gested that these amorphous deposits which are seen in the mitochondrion and the cytoplasm are transported in some way through the cell membrane to be deposited at the appropriate extracellular calcifications site.[37]

The X-ray radial distribution function study[36] on cytoplasmic ACP from the hepatopancreas of the blue crab suggested that, like the synthetic ACP, these granules consist of ion clusters of the order of 10 Å in largest dimension. The same amount of adenosine triphosphate (ATP) found in the cellular amorphous granules was effective in stabilizing synthetic ACP in solution. The high Mg content of these cytoplasmic deposits suggests that the stabilization of this amorphous mineral may be due to some cooperative effect between the Mg and ATP.

Early electron microscope studies on newly forming bone showed that the first mineral to be deposited was morphologically amorphous.[40] A recent electron microscope study showed that a mineral phase in newly formed embryonic chick tibia was morphologically amorphous and gave an electron diffraction pattern identical with that of synthetic ACP; older bone in this embryonic system appeared microcrystalline and gave an apatite electron diffraction pattern.[41] In addition, an electron microscope study[42] on metabolically active medullary bone in pigeon femur, which was shown to be highly amorphous by the X-ray diffraction percent crystallinity method,[19] showed the morphology of a large portion of this bone mineral to be spherical in nature, similar in size and shape to the appearance of synthetic ACP.

From our radial distribution studies it is probable that fully developed bone mineral cannot contain over 10% of a phase which is a close analog of the synthetic amorphous calcium phosphate. It appears that the amorphous phase which is present in the early stages of tissue mineralization does not persist during the biological maturation process. It is probable that the bulk of mature bone mineral is a carbonate-containing analog of hydroxylapatite which is characterized by internal structural disorder.

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(41) W. J. Landis, B. T. Hauschka, and M. C. Paine, Orthopaedic Research Society, San Francisco, Calif., Feb–March 1975, Abstract No. 43.
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Mechanisms of Action of Naturally Occurring Irreversible Enzyme Inhibitors

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Naturally occurring competitive inhibitors which specifically block biological receptors are principally macromolecular in nature. For example, the naturally occurring protease inhibitors are proteins, as are the exceedingly potent snake neurotoxins. The soybean trypsin inhibitors, leupeptin and pepstatin, belong in the former class, and α-bungarotoxin is of the latter class.[1-3]

Since these inhibitors are macromolecular in nature, it is not too difficult to understand, in a general way, the factors to which they owe their remarkable specificity. Simply, these macromolecules can make a large number of noncovalent contacts with the appropriate receptor. This assures tight binding, and thus selectivity.

On the other hand, there is another group of specific toxins[4] functioning principally as noncompetitive inhibitors of enzymes, whose specificity of action cannot be understood in terms of isosterism with normal substrate (effector) with a concomitant large number of noncovalent interactions with the target enzyme. The relatively more complex mechanism of action of this latter class of toxins is the subject of this Account.

I would like to confine myself to an examination of those naturally occurring toxins which act on enzymes as receptors, and show that the specificity of these toxins is a consequence of the fact that they must be converted into the active form of the inhibitor by the target enzyme itself. That is, these molecules are substrates for the target enzymes, and in the process of or as a consequence of this conversion the enzyme becomes irreversibly inhibited. I am interested here not in the molecules of nature that are chemically highly reactive and hence general poisons,

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(1) M. Laskowski and R. W. Sealock, *Enzymes, 3rd ed.*, **3**, 375 (1971).
(2) H. Umezawa, "Enzyme Inhibitors of Microbial Origin", University Park Press, London, 1972, Chapter 4.
(3) C. C. Chang and C. Y. Lee, *Arch. Int. Pharmacodyn.*, 144, 241 (1963).
(4) Toxin is used here in the broad sense without reference to organisms; that is, antibiotics are considered as toxins.

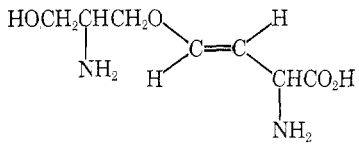
e.g., sarkomycin and patulin,[5] but those which are selective irreversible enzyme inhibitors.

In a general way, the examples of this kind of inhibition fall into two categories. In the first category are those in which the enzyme converts a chemically unreactive substrate into a highly reactive product. Once generated, the product chemically reacts with an active-site residue, resulting in the irreversible inactivation of the enzyme. Inhibitors of this type are called k_{cat} inhibitors, and several synthetic analogs of this type have been described.[6] In the second category are those cases where the product of the enzymatic reaction binds so tightly to the enzyme that the enzyme is apparently irreversibly inactivated, although covalent bond formation between enzyme and inhibitor need not have occurred. It should be noted that the distinction made between the two categories of inhibitors is clearly not a qualitative one. In both cases the proinhibitor to inhibitor transformation must be carried out by the target enzyme. This is the crucial point. In the following sections specific examples of these inhibitors are discussed.

Naturally Occurring k_{cat} Inhibitors

Inhibitors of Vitamin B_6 Containing Enzymes. There are a large number of naturally occurring toxins which function by specifically and irreversibly inhibiting various vitamin B_6 containing enzymes. Since most B_6-cofactored enzymes function in amino acid metabolism, the known inhibitors of these enzymes are almost exclusively amino acid derivatives.

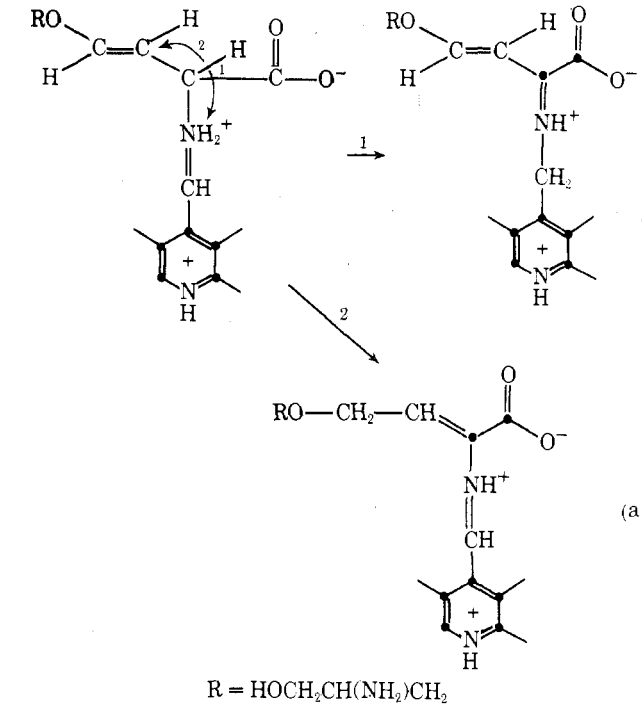
The Unsaturated Amino Acids. Several intriguing β,γ-unsaturated amino acid toxins have been reported in the literature. Rhizobitoxine (I), produced



rhizobitoxine (I)

