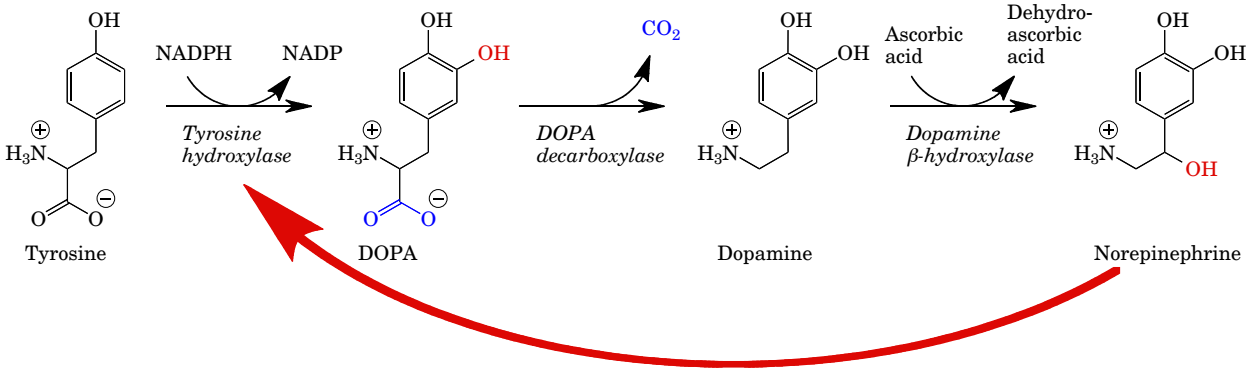
Enzyme Inhibition

Why inhibit enzymes?

Physiological reasons

Enzyme inhibition is a common physiological process. Some aspects are fairly obvious: in tissues that synthesize proteases, inhibitors are necessary to prevent inappropriate proteolysis. A number of serum proteins act as protease inhibitors, including a protein called α_2 macroglobulin, which surrounds proteolytic enzymes and prevents substrate binding. The pancreas has several methods for preventing self-destruction by its secreted digestive proteases. One, discussed earlier, is the production of inactive precursors of the enzymes. Another is the production of potent protease inhibitors, such as a peptide called "pancreatic trypsin inhibitor".

Organisms also use enzyme inhibition as one **method for regulating of metabolic pathways**; reducing the activity of one enzyme in a pathway prevents the reactions from occurring and therefore prevents both substrate utilization and product formation. One common form of this occurs when the final product of a pathway involving several enzymes inhibits the first enzyme in the pathway. As an example, the amino acid tyrosine is converted to the neurotransmitter norepinephrine by a three-enzyme pathway (shown below). The first enzyme in the pathway (*tyrosine hydroxylase*) is inhibited by norepinephrine; when norepinephrine levels are high enough, inhibition by the product acts as a signal to cease additional synthesis. This (combined with other mechanisms for regulation of tyrosine hydroxylase) avoids both overproduction of norepinephrine, and excessive utilization of the valuable amino acid tyrosine.



Understanding this type of regulation can be useful. In Parkinson's syndrome, cells that normally produce and release dopamine are damaged; the symptoms of Parkinson's syndrome are due to lack of dopamine stimulation of cells in parts of the brain. Administering dopamine to individuals with Parkinson's syndrome is ineffective, because dopamine does not cross the blood-brain barrier. Administering additional tyrosine does not result in enhanced dopamine production, because regulation of tyrosine hydroxylase prevents overproduction of dopamine from tyrosine in any individual cell. In contrast, administration of DOPA bypasses the regulated tyrosine hydroxylase step; because DOPA crosses the blood-brain barrier, it results in overproduction of dopamine in the remaining cells, and thereby results

in some alleviation of Parkinson's syndrome symptoms.

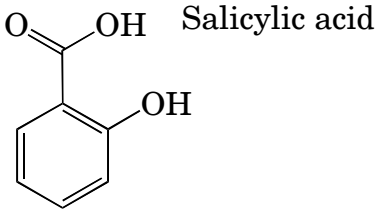
Scientific reasons

The use of inhibitors is an extremely useful method for **studying enzyme mechanisms**. Examining which compounds inhibit an enzyme and which do not can yield information about the binding properties of the enzyme. In addition, inhibitors can yield information about substrate specificity and about the types of reactions that the enzyme can catalyze.

