19.6: Enzyme Regulation - Inhibition

Learning Objectives

- Explain what an enzyme inhibitor is.
- Distinguish between reversible and irreversible inhibitors.
- Distinguish between competitive, noncompetitive, and uncompetitive inhibitors.

Previously, we noted that enzymes can be inactivated at high temperatures and by changes in pH. These are *nonspecific* factors that would inactivate any enzyme. The activity of enzymes can also be regulated by more *specific* **inhibitors** that slow or stop catalysis. Enzyme inhibition can either be *reversible* or *irreversible*. In reversible inhibition, the inhibitor can bind (usually non-covalently) and dissociate, allowing enzyme activity to return back to its original, uninhibited level. Irreversible inhibitors bind to the enzyme permanently and thus permanently inhibit enzyme activity.

Reversible Inhibition

Reversible enzyme inhibition can be *competitive*, *noncompetitive*, or *uncompetitive*, depending on where the inhibitor binds to the enzyme, substrate, or enzyme-substrate complex.

Competitive inhibition is when an inhibitor reversibly binds to an enzyme at the enzyme active site; competing with the substrate for binding. A competitive inhibitor must be a molecule that is *structurally similar* to the substrate molecule, allowing it to interact with the enzyme active site through similar non-covalent interactions, but it does not, or cannot, undergo the same chemical reaction. When the inhibitor is bound to the active site, it blocks the correct substrate from binding and catalysis from occurring. However, as a reversible inhibitor, it can disassociate from the enzyme eventually allowing for the correct substrate to bind and the catalysis to occur. Because the inhibitor and substrate are in competition for the same active site, inhibition is concentration-dependent. As shown in the below plot of rate of reaction vs. substrate concentration (Figure 19.6.1), the competitive inhibitor slows the rate of reaction, but at higher substrate concentrations, the normal maximum rate can be reached.

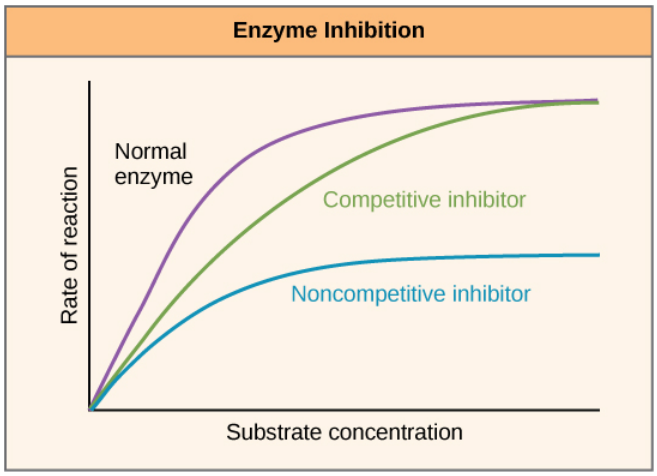


Figure 19.6.1: Plots of reaction rate vs. substrate concentration with and without inhibitors. Competitive inhibitors bind reversibly at the active site and therefore, compete with the substrate for binding. As substrate concentration increases, it can outcompete the inhibitor allowing enzyme activity to reach a normal maximum (green line). A noncompetive inhibitor binds at a site separate from the active site, the enzyme activity can only reach a lower than normal maximum reaction rate even as substrate concentration increases (blue line). Uncompetitive inhibition is not represented on this plot, but would be similar to the noncompetitive inhibitor, reaching a lower maximum rate. (Figure from OpenStax Biology)

Studies of competitive inhibition have provided helpful information about certain enzyme-substrate complexes and the interactions of specific groups at the active sites. As a result, pharmaceutical companies have synthesized drugs that competitively inhibit metabolic processes in bacteria and certain cancer cells. Many drugs are competitive inhibitors of specific enzymes.

A classic example of competitive inhibition is the effect of malonate on the enzyme activity of succinate dehydrogenase (Figure 19.6.2). Malonate and succinate are the anions of dicarboxylic acids and contain three and four carbon atoms, respectively. The malonate molecule binds to the active site because the spacing of its carboxyl groups is not greatly different from that of succinate.

However, no catalytic reaction occurs because malonate does not have a CH_2CH_2 group to convert to CH=CH. This reaction will also be discussed in connection with the Krebs cycle and energy production in a later chapter.