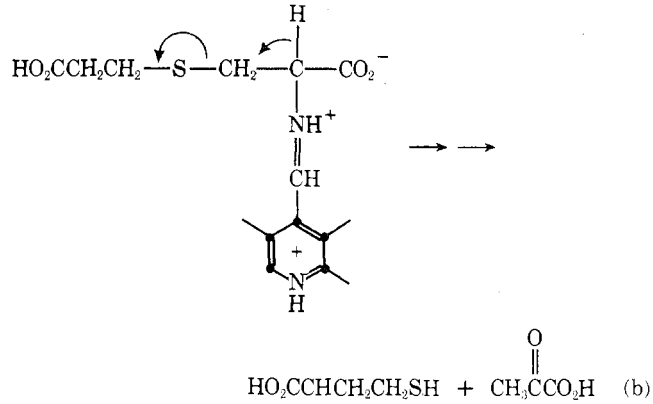
by *Rhizobium japonicum,* is a highly specific and irreversible inhibitor of B_6-linked β-cystathionase from bacteria and plants.[7] When this enzyme is inhibited in plants, production of the plant hormone ethylene is prevented.[8] In Figure 1a, a possible mechanism of action of this inhibitor is shown. In Figure 1b, the mechanism of action of the enzyme with cystathionine, its normal substrate, is shown. In Figure 1a, enzymatic abstraction of the α-CH leads to one or the other of the two reactive Michael acceptor intermediates 1 or 2 which then chemically reacts with some nucleophilic group of the holoenzyme.

Other enol ether containing amino acid toxins have also been found. For instance, L-2-amino-4-methoxy-*trans*-3-butenoic acid (II) and L-2-amino-4-(2-aminoethoxy)-*trans*-3-butenoic acid (III) were both recently isolated.[9,10] Although the exact manner by



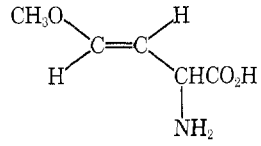
(a)

R = $HOCH_2CH(NH_2)CH_2$

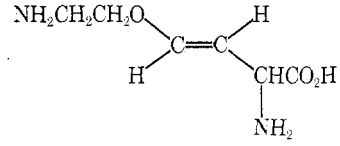


(b)

Figure 1. Mechanism of action of β-cystathionase and its irreversible inhibition by rhizobitoxine.



II



III

which they each function as antibacterial agents is unknown, we have found that L-2-amino-4-methoxy-*trans*-3-butenoic acid irreversibly inhibits B_6-containing cytoplasmic aspartate aminotransferase from pig heart.[11] We have demonstrated that the irreversible inhibition step involves the sequence of events shown in Figure 2. In order to check this mechanism, we synthesized 2-amino-3-butenoic acid and tried to irreversibly inhibit aspartate aminotransferase with it. If the inhibition occurred as in Figure 2, this material should also function as an irreversible inhibitor of the enzyme. This expectation was borne out.[12] In-

(5) D. Gottlieb and P. D. Shaw, Ed., "Antibiotics—Mechanisms of Action", Vol. I, Springer-Verlag, New York, N.Y., 1967.
(6) R. R. Rando, *Science,* **185,** 320 (1974).
(7) L. Giovanelli, L. D. Owens, and S. H. Mudd, *Biochim. Biophys. Acta,* 227, 671 (1971); L. D. Owens, J. F. Thompson, R. G. Pitcher, and T. Williams, *J. Chem. Soc., Chem. Commun.,* 714 (1972).
(8) L. D. Owens, M. Lieberman, and A. T. Kunishi, *Plant Physiol.,* **48,** 1 (1971).
(9) J. P. Scannell, D. L. Preuss, T. C. Demny, L. H. Sello, T. Williams, and A. Stempel, *J. Antibiot.* **25,** 122 (1972); U. Sahn, G. Knoblock, and F. Wagner, *ibid.,* **26,** 389 (1973).
(10) D. L. Preuss, J. P. Scannell, M. Kellett, H. A. Ax, J. Janecek, T. H. Williams, A. Stempel, and J. Berger, *J. Antibiot.,* **27,** 229 (1974).
(11) R. R. Rando, *Nature (London),* **250,** 586 (1974).
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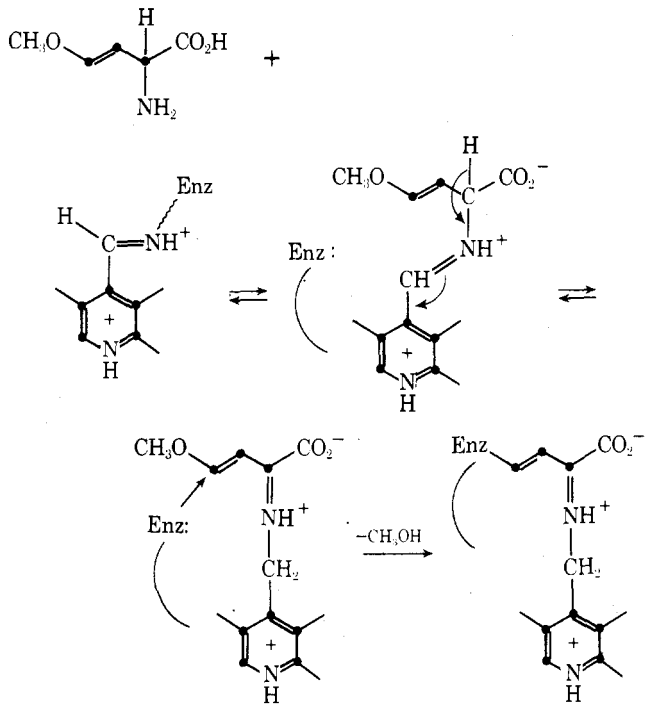
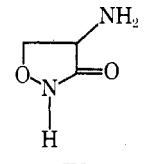


Figure 2. Irreversible inhibition of aspartate aminotransferase by L-2-amino-4-methoxy-*trans*-3-butenoic acid.

terestingly enough, after we had synthesized this inhibitor and studied its mechanism of action, it was reported to be a naturally occurring toxin isolable from plants.[13]

Other unsaturated amino acids which are k_{cat} inhibitors of B_6-linked enzymes have also been reported. In 1949, the synthesis of propargylglycine was reported.[14] It was found that this material inhibited microbial growth, although a mechanism for this inhibition was not discussed. Much later, Scannell and coworkers reported that this material was a natural toxin produced by an unidentified streptomycete.[15] In their test systems they found that the inhibitor was an antagonist of both methionine and leucine. Furthermore, it was recently found that the toxin irreversibly inhibited homoserine dehydratase (Figure 3) by a k_{cat} mechanism.[16] In Figure 3a the mechanism of action of the enzyme and in Figure 3b the putative mechanism of action of the toxin are shown. Presumably the enzymatic formation of the highly reactive allene accounts for the irreversible inhibition observed.

