but rather show sigmoidal (S-shaped) kinetics owing to cooperativity among substrate binding sites, as shown in Figure 2.6. Cooperative enzymes have multiple subunits and multiple active sites. Subunits and enzymes may exist in one of two states: a low-affinity tense state (T) or a high-affinity relaxed state (R). Binding of the substrate encourages the transition of other subunits from the





T state to the R state, which increases the likelihood of substrate binding by these other subunits. Conversely, loss of substrate can encourage the transition from the R state to the T state, and promote dissociation of substrate from the remaining subunits. Think of cooperative enzyme kinetics like a party. As more people start arriving, the atmosphere becomes more relaxed and the party seems more appealing, but as people start going home the party dies down and more people are encouraged to leave so the tense hosts can clean up. Enzymes showing cooperative kinetics are often regulatory enzymes in pathways, like *phosphofructokinase-1* in glycolysis. Cooperative enzymes are also subject to activation and inhibition, both competitively and through allosteric sites.

Cooperativity can also be quantified using a numerical value called **Hill's coefficient**. The value of Hill's coefficient indicates the nature of binding by the molecule:

- If Hill's coefficient > 1 , positively cooperative binding is occurring, such that after one ligand is bound the affinity of the enzyme for further ligand(s) increases.
- If Hill's coefficient < 1 , negatively cooperative binding is occurring, such that after one ligand is bound the affinity of the enzyme for further ligand(s) decreases.
- If Hill's coefficient = 1, the enzyme does not exhibit cooperative binding.

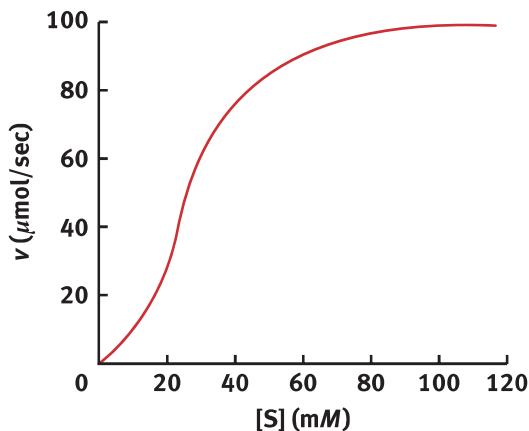


Figure 2.6 Cooperative Enzyme Kinetics



BIOCHEMISTRY GUIDED EXAMPLE WITH EXPERT THINKING

The PH1704 protease from *Pyrococcus horikoshii* OT3 is a hyperthermophilic enzyme that belongs to the DJ-1/ Thij/PfpI superfamily. One of its members, the human protein DJ-1, has recently been reported to cause certain types of early-onset Parkinsonism. Thus, an increasing number of studies on this superfamily are being conducted. Despite this growing interest, few members of this superfamily have been biochemically characterized. PH1704 has both endopeptidase (cleaves nonterminal amino acids) and aminopeptidase (cleaves amino acids from the N-terminus) activity. Further analysis suggests that PH1704 specifically cleaves after arginine. Two 7-amino-4-methylcoumarin (AMC)-linked substrates were used in kinetic assays: R-AMC and AAFLR-AMC.

Predictive substrate docking studies pinpointed Tyr-120 in the active site, possibly involved in aromatic and hydrophobic interaction with the substrate. Therefore, Tyr-120 was chosen for mutation to analyze the effects on PH1704 kinetics, and the results are shown in Table 1 below.

Enzyme	Aminopeptidase (R-AMC)		
	K_{cat} (min $^{-1}$)	K_m (μ M)	K_{cat}/K_m (min $^{-1}$ μ M $^{-1}$)
WT	0.646 \pm 0.05	12 \pm 0.65	0.052 \pm 0.008
Y120S	0.147 \pm 0.01	9.0 \pm 0.43	0.024 \pm 0.004
Y120W	1.2 \pm 0.07	20.6 \pm 0.77	0.066 \pm 0.005
Y120P	4.37 \pm 0.12	11.3 \pm 0.59	0.398 \pm 0.025
Enzyme	Endopeptidase (AAFLR-AMC)		
	K_{cat} (min $^{-1}$)	K_m (μ M)	K_{cat}/K_m (min $^{-1}$ μ M $^{-1}$)
WT	0.11 \pm 0.02	10 \pm 0.2	0.018 \pm 0.004
Y120S	0.084 \pm 0.01	6.5 \pm 0.4	0.017 \pm 0.003
Y120W	0.12 \pm 0.01	16.0 \pm 1.5	0.019 \pm 0.001
Y120P	1.13 \pm 0.07	5.4 \pm 0.3	0.21 \pm 0.03

Table 1

Adapted from Zhan, D., Bai, A., Yu, L., Han, W., & Feng, Y. (2014). Characterization of the PH1704 protease from *Pyrococcus horikoshii* OT3 and the critical functions of Tyr120. *PLoS One*, 9(9), e103902. doi:10.1371/journal.pone.010390

This is just a lot of context; the useful info is that PH1704 is a protease, which means it is an enzyme that cleaves peptide bonds

2 kinds of protease activity for this enzyme

Probably important—cleaves after arginine! AMC sounds like a molecule attached to amino acids or peptides

Y120 seems like an important residue

Trends: catalytic efficiency (k_{cat}/K_m) seems much higher for Y120P, Y120S is much slower at producing product in both cases (lower k_{cat}), and in general efficiency is higher for aminopeptidase than for endopeptidase activity



According to the data, does PH1704 have equivalent aminopeptidase and endopeptidase activity? Why or why not?

