Pergamon

Bioorganic & Medicinal Chemistry 7 (1999) 647–652

BIOORGANIC & MEDICINAL CHEMISTRY

REVIEW ARTICLE

Conformational Aspects of Inhibitor Design: Enzyme–Substrate Interactions in the Transition State

Richard Wolfenden*

Department of Biochemistry and Biophysics, University of North Carolina, Chapel Hill, NC 27599, USA

Received 2 September 1998; accepted 6 October 1998

Abstract—The theory of absolute reaction rates suggests that enzymes, like other catalysts, can enhance the rate of a reaction only to the extent that they bind the altered substrate in the transition state ($S^{\ddagger}$) more tightly than they bind the substrate in the ground state (S). ES dissociation constants commonly fall in the physiological range, but recent kinetic studies indicate that formal $ES^{\ddagger}$ dissociation constants of less than 10^{-20} M are achieved by enzymes of several classes. Studies with stable analogues suggest that these remarkable powers of discrimination involve a tendency of the enzyme to close around $S^{\ddagger}$ in such a way as to maximize binding contacts; that several parts of the substrate contribute to $S^{\ddagger}$ binding; and that their contributions to binding affinity can be strongly synergistic.

Introduction

As he laid the foundations of conformational analysis, Derek Barton identified several useful approaches to producing and understanding the interactions of biologically active compounds. Many of these compounds have enzyme active sites as targets. Because the conformations of enzymatic active sites and substrates are of central importance to catalysis, it seems appropriate to consider some lessons that have been learned about these targets from their behavior in the presence of substrates and inhibitors. These observations seem likely to have a significant bearing on prospects for future improvements in drug design.

Are substrate analogues expected to be effective inhibitors?

Enzyme–substrate interactions have long been recognized as representing the acme of structural complementarity in biological chemistry. One of the earliest observations to emerge from studies of catalysis by enzymes, and from heat inactivation of enzymes in the presence of small molecules, was that enzymes bind substrates reversibly, forming complexes that appear to dissociate at concentrations usually slightly higher than those that are present physiologically. Inhibition is usually observed in the presence of unreactive structural analogues of a substrate. That suggests that substrates and substrate analogues compete for a place on the enzyme, in accord with the possibility that ES complexes are also formed during the catalytic transformation of the substrate (for a review, see ref 1). This view led to the well-known proposal by Emil Fischer that substrates fit enzymes as a key fits a lock. Are substrate-like inhibitors expected to be strong and enzyme-specific, as that simile indicates?

In considering that question, it can be helpful to focus one's attention on structures rather than processes, involving stages in the enzyme's interaction with the altered substrate as the substrate undergoes chemical activation. To enhance the rate of a reaction, a catalyst lowers the equilibrium constant for attainment of the transition state. It was first recognized during the 1920s that a catalyst binds a reactant with increasing affinity as the reactant is distorted toward the structure that it adopts in the transition state.[2] In the words of Schwab, written in 1931:

> "The energy barrier to be overcome is lowered in the adsorption layer because the activated state is strongly adsorbed and, therefore, in the adsorption layer, is less endothermic and therefore more often reached. Hence, it is not that the adsorbate is activated but that the adsorbate is easily activated and is therefore, at equilibrium, present in the activated state to a greater percentage extent than in the free gas."[3]

If 'active site' is substituted for 'adsorption layer', that statement contains the essence of our present view of

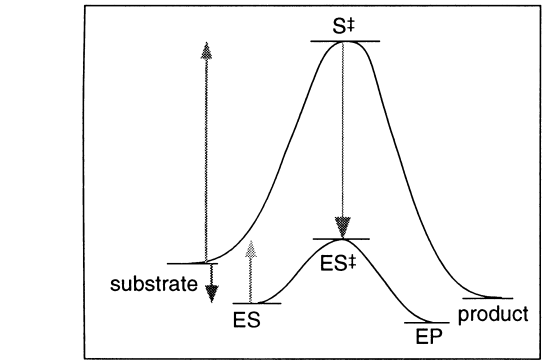
*Corresponding author. Tel.: +919-966-1203; fax: +919-966-2852.

0968-0896/99/$ - see front matter
PII: S0968-0896(98)00247-8

the free energy changes associated with enzyme catalysis (Fig. 1).

Based on similar considerations, Pauling[4] and Jencks[5] went on to suggest, quite independently, that it might be possible in principle to develop a powerful enzyme antagonist in the form of an unreactive compound, analogous in structure to S‡. When it was recognized in 1969 that such a 'transition state analogue' should surpass a conventional substrate in its enzyme affinity by a factor that matches or surpasses the large rate enhancement that the enzyme produces (Fig. 1), only a few months elapsed before the first such analogue was prepared as a potential antagonist of triosephosphate isomerase, and tested with results that seemed promising.[6] Later, ^{13}C NMR showed that 2-phosphoglycolate is bound as a species that is rare in solution, with a dissociation constant from this enzyme that is roughly 5 orders of magnitude lower than the K_m value for glyceraldehyde 3-phosphate.[7]