Cycloserine. Cycloserine (IV) is presumed to exert toxic effects on bacteria by preventing cell wall formation. Cycloserine is an irreversible inhibitor of several pyridoxal-linked enzymes which are capable of abstracting an α-CH bond as part of their mechanisms of action.[17] Indeed, cycloserine can react with pyridoxal phosphate itself under mild conditions.[18] Furthermore, the inhibitor prevents the functioning of alanine racemase, a pyridoxal phosphate linked enzyme responsible for the biosynthesis of D-alanine from its enantiomorph. The "unnatural" D-alanine is a crucial component in cell wall biosynthesis, and the blocking of its formation prevents cell wall formation.



IV
cycloserine

It is natural to ask whether the D-cycloserine irreversibly inhibits this enzyme in the same way that L-cycloserine can irreversibly inhibit other pyridoxal-linked enzymes capable of abstracting an α-CH. Evidence suggests that this is indeed the case. For example, the amount of inhibition obtained is time depen-

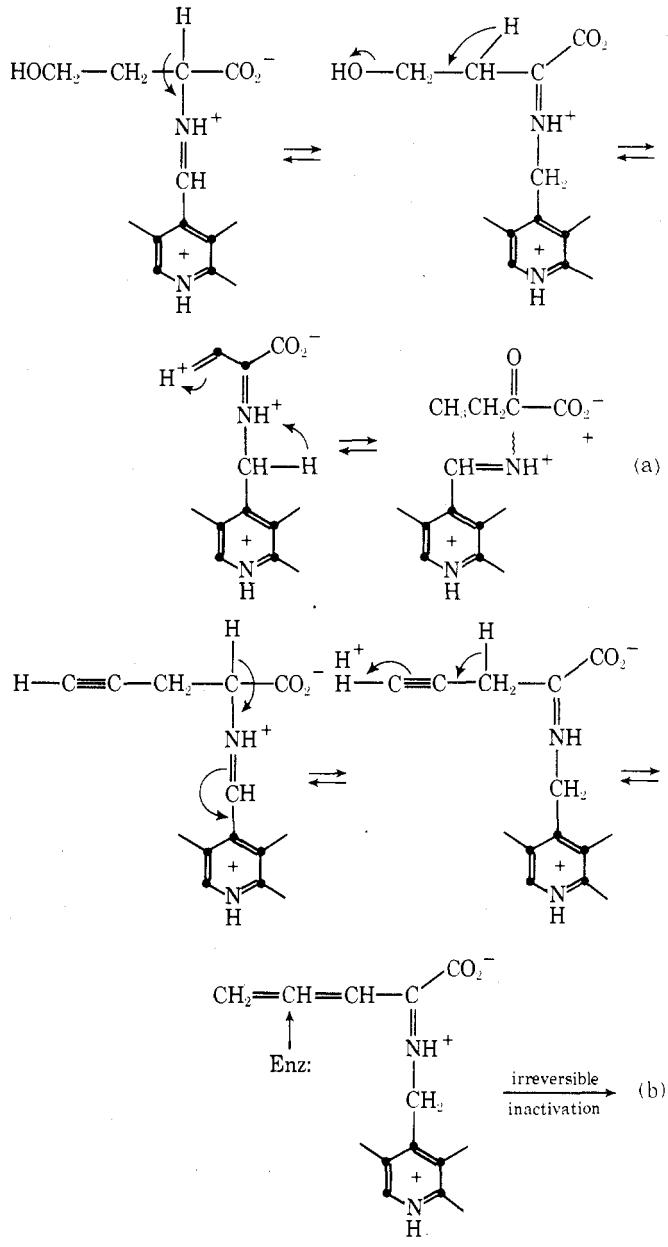


Figure 3. Mechanism of action of homoserine dehydratase and its irreversible inhibition of propargylglycine.

(13) G. Dardenne, J. Casimir, M. Marlier, and P. O. Larsen, *Phytochemistry*, **13**, 1897 (1974).
(14) H. Gershon, J. S. Meek, and K. Kittmer, *J. Am. Chem. Soc.*, **71**, 3573 (1949).
(15) J. P. Scannell, D. L. Preuss, T. C. Demny, F. Weiss, T. Williams, and A. Stempel, *J. Antibiot.*, **24**, 239 (1971).
(16) R. H. Abeles and C. T. Walsh, *J. Am. Chem. Soc.*, **95**, 6124 (1973).
(17) F. C. Meuhaus in ref 5, pp 64–69.
(18) U. Roze and J. L. Strominger, *Fed. Proc. Abstr.*, **22**, 423 (1963).