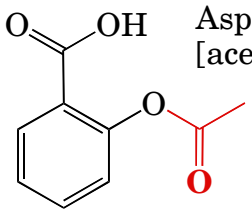
A second major research use of inhibitors is for **studying metabolic pathways**. A full understanding of any metabolic pathway depends on an understanding of each enzyme in the pathway, and how each reaction fits with the others. One method for doing this is to use inhibitors as probes of the role of each enzyme. In cells, the result of enzyme inhibition is accumulation of the physiological substrate, and decreased levels of the physiological product, and of subsequent compounds within the pathway. The use of enough inhibitors allows the entire pathway to be worked out. In whole cells, or whole organisms, the effect of an inhibitor may allow observation of compensatory regulatory changes as the organism attempts to alleviate the effects of decreased levels of products of the pathway.

Therapeutic reasons

Drugs often work by inhibiting an enzyme, and therefore preventing the reaction the enzyme catalyzes. As an example of this common mechanism, the first true drug, aspirin, is an inhibitor of the enzyme *cyclooxygenase*. Aspirin is a derivative of the salicylic acid, a compound found in the bark of some trees. The fact that chewing on the bark of these trees acted as a painkiller was known for centuries. However, people who regularly chewed on the bark frequently developed stomach problems. Acetylating the salicylic acid reduced the undesirable side effects, and made the compound more potent. The reasons for the effectiveness of aspirin are complex, and are left for subsequent courses.



Salicylic acid



Aspirin
[acetyl salicylic acid]

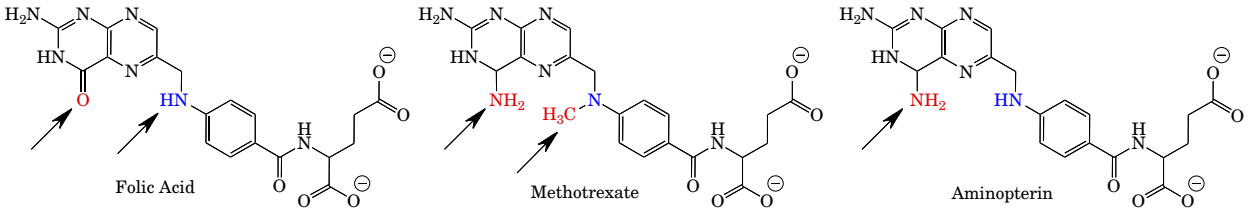
A wide variety of drugs since the invention of aspirin also work by inhibiting specific enzymes.

How does inhibition occur?

Active site occupation

If a molecule that cannot act as a substrate occupies the active site of an enzyme, the enzyme will be unable to catalyze any reaction until the molecule dissociates. This is the mechanistic basis of a major class of inhibitors called "competitive inhibitors".

As an example, folic acid must be converted to dihydrofolate and tetrahydrofolate by *dihydrofolate reductase*. Methotrexate and aminopterin are compounds that closely resemble folic acid; these compounds bind the dihydrofolate reductase active site with high affinity, but do not act as substrates. However, while these compounds are bound to the active site, the enzyme cannot catalyze its reaction.



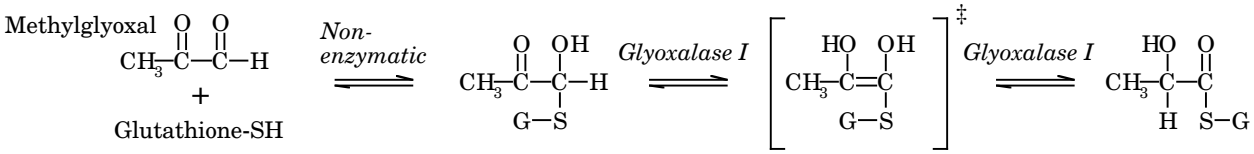
Transition state analogs

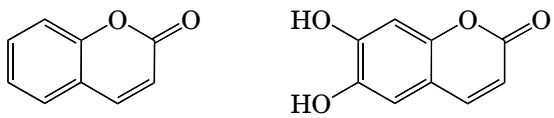
The primary mechanism by which enzymes enhance the rates of reactions is by stabilization of the transition state. Analysis of the principles of transition state theory reveals that the enzyme must bind to the transition state with higher affinity than it binds to the substrate.

Attempting to use an actual transition state species as an inhibitor is impossible: transition states are transient entities that cannot be isolated, and clearly cannot be used as drugs. However, it is possible to find or design stable compounds that are similar to the transition state. These compounds are "transition state analogs" and usually have a much higher affinity interaction with the enzyme than does the substrate. As a result, these compounds typically make very efficient competitive inhibitors.

An example of this is provided by inhibitors of the enzyme *glyoxalase I*. Glyoxalase I catalyzes the conversion of certain ketothioether compounds to the corresponding hydroxythioester. Based on a variety of studies, it is believed that the transition state for the reaction has a planar ene-diol structure.