The question asks us for data analysis, so we know we should start by making sure we understand what the given material is telling us. A quick scan of the passage reveals unfamiliar enzymes, terminology, and a data-packed table, so a careful read of the passage for the big picture will be important in order to relate what the table is trying to prove. We are also definitely going to need to bring in some of our outside knowledge about enzymes. The passage describes an enzyme called PH1704, which has several characteristics outlined in the first half of the paragraph. We're told it's a protease, which means it breaks peptide bonds, and it's hyperthermophilic. Breaking down the term hyperthermophilic, we get *hyper-* (higher) -*thermo-* (heat) -*philic* (love), and we can thus deduce that the enzyme is from a bacterial species that lives in high temperatures. Next, we're told it's an endopeptidase (*endo-* meaning between) and an aminopeptidase with a strong preference for arginine. There are two substrates described in the study. Notice how only R-AMC can be used to discern the aminopeptidase activity, and AAFR-AMC can be used to test endopeptidase activity. Finally, we're told that Tyr-120 can be important for activity, so researchers created mutations of this residue and the kinetic information of the resulting mutants is described in Table 1.

The question asks us to determine whether PH1704 acts equally as an aminopeptidase and an endopeptidase. In order to answer this question, first note that the mutation data for Y120 is not needed since we are only being asked about the wild-type PH1704 enzyme.

All we really need is a comparison between the WT enzyme with R-AMC as a substrate, and WT enzyme with AAFR-AMC as a substrate. Also, we need to determine whether k_{cat} , k_m , or k_{cat}/k_m will be most useful. Recall that k_{cat} provides information about how much product is produced per unit time, and k_m is a measure of substrate affinity. To have an enzyme that is maximally efficient, you want both a high catalytic turnover (high k_{cat}) and a high substrate affinity (low k_m), so the best comparison would be the k_{cat}/k_m data, which is equal to the catalytic efficiency of an enzyme. The catalytic efficiency for PH1704 as an aminopeptidase is $0.052 \text{ min}^{-1}/\mu\text{M}$, and for PH1704 as an endopeptidase efficiency is $0.018 \text{ min}^{-1}/\mu\text{M}$.

Since these values of catalytic efficiency are different when measuring aminopeptidase and endopeptidase activity, we can conclude that PH1704 functions more effectively as an aminopeptidase than as an endopeptidase.



MCAT CONCEPT CHECK 2.3

Before you move on, assess your understanding of the material with these questions.

1. What are the effects of increasing $[S]$ on enzyme kinetics? What about increasing $[E]$?

- Increasing $[S]$:

- Increasing $[E]$:

-
2. How are the Michaelis–Menten and Lineweaver–Burk plots similar? How are they different?

- Similarities:

- Differences:

3. What does K_m represent? What would an increase in K_m signify?

4. What do the x - and y -intercepts in a Lineweaver–Burk plot represent?

- x -intercept:

- y -intercept:

5. What is enzyme cooperativity?



2.4 Effects of Local Conditions on Enzyme Activity

High-Yield

LEARNING OBJECTIVES

After Chapter 2.4, you will be able to:

- Predict how changes to the environment will alter enzyme behavior
- Estimate the ideal pH and temperature for enzymes found in the human body

The activity of an enzyme is heavily influenced by its environment; in particular, temperature, acidity or alkalinity (pH), and high salinity have significant effects on the ability of an enzyme to carry out its function. Note that the terms **enzyme activity**, **enzyme velocity**, and **enzyme rate** are all used synonymously on the MCAT.

Temperature

Enzyme-catalyzed reactions tend to double in velocity for every 10°C increase in temperature until the optimum temperature is reached; for the human body, this is 37°C (98.6°F or 310 K). After this, activity falls off sharply, as the enzyme will denature at higher temperatures, as shown in Figure 2.7. Some enzymes that are overheated may regain their function if cooled. A real-life example of temperature dependence occurs in Siamese cats. Siamese cats are dark on their faces, ears, tails, and feet but white elsewhere. Why? The enzyme responsible for pigmentation, *tyrosinase*, is mutated in Siamese cats. It is ineffective at body temperature but at cooler temperatures becomes active. Thus, only the tail, feet, ears, and face (cooled by air passing through the nose and mouth) have an active form of the enzyme and are dark.

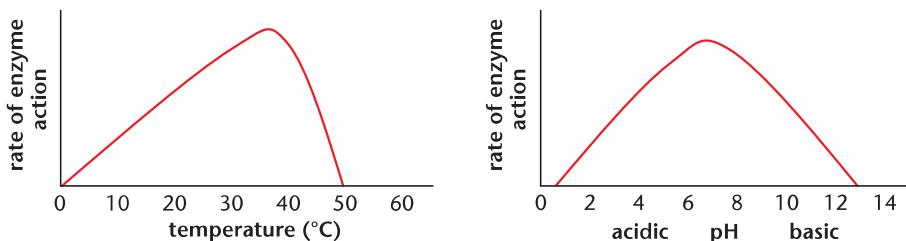


Figure 2.7 Effects of Temperature and pH on the Rate of Enzyme Action

pH

Most enzymes also depend on pH in order to function properly, not only because pH affects the ionization of the active site, but also because changes in pH can lead to denaturation of the enzyme. For enzymes that circulate and function in human blood, this optimal pH is 7.4, as shown in Figure 2.7. A pH < 7.35 in human blood is termed acidemia. Even though it's more basic than *chemically neutral* 7.0, it is more acidic than *physiologically neutral* 7.4. Where might exceptions to this pH 7.4 occur?

BRIDGE

The pH levels in the stomach and intestine, and their effects on these gastric and pancreatic enzymes, are covered in Chapter 9 of *MCAT Biology Review*.



Both are in our digestive tract. Pepsin, which works in the stomach, has maximal activity around pH 2, whereas pancreatic enzymes, which work in the small intestine, work best around pH 8.5.