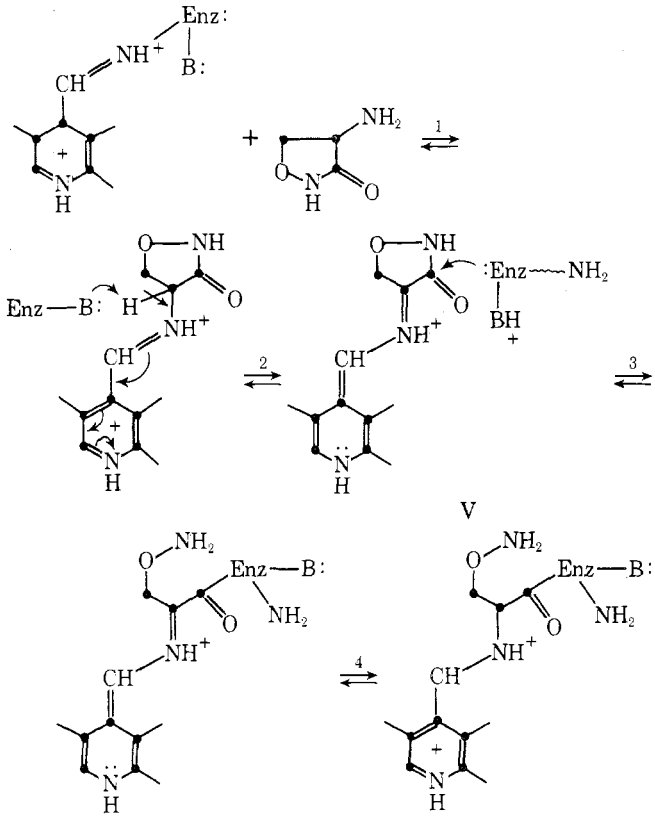


Figure 4. *Irreversible inhibition of pyridoxal-linked enzymes by cycloserine.*

dent.[19] We have recently shown that D-cycloserine is indeed an irreversible inhibitor of *B. subtilis* alanine racemase.[20] The proposed mode of action of this inhibitor is shown in Figure 4. With cycloserine as a substrate, enzymatic cleavage of the α-CH bond would lead to the formation of the highly reactive acylating agent V from its chemically unreactive counterpart. This acylating agent V would then engage in a chemical reaction with a propinquous active-site amino acid residue, resulting in the irreversible inactivation of the enzyme. This same intermediate (V) is thought to be involved in the irreversible inhibition of other vitamin B_6 containing enzymes by cycloserine.[20]

Structure–activity relationships in the cycloserine series also support the validity of this interpretation. For example, alkylation or acylation of the free amino group of cycloserine and cleavage of the ring system lead to compounds that are inactive as irreversible enzyme inhibitors.[21]

Additional Inhibitors of Vitamin B_6 Containing Enzymes. In this section I reexamine some studies on powerful toxins whose structures make them particularly interesting in terms of the mechanisms discussed here.

Osteolathyrism is caused by β-aminoproprionitrile, a plant product usually found as an α-glutamyl derivative.[22] The disease itself, a so-called molecular dis-

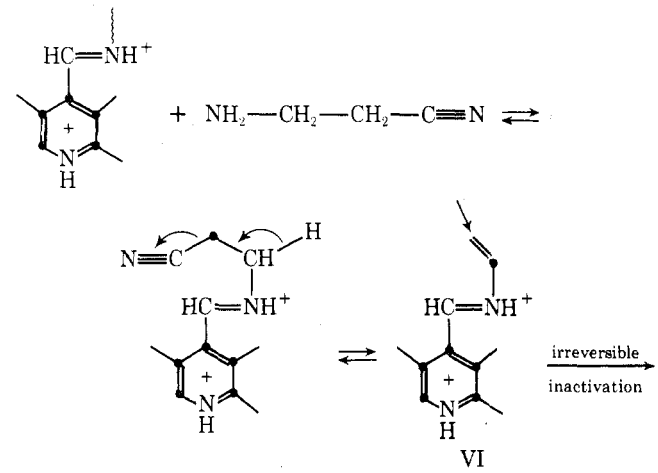


Figure 5. Irreversible inhibition of collagen cross-linking enzyme by β-aminopropionitrile.

ease, is characterized by a structural degeneracy in the collagen and elastin. This degeneracy is thought to be a consequence of an interference with the normal intermolecular cross-linking of protein chains, with a resultant lack of insoluble matrix formation. In collagen, cross-linking is the result of an aldol condensation between two aldehydic groups of adjacent chains, which in turn are derived by oxidation of the ϵ-amino groups of specific lysyl groups on adjacent chains. The oxidation is presumed to be carried out by a specific monoamine oxidase. β-Aminoproprionitrile is thought to inhibit this enzyme. Although several hypotheses for the mechanism of this inhibition have been suggested, one which has not would require an induced irreversible inhibition of the enzyme by β-aminoproprionitrile. This proposal is shown in Figure 5.

The formation of the reactive intermediate VI would lead to irreversible inhibition of the enzyme in this scheme. Three precise analogies for this mode of inhibition exist in the serine-induced irreversible inhibition of threonine dehydratase,[23] in the chloroalanine-induced inhibition of aspartate aminotransferase,[24] and in the ethanolamine *O*-sulfate induced irreversible inhibition of γ-aminobutyric acid–α-ketoglutarate transaminase[25] (Figure 6).

Other toxins of intriguing structures are mimosine and the cyclopropyl-containing amino acids, hypoglycine A and α-(methylenecyclopropyl)glycine[26] (Figure 7). Mimosine appears to be a general antagonist of B_6-linked enzymes, and it has been reported that this toxin can react with pyridoxal itself.[27] Hypoglycine and α-(methylenecyclopropyl)glycine should be exceedingly reactive toward B_6-linked enzymes, although reports to that effect do not yet exist.

The Diazocarbonyl-Containing Antibiotics. Several antibiotics contain the biologically unusual diazo ester and diazo ketone moieties[28] (Figure 8). These inhibitors function by irreversibly inhibiting

(19) F. C. Meuhaus, *Antimicrob. Agents Annu.*, 304 (1968).
(20) R. R. Rando, unpublished experiments.
(21) M. Ya Karpilsky, R. M. Khomutov, E. S. Severin, and Yu N. Bresiv, in "Chemical and Biological Aspects of Pyridoxal Catalysis", E. E. Snell et al., Ed., Wiley, New York, N.Y., 1963, pp 323.
(22) R. M. Khomutov, E. S. Severin, G. K. Kovaleva, M. M. Gulyaev, M. U. Gnuchev, and L. P. Sastchenko, in "Pyridoxal Catalysis: Enzymes and Model Systems", E. E. Snell et al., Ed., Wiley, New York, N.Y. 1968, p 639.
(23) L. A. Emrhurt, S. H. Lipton, and F. M. Strong, *Biochemistry*, **2**, 300 (1963).
(24) W. A. Wood and J. C. Gunsalus, *J. Biol. Chem.*, **181**, 171 (1949); A. T. Philips, *Biochim. Biophys. Acta*, **151**, 171 (1968).
(25) Y. Morino and M. Okamoto, *Biochem. Biophys. Res. Commun.*, **50**, 1061 (1973).
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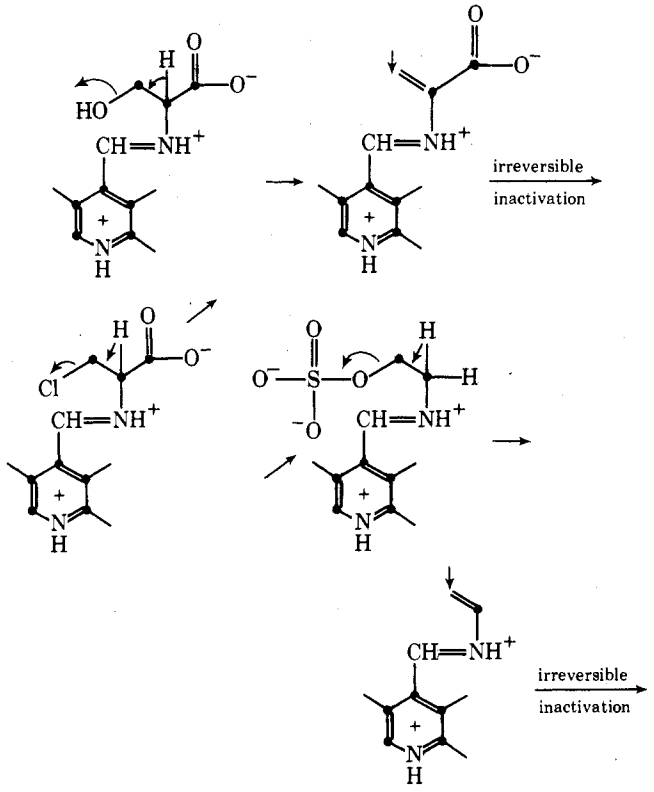