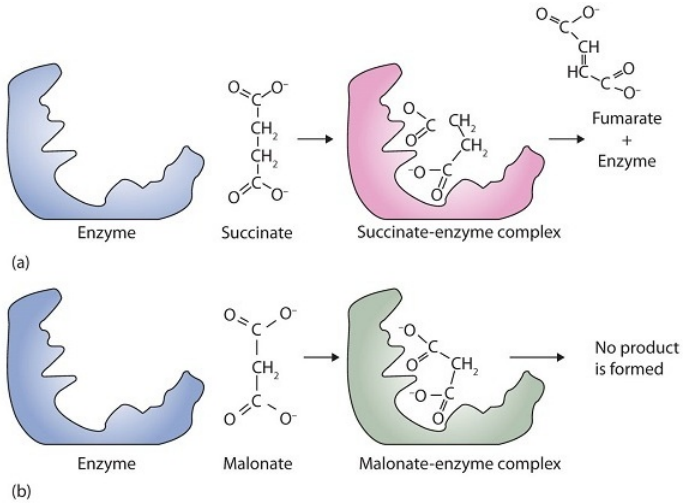


Figure 19.6.2: Competitive Inhibition. (a) Succinate binds to the enzyme succinate dehydrogenase. A dehydrogenation reaction occurs, and the product—fumarate—is released from the enzyme. (b) Malonate, a competitive inhibitor, also binds to the active site of succinate dehydrogenase. In this case, however, no subsequent reaction occurs while malonate remains bound to the enzyme.

In **uncompetitive inhibition**, the inhibitor can only bind the enzyme when the substrate is already bound, in other words it binds the enzyme-substrate complex but not the enzyme alone. The maximum reaction rate in the presence of an uncompetitive inhibitor is lowered, however, unlike with competitive inhibition, the rate cannot be increased by adding more substrate. This type of inhibition is most commonly seen when the enzyme reaction involves two substrates and as long as the concentration of inhibitor remains constant, the maximum reaction rate does not change.

A **noncompetitive inhibitor** can bind to either the free enzyme or the enzyme-substrate complex because its binding site on the enzyme is distinct from the active site. Binding of this kind of inhibitor alters the three-dimensional conformation of the enzyme, changing the configuration of the active site with one of two results. Either the enzyme-substrate complex does not form at its normal rate, or, once formed, it does not yield products at the normal rate (see Figure 19.6.1). Because the inhibitor does not structurally resemble the substrate, nor is it competing with the substrate for the active site, the addition of excess substrate does *not* reverse the inhibitory effect.

Chemotherapy is the strategic use of chemicals (that is, drugs) to destroy infectious microorganisms or cancer cells without causing excessive damage to the other, healthy cells of the host. From bacteria to humans, the metabolic pathways of all living organisms are quite similar, so the search for safe and effective chemotherapeutic agents is a formidable task. Many well-established chemotherapeutic drugs function by inhibiting a critical enzyme in the cells of the invading organism.

An *antibiotic* is a compound that kills bacteria; it may come from a natural source such as molds or be synthesized with a structure analogous to a naturally occurring antibacterial compound. Antibiotics constitute no well-defined class of chemically related substances, but many of them work by effectively inhibiting a variety of enzymes essential to bacterial growth.

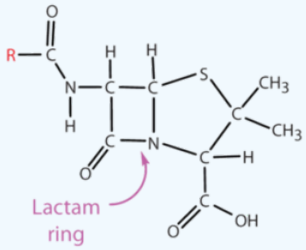
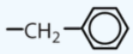
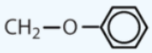
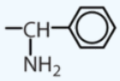
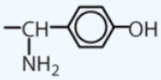
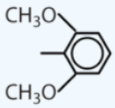
To Your Health: Penicillin

Penicillin, one of the most widely used antibiotics in the world, was fortuitously discovered by Alexander Fleming in 1928, when he noticed antibacterial properties in a mold growing on a bacterial culture plate. In 1938, Ernst Chain and Howard Florey began an intensive effort to isolate penicillin from the mold and study its properties. The large quantities of penicillin needed for this research became available through development of a corn-based nutrient medium that the mold loved and through the discovery of a higher-yielding strain of mold at a United States Department of Agriculture research center near Peoria, Illinois. Even so, it was not until 1944 that large quantities of penicillin were being produced and made available for the treatment of bacterial infections.

Penicillin functions by interfering with the synthesis of cell walls of reproducing bacteria. It does so by inhibiting an enzyme—transpeptidase—that catalyzes the last step in bacterial cell-wall biosynthesis. The defective walls cause bacterial cells to burst. Human cells are not affected because they have cell membranes, not cell walls.

Several naturally occurring penicillins have been isolated. They are distinguished by different R groups connected to a common structure: a four-member cyclic amide (called a lactam ring) fused to a five-member ring. The addition of appropriate organic compounds to the culture medium leads to the production of the different kinds of penicillin.