Salinity

While the effect of salinity or osmolarity is not generally of physiologic significance, altering the concentration of salt can change enzyme activity *in vitro*. Increasing levels of salt can disrupt hydrogen and ionic bonds, causing a partial change in the conformation of the enzyme, and in some cases causing denaturation.

MCAT CONCEPT CHECK 2.4

Before you move on, assess your understanding of the material with these questions.

1. What are the effects of temperature, pH, and salinity on the function of enzymes?
 - Temperature:

- pH:

- Salinity:

2. What is the ideal temperature for most enzymes in the body? The ideal pH?

- Ideal temperature: _____ °C = _____ °F = _____ K
- Ideal pH (most enzymes): _____
- Ideal pH (gastric enzymes): _____
- Ideal pH (pancreatic enzymes): _____



2.5 Regulation of Enzyme Activity

High-Yield

LEARNING OBJECTIVES

After Chapter 2.5, you will be able to:

- Explain feedback inhibition and irreversible inhibition
- Differentiate between the four types of reversible inhibition
- Differentiate between transient and covalent enzyme modifications
- Recall the traits of zymogens

Although enzymes are useful, the body must be able to control when they work; for example, enzymes involved in mitosis should be shut off when cells are no longer dividing (in the G₀ phase). This may be accomplished in a variety of ways, as described below.

Feedback Regulation

Enzymes are often subject to regulation by products further down a given metabolic pathway, a process called **feedback regulation**. Less often, enzymes may be regulated by intermediates that precede the enzyme in the pathway, also called **feed-forward regulation**. This is clearly evident in the study of metabolism, as discussed in Chapters 9 through 12 of *MCAT Biochemistry Review*. While there are some examples of feedback activation, feedback inhibition is far more common. Feedback inhibition, or **negative feedback**, helps maintain homeostasis: once we have enough of a given product, we want to turn off the pathway that creates that product, rather than creating more. In feedback inhibition, the product may bind to the active site of an enzyme or multiple enzymes that acted earlier in its biosynthetic pathway, thereby competitively inhibiting these enzymes and making them unavailable for use. This is schematically represented in Figure 2.8, as we see product D feeding back to inhibit the first enzyme in the pathway.

BRIDGE

Negative feedback is an important topic in both enzymology and the endocrine system. Remember that most hormonal feedback loops are also inhibited by negative feedback. The endocrine system is discussed in Chapter 5 of *MCAT Biology Review*.

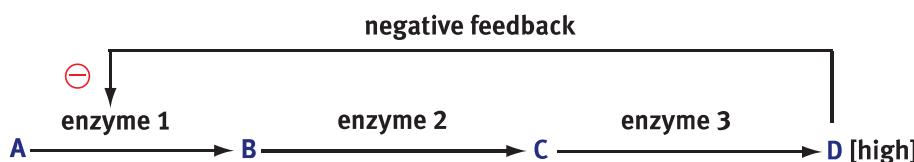


Figure 2.8 Feedback Inhibition by the Product of a Metabolic Pathway

A high concentration of the product, D, inhibits enzyme 1, slowing the entire pathway.



Reversible Inhibition

There are four types of reversible inhibition: competitive, noncompetitive, mixed, and uncompetitive. On Test Day, you will most often encounter competitive and noncompetitive inhibition, but be aware of the differences in the four types. Table 2.2 at the end of this section summarizes the features of each type of reversible inhibition.

Competitive Inhibition

REAL WORLD

The concept of competitive inhibition has relevance in the clinical setting. For example, methanol (wood alcohol), if ingested, is enzymatically converted to toxic metabolites, which can cause blindness and even death. Administration of intravenous ethanol is the treatment of choice for a patient suffering from methanol poisoning. Ethanol works by competing with methanol for the active sites of the enzymes involved.

Competitive inhibition simply involves occupancy of the active site. Substrates cannot access enzymatic binding sites if there is an inhibitor in the way. Competitive inhibition can be overcome by adding more substrate so that the substrate-to-inhibitor ratio is higher. If more molecules of substrate are available than molecules of inhibitor, then the enzyme will be more likely to bind substrate than inhibitor (assuming the enzyme has equal affinity for both molecules). Adding a competitive inhibitor does not alter the value of v_{\max} because if enough substrate is added, it will outcompete the inhibitor and be able to run the reaction at maximum velocity. A competitive inhibitor does increase the measured value of K_m . This is because the substrate concentration has to be higher to reach half the maximum velocity in the presence of the inhibitor. A Lineweaver–Burk plot comparing an enzyme with and without a competitive inhibitor is shown in Figure 2.9.

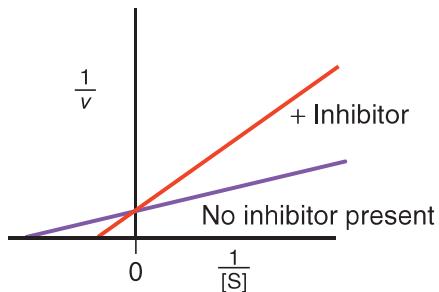


Figure 2.9 Lineweaver–Burk Plot of Competitive Inhibition

Noncompetitive Inhibition

Noncompetitive inhibitors bind to an allosteric site instead of the active site, which induces a change in enzyme conformation. Allosteric sites are non-catalytic regions of the enzyme that bind regulators. Because the two molecules do not compete for the same site, inhibition is considered noncompetitive and cannot be overcome by adding more substrate. Noncompetitive inhibitors bind equally well to the enzyme and the enzyme–substrate complex, unlike mixed inhibitors. Once the enzyme's conformation is altered, no amount of extra substrate will be conducive to forming an enzyme–substrate complex. Adding a noncompetitive inhibitor decreases the measured value of v_{\max} because there is less enzyme available to react; it does not, however, alter the value of K_m because any copies of the enzyme that are still active maintain the same affinity for their substrate. A Lineweaver–Burk plot of an enzyme with and without a noncompetitive inhibitor is shown in Figure 2.10.