Figure 6. Irreversible inhibition of pyridoxal-linked enzymes.

key enzymes in the metabolic pathways involving either glutamine or asparagine.

The diazo moiety is crucial to their mechanisms of action. Since amino acid residues likely to be alkylated or acylated by inhibitors are all Lewis bases, it is not surprising that the inhibitors are uniformly Lewis acids.[29] Indeed, this holds true with the diazo-containing antibiotics also, since these molecules are enzymatically protonated by the target enzyme, generating highly reactive diazonium ions, which then inactivate the enzyme. This principle is clearly laid out in the work of Buchanan and coworkers on the azaserine-induced irreversible inhibition of the enzyme which converts formylglycinamide ribonucleotide (FGAR) to formylglycinamidine ribonucleotide (FGAM).[30] The conversion requires L-glutamine and adenosine triphosphate (ATP) in addition to FGAR, and its interruption prevents purine biosynthesis, inasmuch as the conversion is an integral part of inosinic acid biosynthesis. The mechanism of action of induced irreversible inhibition of formylglycinamide ribonucleotide amidotransferase by azaserine involves the sequence of events shown in Figure 9a. The initial steps of the enzymatic conversion of the substrate glutamine are shown in Figure 9b. Note that azaserine serves as a substrate and is converted into the highly reactive diazonium ion which alkylates an active-site residue, presumably a cysteine residue. The crucial point is that the enzyme must convert the azaserine to the diazonium ion inhibitor before a chemical reaction can occur. As expected, diazo ketones and esters have been shown to be general active-site directed irreversible inhibitors of enzymes

(29) R. B. Livingston, J. M. Verditti, D. A. Cooney, and S. K. Carter, in *Adv. Pharm. Chemother.*, 8, 57 (1970).

(30) G. E. Means and R. E. Feeney, "Chemical Modification of Proteins", Holden-Day, Inc., San Francisco, Calif., 1971.

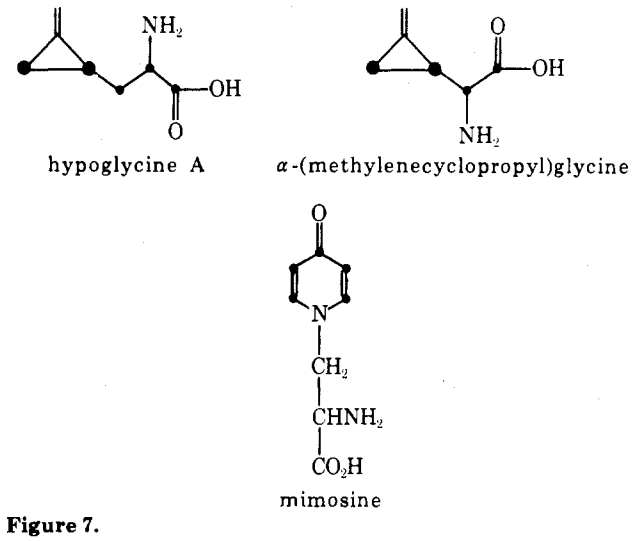


Figure 7.

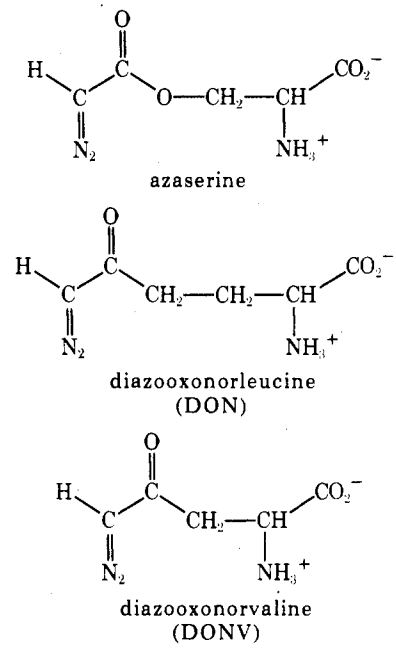


Figure 8.

which function by acid catalysis.[31]

Penicillin and Cephalosporins. The penicillin and cephalosporin derivatives prevent cell wall formation in bacteria.[32] Specifically, they are thought to prevent cross-linking of the peptidoglycan peptide chains, the terminal reaction in cell wall biosynthesis. These drugs are assumed to inactivate irreversibly the as yet unisolated membrane-bound transpeptidase enzyme.[33]

The active penicillin derivatives are all derivatives of 6-aminopenicillanic acid and the active cephalosporins of Δ^3-7-aminocephalosporinic acid[32] (Figure 10). Both series of drugs are remarkably nontoxic as a consequence of their great specificity.

Early attempts to explain this specificity were

(31) T. C. French, I. B. Dawid, R. A. Day, and J. M. Buchanan, *J. Biol. Chem.*, **238**, 2171 (1963); I. B. Dawid, T. C. French, and J. M. Buchanan, *ibid.*, **238**, 2186 (1963).

(32) B. F. Erlanger, S. M. Vratsanos, M. Wassermann, and A. G. Cooper, *Biochem. Biophys. Res. Commun.*, **23**, 243 (1966); T. G. Rajagopalan, W. H. Stein, and S. Moore, *J. Biol. Chem.*, **241**, 4295 (1966).