Based on this concept of the likely transition state, the compounds coumarin and esculetin (among others) were tested as inhibitors. As would be expected, coumarin, which does not contain hydroxyl groups, failed to bind to the enzyme. In contrast, esculetin, which differs from coumarin only by the presence of hydroxyl groups on adjacent carbons in the aromatic ring, binds to the enzyme with considerably higher affinity than does the normal substrate.



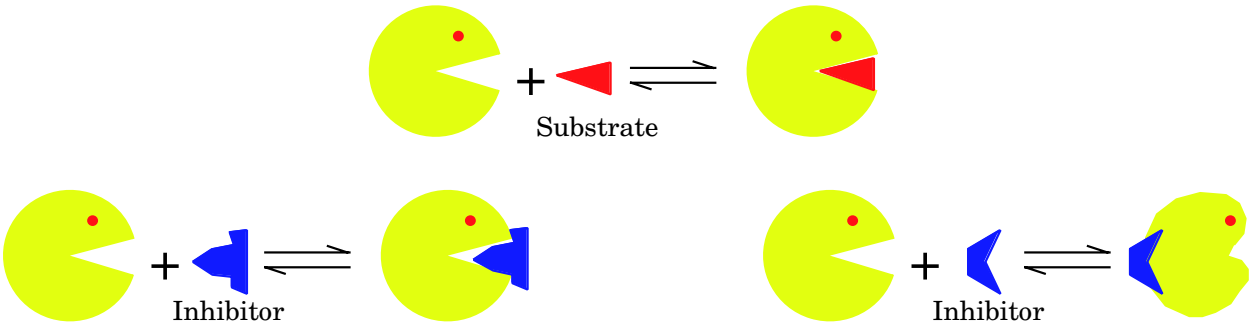


This is an example of using an understanding of the enzyme mechanism to design an inhibitor. In addition, the successful prediction of the high affinity interaction of esculetin with glyoxalase I lends additional experimental support to the proposed mechanism for the enzyme.

Conformational alterations

As discussed earlier, some enzymes undergo conformational changes when they interact with small molecules. These conformational changes alter enzymatic activity. Note that these allosteric effector molecules types are rarely similar in structure to the substrate.

A cartoon of the major types of enzyme inhibitor mechanisms is shown below. Normally enzyme substrates bind to the active site. Some types of inhibitors also bind to the active site, and therefore prevent catalysis by preventing substrate binding. Some types of inhibitors bind to sites on the enzyme other than the active site. The binding of the inhibitor results in a conformational change that prevents catalysis.

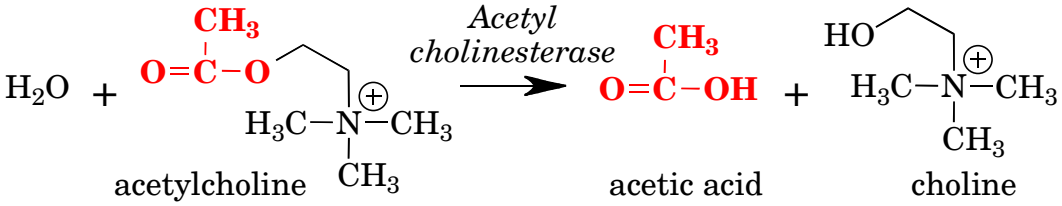


Suicide inhibition

The inhibitors in the above cartoon bind the enzyme reversibly. Some compounds can form covalent bonds to proteins. An ability to form indiscriminate covalent bonds, however, is not necessarily a good feature in an inhibitor, because the compound is likely to form covalent bonds with most proteins, and therefore is unlikely to be specific for a given enzyme.

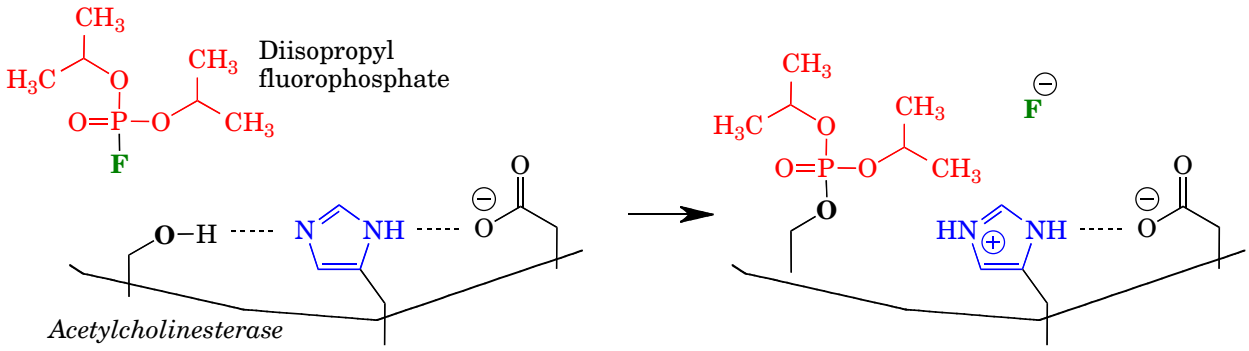
One method for making a specific irreversible inhibitor is to use a compound that will not form covalent bonds to proteins unless activated first. The compound is transformed into an activated intermediate following initiation of the catalytic process, and then forms an irreversible bond to the enzyme. The enzyme in effect commits suicide by activating the inhibitor. Intentionally creating a suicide inhibitor requires understanding the reaction catalyzed by the enzyme, and requires the creation of a compound that is reasonably inert unless exposed to the enzyme.