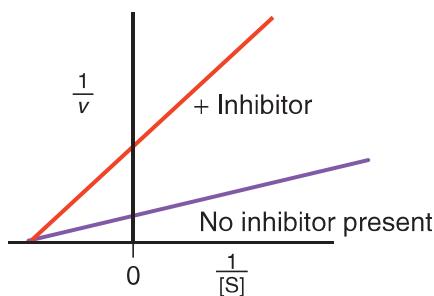


Figure 2.10 Lineweaver–Burk Plot of Noncompetitive Inhibition

Mixed Inhibition

Mixed inhibition results when an inhibitor can bind to either the enzyme or the enzyme–substrate complex, but has different affinity for each. If the inhibitor had the same affinity for both, it would be a noncompetitive inhibitor. Mixed inhibitors do not bind at the active site, but at an allosteric site. Mixed inhibition alters the experimental value of K_m depending on the preference of the inhibitor for the enzyme *vs.* the enzyme–substrate complex. If the inhibitor preferentially binds to the enzyme, it increases the K_m value (lowers affinity); if the inhibitor binds to the enzyme–substrate complex, it lowers the K_m value (increases affinity). In either case, v_{\max} is decreased. On a Lineweaver–Burk plot, the curves for the activity with and without the inhibitor intersect at a point that is not on either axis.

Uncompetitive Inhibition

Uncompetitive inhibitors bind only to the enzyme–substrate complex and essentially lock the substrate in the enzyme, preventing its release. This can be interpreted as increasing affinity between the enzyme and substrate. Because the enzyme–substrate complex has already formed upon binding, uncompetitive inhibitors must bind at an allosteric site; in fact, it is the formation of the enzyme–substrate complex that creates a conformational change that allows the uncompetitive inhibitor to bind. Thus, uncompetitive inhibitors lower K_m and v_{\max} . On a Lineweaver–Burk plot, the curves for activity with and without an uncompetitive inhibitor are parallel.

	Competitive	Noncompetitive	Mixed	Uncompetitive
Binding Site	Active site	Allosteric site	Allosteric site	Allosteric site
Impact on K_m	Increases	Unchanged	Increases or Decreases	Decreases
Impact on v_{\max}	Unchanged	Decreases	Decreases	Decreases

Table 2.2 Comparison of Reversible Inhibitors



Irreversible Inhibition

In this type of inhibition, the active site is made unavailable for a prolonged period of time, or the enzyme is permanently altered. In other words, this type of inhibition is not easily overcome or reversed. A real-world example is aspirin. Acetylsalicylic acid (aspirin) irreversibly modifies *cyclooxygenase-1*. The enzyme can no longer bind its substrate (*arachidonic acid*) to make its products (*prostaglandins*), which are involved in modulating pain and inflammatory responses. To make more prostaglandins, new cyclooxygenase-1 will have to be synthesized through transcription and translation. Irreversible inhibition is a prime drug mechanism, and something that you will come across often in medical school.

Regulated Enzymes

Allosteric Enzymes

Enzymes that are allosteric have multiple binding sites. The active site is present, as well as at least one other site that can regulate the availability of the active site. These are known as **allosteric sites**. **Allosteric enzymes** alternate between an active and an inactive form. The inactive form cannot carry out the enzymatic reaction. Molecules that bind to the allosteric site may be either **allosteric activators** or **allosteric inhibitors**. Binding of either causes a conformational shift in the protein; however, the effect differs. An activator will result in a shift that makes the active site more available for binding to the substrate, whereas an inhibitor will make it less available. In addition to being able to alter the conformation of the protein, binding of activators or inhibitors may alter the activity of the enzyme. As shown in Figure 2.6 above, Michaelis–Menten plots of cooperative allosteric enzyme kinetics often have a sigmoidal (S-shaped) curve.

Covalently Modified Enzymes

In addition to transient interactions, enzymes are often subject to covalent modification. Enzymes can be activated or deactivated by **phosphorylation** or **dephosphorylation**. One cannot predict whether phosphorylation or dephosphorylation will activate an enzyme without experimental determination. **Glycosylation**, the covalent attachment of sugar moieties, is another covalent enzyme modification. Glycosylation can tag an enzyme for transport within the cell, or can modify protein activity and selectivity. Specific mechanisms for the modification of enzymes by glycosylation are still being studied, and are beyond the scope of the MCAT.

BRIDGE

Consider that digestive enzymes, discussed in Chapter 9 of *MCAT Biology Review*, chew up fats, proteins, and carbohydrates—the very same compounds of which our body is made. How do these enzymes know to digest your food but not your body? Simply put, they don't! So, we regulate their activity in a coordinated manner using feedback mechanisms and other substances.

Zymogens

Certain enzymes are particularly dangerous if they are not tightly controlled. These include the digestive enzymes like *trypsin*, which, if released from the pancreas in an uncontrolled manner, would digest the organ itself. To avoid this danger, these enzymes and many others are secreted as inactive **zymogens** like *trypsinogen*. Zymogens contain a catalytic (active) domain and regulatory domain. The regulatory domain must be either removed or altered to expose the active site. Apoptotic enzymes (*caspases*) exhibit similar regulation. Most zymogens have the suffix **-ogen**.



**MCAT CONCEPT CHECK 2.5**

Before you move on, assess your understanding of the material with these questions.