(33) E. F. Gall, E. Curdliffe, P. H. Reynolds, M. H. Richmond, and M. J. Waring, "The Molecular Basis of Antibiotic Action", Wiley, New York, N.Y., 1972, Chapter 3.

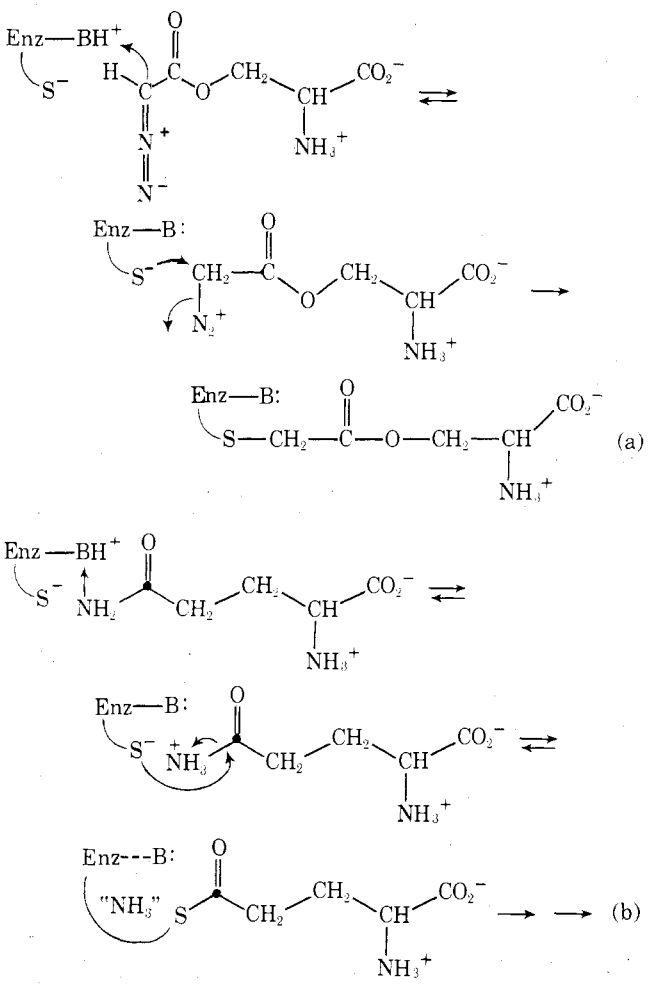
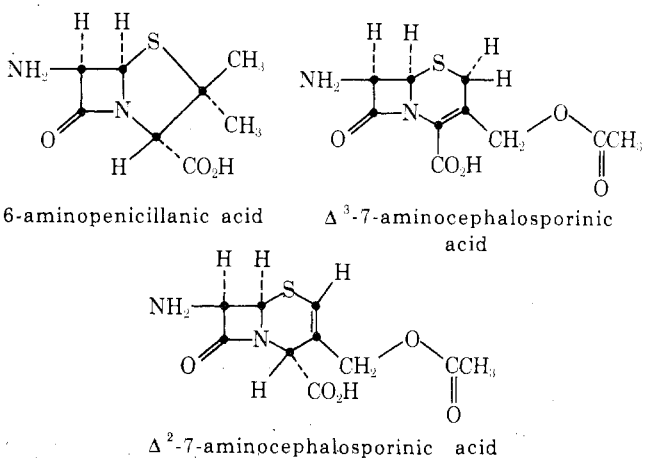


Figure 9. Partial mechanism of action of formylglycinamide ribonucleotide amidotransferase and its irreversible inhibition of azaserine.



6-aminopenicillanic acid

Δ^3-7-aminocephalosporinic acid

Δ^2-7-aminocephalosporinic acid

Figure 10.

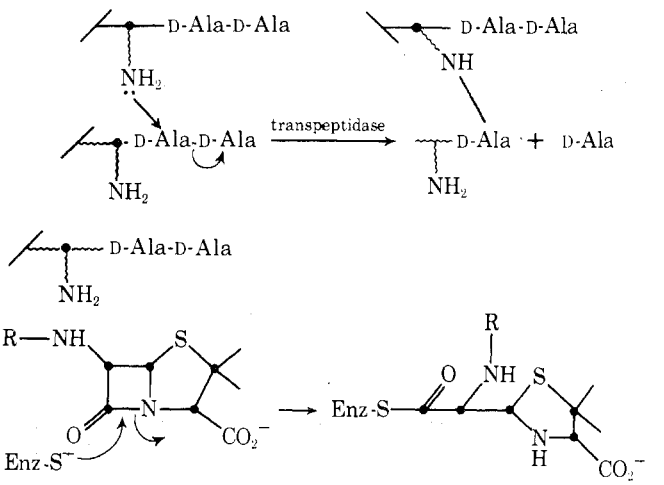


Figure 11. Mechanism of transpeptidase action and its irreversible inhibition by penicillin.

based on the idea that penicillin is isosteric with the terminal D-alanyl dipeptide.[34] Once bound to the transpeptidase, a chemical reaction could then ensue between the antibiotic and an active-site residue of the enzyme, resulting in its irreversible inhibition[34] (Figure 11). The strained β-lactam portions of the antibiotics are presumably the sites of the chemical reactions. Thus in this scheme penicillin would be considered to be an affinity-labeling agent.[35]

The crucial question about the mechanism of action of penicillin (and by analogy of the cephalosporins) is: to what factor(s) does this drug owe its remarkable specificity? The argument about isosterism alluded to earlier cannot be the whole answer, for several reasons. First and foremost, penicillin is hardly isosteric with the terminal D-alanyl dipeptide[32] (Figure 12). The main reasons are the differences in the bond angles and the lengths of the β-lactam bonds compared to the peptide bonds of the dipeptide.[32] Furthermore, what prevents the β-lactam from reacting with a whole host of other biomolecules? On the other hand, the bond angle and bond length differences alluded to may hold the key to the mechanism of action of penicillin. If the enzyme were to bind penicillin to its active site in a conformation

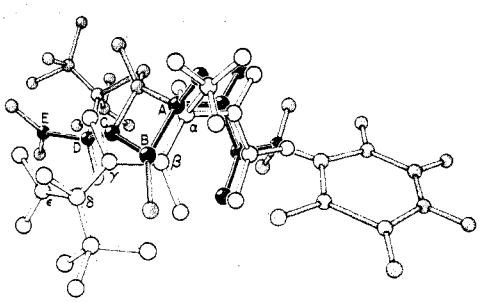


Figure 12. Comparison of D-alanyl dipeptide with benzylpenicillin. The molecule labeled α, β, γ, δ, ϵ is the dipeptide and A, B, C, D, E, the benzylpenicillin.