An example of an enzyme that has been the target of suicide inhibitor design is *acetylcholinesterase*, a serine esterase enzyme that inactivates the neurotransmitter acetylcholine by hydrolyzing the compound to acetic acid and choline.

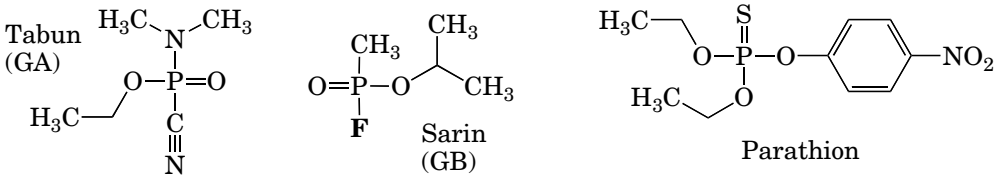


Inability to inactivate the neurotransmitter results in constant stimulation of cholinergic neurons, which has potentially lethal consequences. Inhibitors of acetylcholinesterase are therefore extremely toxic.

One suicide inhibitor of acetylcholinesterase is the compound diisopropyl fluorophosphate, which inhibits both serine proteases and serine esterases. These enzymes form covalent intermediates with the substrate; the diisopropyl fluorophosphate-enzyme covalent intermediate does not hydrolyze readily, and therefore permanently inactivates the enzyme by forming a covalent bond to the catalytic serine.



Nerve gasses such as tabun and sarin (below) are more specific for serine esterases (especially acetylcholinesterase), and kill affected individuals by a similar mechanism.



The tremendous advantage of suicide inhibitors is their specificity. A number of insecticides, such as parathion, irreversibly inactivate insect acetylcholinesterase, but are considerably less effective at inhibiting human acetylcholinesterase. This is because the compounds only form covalent bonds when activated, and the human enzyme is inefficient at activating these compounds.

Catalytic antibodies

Antibodies are immune system molecules that bind antigens (any molecule that stimulates an immune response) with high affinity. Transition state theory predicts that *any* molecule capable of binding to a transition state with high affinity should be able to catalyze reactions. The implication is that if an antibody binds to a transition state with high affinity, it should be able to catalyze a reaction.

This hypothesis was tested by generating antibodies against transition state analogs. Antibodies that bound the transition state analog tightly were then tested with substrate molecules; many of these antibodies were found to catalyze reactions using the substrate. While catalytic antibodies are, in most cases, far inferior to actual enzymes, some are capable of remarkable rate enhancements (with some examples of ~10^7-fold increased rates compared to the uncatalyzed reactions).

Catalytic antibodies were initially generated as a laboratory experiment to examine the role of transition state stabilization in enzyme mechanisms, but some successful attempts have been made to generate catalytic antibodies (sometimes called "abzymes") to catalyze reactions for which enzymes do not exist in nature. Note, however, that catalytic antibodies are somewhat limited; catalytic antibodies for reactions requiring prosthetic groups or covalent intermediates are unlikely to be readily generated.

Summary

The binding of molecules to enzymes may alter the activity of the enzyme. If this alteration is a decrease in activity, the compound is called an inhibitor.

Inhibition is one major mechanism for physiological enzyme regulation. In addition, enzyme inhibition has a number of scientific uses, and large numbers of drugs act by inhibiting enzymes.

Inhibition occurs either as the result of competition for the active site of the enzyme or as the result of a conformational alteration of the enzyme.

While some competitive inhibitors are substrate analogs, the most effective competitive inhibitors tend to be transition state analogs, which usually bind enzyme with much higher affinity than does the substrate.

Suicide inhibitors take advantage of the enzyme mechanism to activate an innocuous compound and irreversibly inactivate the enzyme. While these compounds tend to be difficult to design, they can be extremely effective drugs.

Antibodies that tightly bind to transition states are capable of catalyzing reactions; this is another example of the usefulness of transition state theory in understanding enzyme action.