1. What is feedback inhibition?

2. Of the four types of reversible inhibitors, which could potentially increase K_m ?

3. What is irreversible inhibition?

4. What are some examples of transient and covalent enzyme modifications?

- Transient:
-

- Covalent:
-

5. Why are some enzymes released as zymogens?

Conclusion

Our current chapter focused on the way in which cells are able to carry out the reactions necessary for life. We began with a discussion of the types of enzymes that you are likely to encounter on Test Day before reviewing thermodynamics and kinetics in relation to enzymes, which are biological catalysts. We went on to discuss the analysis of kinetic data with two different types of graphs, and talked about cooperativity. Because catalysts are generally most active in their native environment, we considered the impact of temperature, pH, and salinity on their activity. All of these are likely to appear on Test Day.

Enzymes need to be regulated; we analyzed the basics of feedback mechanisms. We talked about inhibitors of enzymes, which may be reversible or irreversible. The difference between the types of reversible inhibition is a key Test Day concept. Finally, we discussed changes in enzyme activity that may include allosteric activation, covalent modification, or cleavage of inactive zymogens. Let's move on now to discuss the nonenzymatic functions of proteins. You will notice many parallels between the new material and the concepts described in this chapter, like binding affinity. By the end of the next chapter, you'll be ready to face any protein question the MCAT can throw at you!



Concept Summary

Enzymes as Biological Catalysts

- Enzymes are biological catalysts that are unchanged by the reactions they catalyze and are reusable.
- Each enzyme catalyzes a single reaction or type of reaction with high specificity.
 - Oxidoreductases catalyze oxidation–reduction reactions that involve the transfer of electrons.
 - Transferases move a functional group from one molecule to another molecule.
 - Hydrolases catalyze cleavage with the addition of water.
 - Lyases catalyze cleavage without the addition of water and without the transfer of electrons. The reverse reaction (synthesis) is often more important biologically.
 - Isomerases catalyze the interconversion of isomers, including both constitutional isomers and stereoisomers.
 - Ligases are responsible for joining two large biomolecules, often of the same type.
- Exergonic reactions release energy; ΔG is negative.
- Enzymes lower the activation energy necessary for biological reactions.
- Enzymes do not alter the free energy (ΔG) or enthalpy (ΔH) change that accompanies the reaction nor the final equilibrium position; rather, they change the rate (kinetics) at which equilibrium is reached.

Mechanisms of Enzyme Activity

- Enzymes act by stabilizing the transition state, providing a favorable microenvironment, or bonding with the substrate molecules.
- Enzymes have an active site, which is the site of catalysis.
- Binding to the active site is explained by the lock and key theory or the induced fit model.
 - The lock and key theory hypothesizes that the enzyme and substrate are exactly complementary.
 - The induced fit model hypothesizes that the enzyme and substrate undergo conformational changes to interact fully.
- Some enzymes require metal cation cofactors or small organic coenzymes to be active.



Enzyme Kinetics

- Enzymes experience **saturation kinetics**: as substrate concentration increases, the reaction rate does as well until a maximum value is reached.
- Michaelis-Menten** and **Lineweaver-Burk** plots represent this relationship as a hyperbola and line, respectively.
- Enzymes can be compared on the basis of their K_m and v_{max} values.
- Cooperative enzymes** display a sigmoidal curve because of the change in activity with substrate binding.

Effects of Local Conditions on Enzyme Activity

- Temperature and pH affect an enzyme's activity *in vivo*; changes in temperature and pH can result in denaturing of the enzyme and loss of activity due to loss of secondary, tertiary, or, if present, quaternary structure.
- In vitro*, salinity can impact the action of enzymes.

Regulation of Enzyme Activity

- Enzyme pathways are highly regulated and subject to inhibition and activation.
- Feedback inhibition** is a regulatory mechanism whereby the catalytic activity of an enzyme is inhibited by the presence of high levels of a product later in the same pathway.
- Reversible inhibition** is characterized by the ability to replace the inhibitor with a compound of greater affinity or to remove it using mild laboratory treatment.
 - Competitive inhibition** results when the inhibitor is similar to the substrate and binds at the active site. Competitive inhibition can be overcome by adding more substrate. v_{max} is unchanged, K_m increases.
 - Noncompetitive inhibition** results when the inhibitor binds with equal affinity to the enzyme and the enzyme–substrate complex. v_{max} is decreased, K_m is unchanged.
 - Mixed inhibition** results when the inhibitor binds with unequal affinity to the enzyme and the enzyme–substrate complex. v_{max} is decreased, K_m is increased or decreased depending on if the inhibitor has higher affinity for the enzyme or enzyme–substrate complex.
 - Uncompetitive inhibition** results when the inhibitor binds only with the enzyme–substrate complex. K_m and v_{max} both decrease.



- **Irreversible inhibition** alters the enzyme in such a way that the active site is unavailable for a prolonged duration or permanently; new enzyme molecules must be synthesized for the reaction to occur again.
- Regulatory enzymes can experience activation as well as inhibition.
 - **Allosteric** sites can be occupied by activators, which increase either affinity or enzymatic turnover.
 - **Phosphorylation** (covalent modification with phosphate) or **glycosylation** (covalent modification with carbohydrate) can alter the activity or selectivity of enzymes.
 - **Zymogens** are secreted in an inactive form and are activated by cleavage.



Answers to Concept Checks

2.1

1. Catalysts are characterized by two main properties: they reduce the activation energy of a reaction, thus speeding up the reaction, and they are not used up in the course of the reaction. Enzymes improve the environment in which a particular reaction takes place, which lowers its activation energy. They are also regenerated at the end of the reaction to their original form.
2. Enzyme specificity refers to the idea that a given enzyme will only catalyze a given reaction or type of reaction. For example, *serine/threonine-specific protein kinases* will only place a phosphate group onto the hydroxyl group of a serine or threonine residue.

3.