The penicillins are effective against gram-positive bacteria (bacteria capable of being stained by Gram's stain) and a few gram-negative bacteria (including the intestinal bacterium *Escherichia coli*). They are effective in the treatment of diphtheria, gonorrhea, pneumonia, syphilis, many pus infections, and certain types of boils. Penicillin G was the earliest penicillin to be used on a wide scale. However, it cannot be administered orally because it is quite unstable; the acidic pH of the stomach converts it to an inactive derivative. The major oral penicillins—penicillin V, ampicillin, and amoxicillin—on the other hand, are acid stable.

Penicillin Structure	R Group	Drug Name
		penicillin G
		penicillin V
		ampicillin
		amoxicillin
		methicillin

Some strains of bacteria become resistant to penicillin through a mutation that allows them to synthesize an enzyme—penicillinase—that breaks the antibiotic down (by cleavage of the amide linkage in the lactam ring). To combat these strains, scientists have synthesized penicillin analogs (such as methicillin) that are not inactivated by penicillinase.

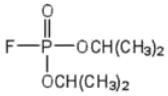
Some people (perhaps 5% of the population) are allergic to penicillin and therefore must be treated with other antibiotics. Their allergic reaction can be so severe that a fatal coma may occur if penicillin is inadvertently administered to them. Fortunately, several other antibiotics have been discovered. Most, including aureomycin and streptomycin, are the products of microbial synthesis. Others, such as the semisynthetic penicillins and tetracyclines, are made by chemical modifications of antibiotics; and some, like chloramphenicol, are manufactured entirely by chemical synthesis. They are as effective as penicillin in destroying infectious microorganisms. Many of these antibiotics exert their effects by blocking protein synthesis in microorganisms.

Initially, antibiotics were considered miracle drugs, substantially reducing the number of deaths from blood poisoning, pneumonia, and other infectious diseases. Some seven decades ago, a person with a major infection almost always died. Today, such deaths are rare. Seven decades ago, pneumonia was a dreaded killer of people of all ages. Today, it kills only the very old or those ill from other causes. Antibiotics have indeed worked miracles in our time, but even miracle drugs have limitations. Not long after the drugs were first used, disease organisms began to develop strains resistant to them. In a race to stay ahead of resistant bacterial strains, scientists continue to seek new antibiotics. The penicillins have now been partially displaced by related compounds, such as the cephalosporins and vancomycin. Unfortunately, some strains of bacteria have already shown resistance to these antibiotics.

Irreversible Inhibition

An **irreversible inhibitor** inactivates an enzyme by bonding covalently to a particular group at the active site. When the inhibitor is bound, the enzyme active site is blocked, the substrate does not bind, and catalysis cannot occur, similar to competitive inhibition. The difference here is that the inhibition is *irreversible*, meaning that the inhibitor remains bound and does not dissociate from the enzyme because the enzyme-inhibitor covalent bonds are not easily broken. In the presence of an irreversible inhibitor, the substrate cannot bind the active site at all, nor can high substrate concentrations outcompete the inhibitor, hence the enzyme is completely inactivated. Many of the known irreversible inhibitors are *poisons* because they inactivate an enzyme completely. Some examples are provided in Table 19.6.1 below.

Table 19.6.1: Poisons as Enzyme Inhibitors

Poison	Formula	Example of Enzyme Inhibited	Action
arsenate	AsO_4^{3-}	glyceraldehyde 3-phosphate dehydrogenase	substitutes for phosphate
iodoacetate	ICH_2COO^-	triose phosphate dehydrogenase	binds to cysteine SH group
diisopropylfluoro-phosphate (DIFP; a nerve poison)		acetylcholinesterase	binds to serine OH group

Summary

An irreversible inhibitor inactivates an enzyme by bonding covalently to a particular group at the active site. A reversible inhibitor inactivates an enzyme through noncovalent, reversible interactions. A competitive inhibitor competes with the substrate for binding at the active site of the enzyme. A noncompetitive inhibitor binds at a site distinct from the active site.

Concept Review Exercises

1. What are the characteristics of an irreversible inhibitor?
2. In what ways does a competitive inhibitor differ from a noncompetitive inhibitor?

Answers

1. It inactivates an enzyme by bonding covalently to a particular group at the active site.
2. A competitive inhibitor structurally resembles the substrate for a given enzyme and competes with the substrate for binding at the active site of the enzyme. A noncompetitive inhibitor binds at a site distinct from the active site and can bind to either the free enzyme or the enzyme-substrate complex.

Exercises

1. What amino acid is present in the active site of all enzymes that are irreversibly inhibited by nerve gases such as DIFP?
2. Oxaloacetate (OOCCH2COCOO) inhibits succinate dehydrogenase. Would you expect oxaloacetate to be a competitive or noncompetitive inhibitor? Explain.

Answer

1. serine