that approximated the dipeptide conformation, we would have a mechanism by which the enzyme could chemically activate the penicillin. Penicillin is already a strained molecule, having the β-lactam ring fused to a five-membered ring. As Figure 12 shows, the rings are strongly puckered. Now if the rings were flattened out (i.e., ring N hybridization goes from sp^3 to sp^2) at the active site of the enzyme, penicillin would "look like" the dipeptide substrate, i.e., be more closely isosteric to the dipeptide. However, if penicillin were distorted in this way by the enzyme, its chemical reactivity would be enormously increased, thereby greatly increasing the probability of a chemical reaction with an active-site residue. The net result of this would be that the binding forces of

(34) O. M. Blumberg and J. L. Strominger, *Bact. Rev.*, 38, 291 (1974).
(35) D. J. Tipper and J. L. Strominger, *Proc. Nat. Acad. Sci. U.S.A.*, **54**, 1133 (1965).

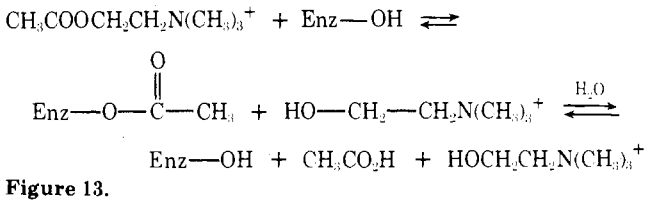


Figure 13.

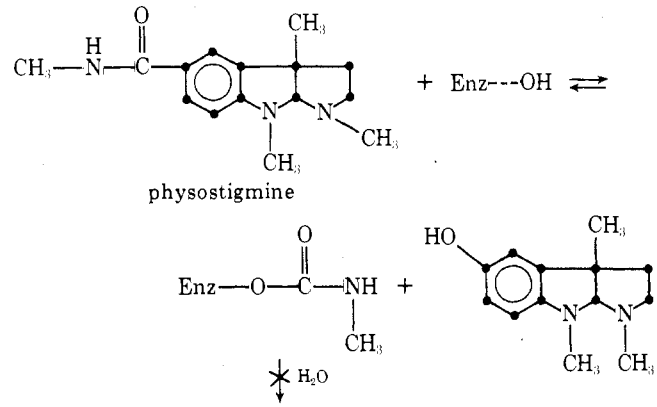


Figure 14. Inhibition of acetylcholinesterase by physostigmine.

the enzyme would be used to activate the irreversible inhibitor.

Two observations in the literature are consistent with this hypothesis. First, the Δ^2-cephalosporins are virtually inactive as cell wall inhibitors[36] (Figure 10). Models show that the Δ^2 derivatives are more closely isosteric to penicillin than the active Δ^3 derivatives.[36] However, the chemical reactivity of the Δ^2-cephalosporins is low.[37] This observation clearly shows that chemical reactivity is much more crucial than any proposed isosterism for antibiotic activity.[38]

Secondly, those isolated penicillin-binding enzymes which bind penicillin in a reversible manner do so with low dissociation constants ($K_I \sim 10^{-8}$ M),[39] whereas those which bind penicillin irreversibly have a relatively high dissociation constant ($K_I \sim 10^{-3}$ M).[39] This is consistent with the view that the binding forces are utilized to distort the penicillin, which would result in a higher measured K_I.

Naturally Occurring Quasi-Substrate Inhibitors

In this section, I discuss those natural toxins which function as substrates for the target enzymes, but for which the product, instead of chemically reacting with the enzyme, is bound so tightly that further access to the active site by other substrate molecules is prevented.

Some of the earliest toxins found to function by this mechanism were inhibitors of the cholinesterases.[40] These enzymes are serine proteases which hydrolyze acetylcholine by means of a well-understood mechanism (Figure 13). This hydrolysis terminates the action of the neurotransmitter acetylcholine at cholinergic neurons. The inhibition of this enzyme leads to the eventual death of the organism. One of

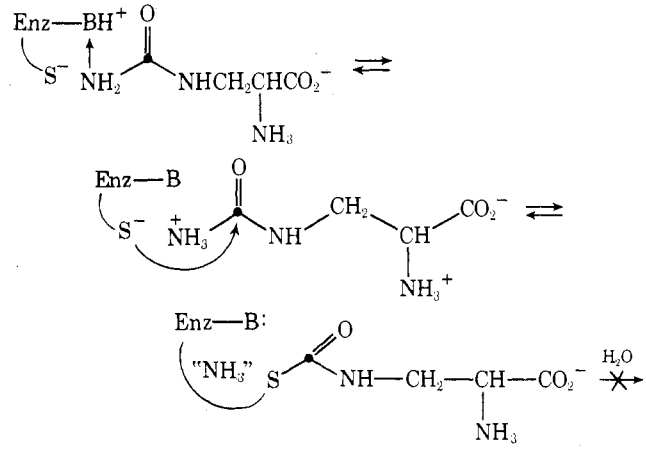


Figure 15. Irreversible inhibition of formylglycinamide ribonucleotide amidotransferase by albizziin.

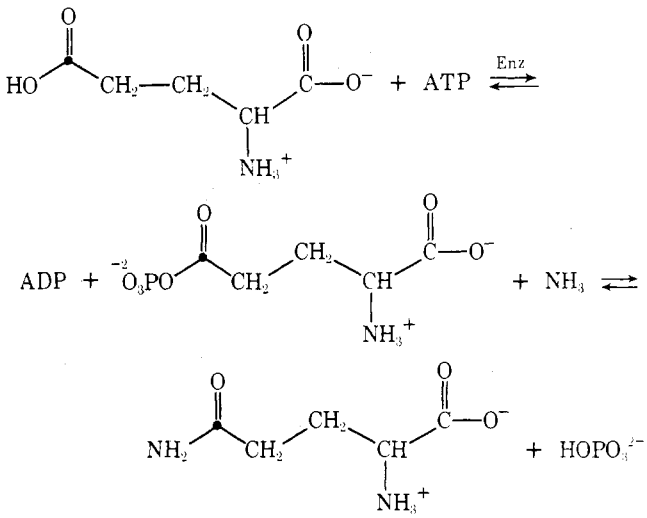


Figure 16. Mechanisms of glutamine synthetase action.