Name	Function
Ligase	Addition or synthesis reactions, generally between large molecules, often require ATP
Isomerase	Rearrangement of bonds within a compound
Lyase	Cleavage of a single molecule into two products, or synthesis of small organic molecules
Hydrolase	Breaking of a compound into two molecules using the addition of water
Oxidoreductase	Oxidation–reduction reactions (transferring electrons)
Transferase	Movement of a functional group from one molecule to another

4. Enzymes have no effect on the overall thermodynamics of the reaction; they have no effect on the ΔG or ΔH of the reaction, although they do lower the energy of the transition state, thus lowering the activation energy. However, enzymes have a profound effect on the kinetics of a reaction. By lowering activation energy, equilibrium can be achieved faster (although the equilibrium position does not change).

2.2

1.

Lock and Key	Induced Fit
<ul style="list-style-type: none">• Active site of enzyme fits exactly around substrate• No alterations to tertiary or quaternary structure of enzyme• Less accurate model	<ul style="list-style-type: none">• Active site of enzyme molds itself around substrate only when substrate is present• Tertiary and quaternary structure is modified for enzyme to function• More accurate model



2. Cofactors and coenzymes both act as activators of enzymes. Cofactors tend to be inorganic (minerals), while coenzymes tend to be small organic compounds (vitamins). In both cases, these regulators induce a conformational change in the enzyme that promotes its activity. Tightly bound cofactors or coenzymes that are necessary for enzyme function are termed prosthetic groups.

2.3

1. Increasing $[S]$ has different effects, depending on how much substrate is present to begin with. When the substrate concentration is low, an increase in $[S]$ causes a proportional increase in enzyme activity. At high $[S]$, however, when the enzyme is saturated, increasing $[S]$ has no effect on activity because v_{\max} has already been attained.

Increasing $[E]$ will always increase v_{\max} , regardless of the starting concentration of enzyme.

2. Both the Michaelis–Menten and Lineweaver–Burk relationships account for the values of K_m and v_{\max} under various conditions. They both provide simple graphical interpretations of these two variables and are derived from the Michaelis–Menten equation. However, the axes of these graphs and visual representation of this information is different between the two. The Michaelis–Menten plot is v vs. $[S]$, which creates a hyperbolic curve for monomeric enzymes. The Lineweaver–Burk plot, on the other hand, is $\frac{1}{v}$ vs. $\frac{1}{[S]}$, which creates a straight line.
3. K_m is a measure of an enzyme's affinity for its substrate, and is defined as the substrate concentration at which an enzyme is functioning at half of its maximal velocity. As K_m increases, an enzyme's affinity for its substrate decreases.
4. The x -intercept represents $-\frac{1}{K_m}$; the y -intercept represents $\frac{1}{v_{\max}}$.
5. Cooperativity refers to the interactions between subunits in a multisubunit enzyme or protein. The binding of substrate to one subunit induces a change in the other subunits from the T (tense) state to the R (relaxed) state, which encourages binding of substrate to the other subunits. In the reverse direction, the unbinding of substrate from one subunit induces a change from R to T in the remaining subunits, promoting unbinding of substrate from the remaining subunits.



2.4

1. As temperature increases, enzyme activity generally increases (doubling approximately every 10°C). Above body temperature, however, enzyme activity quickly drops off as the enzyme denatures. Enzymes are maximally active within a small pH range; outside of this range, activity drops quickly with changes in pH as the ionization of the active site changes and the protein is denatured. Changes in salinity can disrupt bonds within an enzyme, causing disruption of tertiary and quaternary structure, which leads to loss of enzyme function.
2. Ideal temperature: $37^{\circ}\text{C} = 98.6^{\circ}\text{F} = 310\text{ K}$
Ideal pH for most enzymes is 7.4; for gastric enzymes, around 2; for pancreatic enzymes, around 8.5.

2.5

1. Feedback inhibition refers to the product of an enzymatic pathway turning off enzymes further back in that same pathway. This helps maintain homeostasis: as product levels rise, the pathway creating that product is appropriately downregulated.
2. A competitive inhibitor increases K_m because the substrate concentration has to be higher to reach half the maximum velocity in the presence of the inhibitor. A mixed inhibitor will increase K_m only if the inhibitor preferentially binds to the enzyme over the enzyme–substrate complex.
3. Irreversible inhibition refers to the prolonged or permanent inactivation of an enzyme, such that it cannot be easily renatured to gain function.
4. Examples of transient modifications include allosteric activation or inhibition. Examples of covalent modifications include phosphorylation and glycosylation.
5. Zymogens are precursors of active enzymes. It is critical that certain enzymes (like the digestive enzymes of the pancreas) remain inactive until arriving at their target site.



Equations to Remember



(2.2) Michaelis–Menten equation: $v = \frac{v_{\max} [S]}{K_m + [S]}$

(2.3) Turnover number (k_{cat}): $v_{\max} = [E]k_{\text{cat}}$

Shared Concepts

Biochemistry Chapter 1

Amino Acids, Peptides, and Proteins

Biochemistry Chapter 12

Bioenergetics and Regulation of Metabolism

Biology Chapter 5

The Endocrine System

Biology Chapter 9

The Digestive System

General Chemistry Chapter 5

Chemical Kinetics

General Chemistry Chapter 7

Thermochemistry

General Chemistry Chapter 11

Oxidation–Reduction Reactions

DISCRETE PRACTICE QUESTIONS

Consult your online resources for additional practice.

GO ONLINE

1. Consider a biochemical reaction $A \rightarrow B$, which is catalyzed by *A-B dehydrogenase*. Which of the following statements is true?
 - A. The reaction will proceed until the enzyme concentration decreases.
 - B. The reaction will be most favorable at 0°C .
 - C. A component of the enzyme is transferred from A to B.
 - D. The free energy change (ΔG) of the catalyzed reaction is the same as for the uncatalyzed reaction.
2. Which of the following statements about enzyme kinetics is FALSE?
 - A. An increase in the substrate concentration (at constant enzyme concentration) leads to proportional increases in the rate of the reaction.
 - B. Most enzymes operating in the human body work best at a temperature of 37°C .
 - C. An enzyme-substrate complex can either form a product or dissociate back into the enzyme and substrate.
 - D. Maximal activity of many human enzymes occurs around pH 7.4.
3. Some enzymes require the presence of a nonprotein molecule to behave catalytically. An enzyme devoid of this molecule is called a(n):
 - A. holoenzyme.
 - B. apoenzyme.
 - C. coenzyme.
 - D. zymoenzyme.
4. Which of the following factors determine an enzyme's specificity?
 - A. The three-dimensional shape of the active site
 - B. The Michaelis constant
 - C. The type of cofactor required for the enzyme to be active
 - D. The prosthetic group on the enzyme
5. Enzymes increase the rate of a reaction by:
 - A. decreasing the activation energy.
 - B. decreasing the overall free energy change of the reaction.
 - C. increasing the activation energy.
 - D. increasing the overall free energy change of the reaction.
6. In the equation below, substrate C is an allosteric inhibitor to enzyme 1. Which of the following is another mechanism necessarily caused by substrate C?
$$A \xrightarrow{\text{enzyme 1}} B \xrightarrow{\text{enzyme 2}} C$$
 - A. Competitive inhibition
 - B. Irreversible inhibition
 - C. Feedback enhancement
 - D. Negative feedback



7. The activity of an enzyme is measured at several different substrate concentrations, and the data are shown in the table below.

[S] (mM)	v (mmol/sec)
0.01	1
0.05	9.1
0.1	17
0.5	50
1	67
5	91
10	95
50	99
100	100

K_m for this enzyme is approximately:

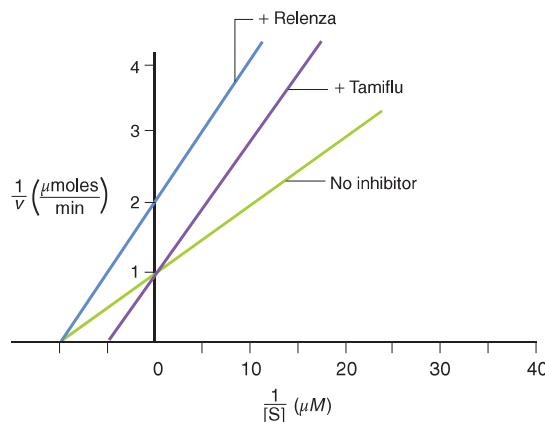
- A. 0.5.
- B. 1.
- C. 10.
- D. 50.

Questions 8 and 9 refer to the following statement:

Consider a reaction catalyzed by enzyme A with a K_m value of $5 \times 10^{-6} M$ and v_{max} of $20 \frac{\text{mmol}}{\text{min}}$.

8. At a concentration of $5 \times 10^{-6} M$ substrate, the rate of the reaction will be:
- A. $10 \frac{\text{mmol}}{\text{min}}$.
 - B. $20 \frac{\text{mmol}}{\text{min}}$.
 - C. $30 \frac{\text{mmol}}{\text{min}}$.
 - D. $40 \frac{\text{mmol}}{\text{min}}$.
9. At a concentration of $5 \times 10^{-4} M$ substrate, the rate of the reaction will be:
- A. $10 \frac{\text{mmol}}{\text{min}}$.
 - B. $15 \frac{\text{mmol}}{\text{min}}$.
 - C. $20 \frac{\text{mmol}}{\text{min}}$.
 - D. $30 \frac{\text{mmol}}{\text{min}}$.

10. The graph below shows kinetic data obtained for flu virus enzyme activity as a function of substrate concentration in the presence and absence of two antiviral drugs.



Based on the graph, which of the following statements is correct?

- A. Both drugs are noncompetitive inhibitors of the viral enzyme.
 - B. Tamiflu increases the K_m value for the substrate compared to Relenza.
 - C. Relenza increases the v_{max} value for the substrate compared to Tamiflu.
 - D. Both drugs are competitive inhibitors of the viral enzyme.
11. The conversion of ATP to cyclic AMP and inorganic phosphate is most likely catalyzed by which class of enzyme?
- A. Ligase
 - B. Hydrolase
 - C. Lyase
 - D. Transferase
12. Which of the following is NOT a method by which enzymes decrease the activation energy for biological reactions?
- A. Modifying the local charge environment
 - B. Forming transient covalent bonds
 - C. Acting as electron donors or receptors
 - D. Breaking bonds in the enzyme irreversibly to provide energy



- 13.** A certain cooperative enzyme has four subunits, two of which are bound to substrate. Which of the following statements can be made?
- A.** The affinity of the enzyme for the substrate has just increased.
 - B.** The affinity of the enzyme for the substrate has just decreased.
 - C.** The affinity of the enzyme for the substrate is at the average for this enzyme class.
 - D.** The affinity of the enzyme for the substrate is greater than with one substrate bound.
- 14.** Which of the following is LEAST likely to be required for a series of metabolic reactions?
- A.** Triacylglycerol acting as a coenzyme
 - B.** Oxidoreductase enzymes
 - C.** Magnesium acting as a cofactor
 - D.** Transferase enzymes
- 15.** How does the ideal temperature for a reaction change with and without an enzyme catalyst?
- A.** The ideal temperature is generally higher with a catalyst than without.
 - B.** The ideal temperature is generally lower with a catalyst than without.
 - C.** The ideal temperature is characteristic of the reaction, not the enzyme.
 - D.** No conclusion can be made without knowing the enzyme type.