the earliest known natural antagonists of this enzyme is the alkaloid physostigmine[41] (Figure 14). This molecule is a substrate for the enzyme. Physostigmine is an inhibitor of the enzyme by virtue of the fact that the carbamate–enzyme intermediate is only very slowly hydrolyzed to yield the free enzyme and N-methyl acid, thus tying up the enzyme[41] (Figure 14). By now there are literally hundreds of this kind of serine protease inhibitor in the literature.[41] For example, the phosphorus-containing insecticides and nerve gases such as diisopropyl phosphorofluoridate all fall into this class. Inhibitors of this type have been termed quasi-substrate inhibitors.[42]

A similar kind of inhibition has been found in studies on the effect of albizziin (Figure 15) on formylglycinamide ribonucleotide amidotransferase, the same enzyme alluded to earlier in the diazo ketone and ester section.[43] Albizziin is a constituent of several plants belonging to the family *Mimosaceae*.[43] This agent functions as an irreversible inhibitor of the enzyme in the way shown in Figure 15. The thiocarbamate intermediate is not further hydrolyzed by the enzyme; hence the enzyme is irreversibly inhibited.

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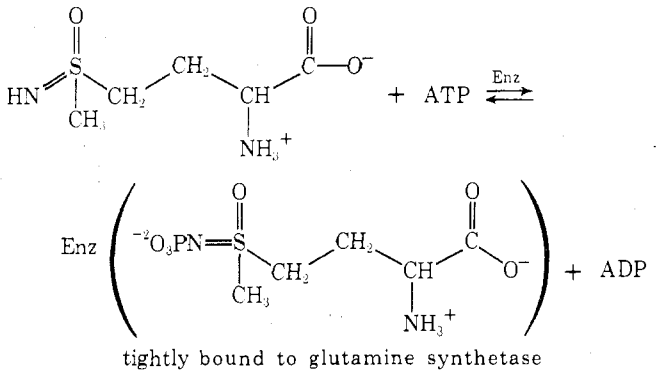

Figure 17. Mechanism of the irreversible inhibition of glutamine synthetase by methionine sulfoximine.

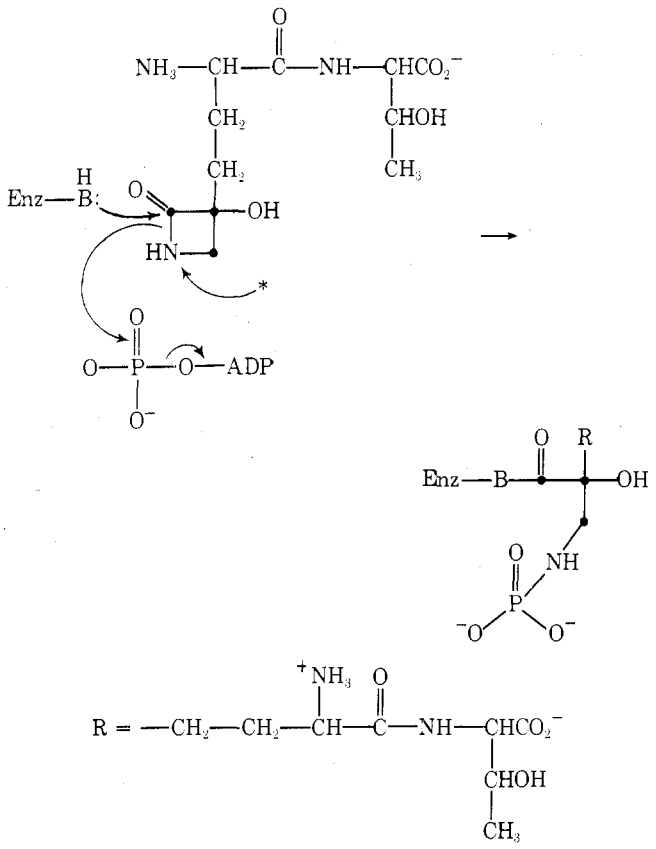

Figure 18. Inhibition of glutamine synthetase by wildfire toxin.

Glutamine synthetase is also profoundly inhibited by several naturally occurring toxins which serve as substrates for the enzyme. The mechanism of action of the enzyme is shown in Figure 16. Methionine sulfoximine, a methionine analog found in bleached flour, was shown to be an irreversible inhibitor of the enzyme.[44] The mechanism of inhibition involves the following sequence of steps (Figure 17). The phosphorylated intermediate is bound so tightly to the enzyme that it can be removed neither by gel filtration nor by continued dialysis. This tight binding fits in very nicely with the ideas developed from studies on the transition-state inhibitors.[45] Interestingly enough, the actual inhibitor itself, phosphorylated methionine sulfoximine, has been found to be a naturally occurring bacterial toxin.[46]

Glutamine synthetase has also been found to be the site of action of wildfire toxin[47] (Figure 18). The mechanism of action of this inhibitor is thought to involve the steps shown in Figure 18.[48] In this instance the activation of the β-lactam ring is coupled with the enzymatic cleavage of ATP.

Conclusion

I have tried to show here that the small molecule toxins generally function by mechanisms which require them to be substrates for the target enzymes. These molecules almost never function by being simple isosteric competitive inhibitors of the enzymes. It is clear why this should be. First, a simple competitive inhibitor will only have a profound effect on a pathway if it happens to work on a rate-limiting enzyme, and then only if the activity of this particular enzyme is reduced to virtually zero. In many cases even reducing the enzyme's activity by 95% has little effect. Secondly, simple isosteric inhibitors of an enzyme must achieve concentrations far in excess of the substrate's physiological concentration in order to be at all effective. For example, it is hard to see how a simple competitive inhibitor could effectively interfere with an enzyme involved in glucose metabolism.

On the other hand, small molecule inhibitors which are noncompetitive and irreversible inhibitors of enzymes can overcome the aforementioned difficulties. For reasons already discussed, the simple attachment of a chemically reactive group to a molecule roughly isosteric with substrate will not give rise to sufficiently specific reagents.[6]

The studies discussed here are of further interest because they allow one to classify toxins and antibiotics which act in enzymes in terms of well-known organic reaction mechanisms. The inhibitor (toxin) is introduced in an unreactive form, which allows it to circumvent reactions with foreign biomolecules. The target enzyme itself will then convert this proinhibitor into the actual inhibitor, which immediately inactivates the enzyme without diffusing into solution. It is important to note that this mode of inactivation is completely different from the well-known examples of drugs (e.g., mitomycin) being enzymatically activated *in vivo* only to then diffuse away from the enzyme and react with another receptor distinct from the enzyme.

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