DISCRETE PRACTICE ANSWERS

1. D

Enzymes catalyze reactions by lowering their activation energy, and are not changed or consumed during the course of the reaction. While the activation energy is lowered, the free energy of the reaction, ΔG , remains unchanged in the presence of an enzyme. A reaction will continue to occur in the presence or absence of an enzyme; it simply runs slower without the enzyme, eliminating (A). Most physiological reactions are optimized at body temperature, 37°C, eliminating (B). Finally, dehydrogenases catalyze oxidation-reduction reactions, not transfer reactions, eliminating (C).

2. A

Most enzymes in the human body operate at maximal activity around a temperature of 37°C and a pH of 7.4, which is the pH of most body fluids. In addition, as characterized by the Michaelis–Menten equation, enzymes form an enzyme-substrate complex, which can either dissociate back into the enzyme and substrate or proceed to form a product. So far, we can eliminate (B), (C), and (D), so let's check (A). An increase in the substrate concentration, while maintaining a constant enzyme concentration, leads to a proportional increase in the rate of the reaction only initially. However, once most of the active sites are occupied, the reaction rate levels off, regardless of further increases in substrate concentration. At high concentrations of substrate, the reaction rate approaches its maximal velocity and is no longer changed by further increases in substrate concentration.

3. B

An enzyme devoid of its necessary cofactor is called an apoenzyme and is catalytically inactive.

4. A

An enzyme's specificity is determined by the three-dimensional shape of its active site. Regardless of which explanation for enzyme specificity we are discussing (lock and key or induced fit), the active site determines which substrate the enzyme will react with.

5. A

Enzymes increase the rate of a reaction by decreasing the activation energy. They do not affect the overall free energy, ΔG , of the reaction.

6. D

By limiting the activity of enzyme 1, the rest of the pathway is slowed, which is the definition of negative feedback. (A) is incorrect because there is no competition for the active site with allosteric interactions. While many products do indeed competitively inhibit an enzyme in the pathway that creates them, this is an example of an allosterically inhibited enzyme. There is not enough information for (B) to be correct because we aren't told whether the inhibition is reversible. In general, allosteric interactions are temporary. (C) is incorrect because it is the opposite of what occurs when enzyme 1 activity is reduced.

7. A

While the equations given in the text are useful, recognizing relationships is even more important. You can see that as substrate concentration increases significantly, there is only a small change in the rate. This occurs as we approach v_{\max} . Because the v_{\max} is near 100 $\frac{\text{mmol}}{\text{min}}$, $\frac{v_{\max}}{2}$ equals 50 $\frac{\text{mmol}}{\text{min}}$. The substrate concentration giving this rate is 0.5 mM and corresponds to K_m ; therefore, (A) is correct.

8. A

As with the last question, relationships are important. At a concentration of $5 \times 10^{-6} M$, enzyme A is working at one-half of its v_{\max} because the concentration is equal to the K_m of the enzyme. Therefore, one-half of $20 \frac{\text{mmol}}{\text{min}}$ is $10 \frac{\text{mmol}}{\text{min}}$, which corresponds to (A).

9. **C**

At a concentration of $5 \times 10^{-4} M$, there is 100 times more substrate than present at half maximal velocity. At high values (significantly larger than the value of K_m), the enzyme is at or near its v_{max} , which is $20 \frac{\text{mmol}}{\text{min}}$.

10. **B**

Based on the graph, when the substrate is present, Tamiflu results in the same v_{max} and a higher K_m compared to when no inhibitor is added. These are hallmarks of competitive inhibitors. Noncompetitive inhibitors result in decreased v_{max} and the same K_m as the uninhibited reaction, which is shown by the Relenza line in the graph. Because the question is only comparing the values between the two inhibitors, and not the enzyme without inhibitor, the mechanism of inhibition is less important to determine than the values of K_m and v_{max} . This is a great example of why previewing the answer choices works well in the sciences.

11. **C**

Lyases are responsible for the breakdown of a single molecule into two molecules without the addition of water or the transfer of electrons. Lyases often form cyclic compounds or double bonds in the products to accommodate this. Water was not a reactant, and no cofactor was mentioned; thus lyase, (C), remains the best answer choice.

12. **D**

Enzymes are not altered by the process of catalysis. A molecule that breaks intramolecular bonds to provide activation energy would not be able to be reused.

13. **D**

Cooperative enzymes demonstrate a change in affinity for the substrate depending on how many substrate molecules are bound and whether the last change was accomplished because a substrate molecule was bound or left the active site of the enzyme. Because we cannot determine whether the most recent reaction was binding or dissociation, (A) and (B) are eliminated. We can make absolute comparisons though. The unbound enzyme has the lowest affinity for substrate, and the enzyme with all but one subunit bound has the highest. The increase in affinity is not linear, and therefore (C) is not necessarily true. An enzyme with two subunits occupied must have a higher affinity for the substrate than the same enzyme with only one subunit occupied; thus, (D) is correct.

14. **A**

Triglycerides are unlikely to act as coenzymes for a few reasons, including their large size, neutral charge, and ubiquity in cells. Cofactors and coenzymes tend to be small in size, such as metal ions like (C) or small organic molecules. They can usually carry a charge by ionization, protonation, or deprotonation. Finally, they are usually in low, tightly regulated concentrations within cells. Metabolic pathways would be expected to include both oxidation–reduction reactions and movement of functional groups, thus eliminating (B) and (D).

15. **B**

The rate of reaction increases with temperature because of the increased kinetic energy of the reactants, but reaches a peak temperature because the enzyme denatures with the disruption of hydrogen bonds at excessively high temperatures. In the absence of enzyme, this peak temperature is generally much hotter. Heating a reaction provides molecules with an increased chance of achieving the activation energy, but the enzyme catalyst would typically reduce activation energy. Keep in mind that thermodynamics and kinetics are not interchangeable, so we are not considering the impact of heat on the equilibrium position.