As early as 1976, more than 60 such inhibitors had been identified, including enzymes of every mechanistic class that was then recognized. These inhibitors offered a new way of testing the general mechanism on which their design had been predicated, and a tool by which structural methods of analysis could be used to uncover the structural details of enzyme–substrate interaction. The continuing successes of this method in generating powerful enzyme inhibitors[8] have been matched by practical applications exemplified by herbicides (glyphosate, an inhibitor of aromatic amino acid biosynthesis), agents for the control of high blood pressure (Capoten and Vasotec, inhibitors of the angiotensin-converting enzyme),[9] and a group of drugs that are presently being used to control the spread of HIV infection (statine-like inhibitors of the HIV-1 protease).[10] Some of these inhibitors have K_i values of less than 10^{-12} M, and in the remarkable case of methionine sulfoximine phosphate (an inhibitor of glutamine synthetase), exchange experiments have placed an upper limit of 10^{-18} M on the dissociation constant of the E–I complex.[11]



if an enzyme reaction proceeds more rapidly than the uncatalyzed reaction ($k_{cat} > k_{non}$), by some factor

then the equilibrium of activation is more favorable on the enzyme than in free solution ($K_{ES}^{\ddagger} > K_S^{\ddagger}$), by this same factor

and the enzyme binds S‡ more tightly than S, by the same factor, i. e. the binding discrimination matches the rate enhancement

$$E + S \overset{k_{non}}{\rightleftharpoons} S^{\ddagger} + E \qquad K_m \Updownarrow k_{cat} \Updownarrow K_{tx} \qquad ES \rightleftharpoons ES^{\ddagger}$$

$$\frac{K_{tx}}{K_m} = \frac{K_S^{\ddagger}}{K_{ES}^{\ddagger}} = \frac{k_{non}}{k_{cat}}$$

Figure 1. Free energy changes associated with substrate and transition state binding (refs 3 and 6).

Because biological reactions take place very slowly in the absence of enzymes (see below), their transition states would be expected to differ markedly in energy and structure from substrates in the ground state. Their conformational properties might be expected to be important in generating the correct products from the reactants, but also because the tight binding that is required for catalysis involves nearly exact structural complementarity to an enzyme's active site, which is itself asymmetric. In displacement reactions at sp^2-hybridized carbon atoms, transition states with tetrahedral-like carbon atoms are generated, with a transient chirality that is not present in reactants or products. That chirality can sometimes be detected and exploited by comparing the effectiveness of two diastereomeric transition state analogue inhibitors. A dramatic example is furnished by 2′-deoxycoformycin, whose 8*R*-form is bound by adenosine deaminase 7 orders of magnitude more tightly than the substrate, whereas the 8*S*-isomer is less tightly bound than the substrate.[12] Figure 2 shows these inhibitors, along with the 1,6-hydrate of inosine, which is even more tightly bound,[13] presumably because it has the correct ring size. The chirality of the bound hydrate, like that of the 8*R* form of deoxycoformycin, reflects the aspect of the purine ring from which zinc-bound hydroxide ion is believed to mount its attack.[14]

In contrast to the tight binding of the substrate in the transition state, the substrate in the ground state and its analogues are expected to be relatively weakly bound. Tight binding of the substrate is actually counterproductive to efficient catalysis, because an enzyme can enhance the rate of reaction only to the extent that it

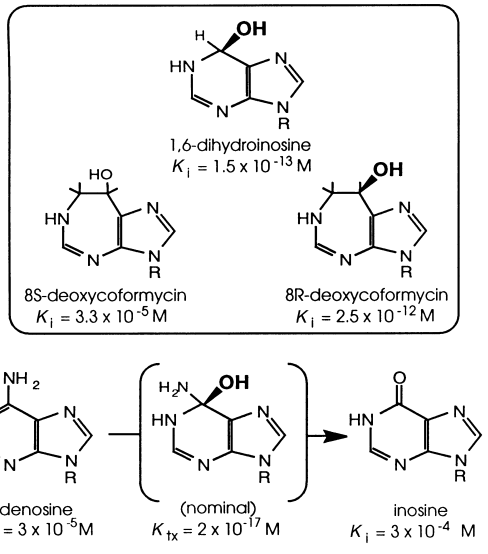


Figure 2. Inhibitors of adenosine deaminase, designed to resemble a tetrahedral intermediate (in brackets) that approaches the transition state in structure (refs 12–14).

binds S‡ more tightly than S. NMR experiments suggest that substrates often appear to be bound by enzymes, initially, in forms that are closely related to the forms that are most abundant in free solution.[15] Accordingly, substrate analogues are expected to be relatively weak inhibitors. Indeed, very few substrate analogues had been reported by 1970 that were more tightly bound than the substrates themselves.[16] Moreover, substrate analogues are expected to inhibit any of the enzymes with which a particular substrate may interact, including those involved in its formation and breakdown. In contrast, transition state analogue inhibitors tend to be specific for the particular reaction whose unique transition state they resemble.

Does an enzyme's native structure furnish an ideal template for inhibitor design?

The affinity of an enzyme for the altered substrate in the transition state, and its ability to distinguish between S and S‡, presumably depend on structural complementarity between the host and its guest. Upon first glance, one might guess that optimal affinity would be observed if the enzyme's active site, in its native or most stable form, were rigidly designed to form a perfectly-fitting template for S‡. It was therefore of interest when, in 1970, the crystal structure of one of the first transition state complexes revealed a tendency of the enzyme's structure to change with inhibitor binding. Crystals of triosephosphate isomerase contracted by 7% along their major axis when 2-phosphoglycolate was bound, and expanded to their original size when the inhibitor was removed by dialysis,[17] and the crystal structures of other enzyme complexes with transition state analogue inhibitors exhibit a similar tendency of the enzyme to surround the substrate in its activated forms.[18,19]

Figure 3 suggests a possible reason for this tendency.[20] When k_{cat}/K_m is measured, many enzymes are found to act with apparent second order rate constants that approach the limits imposed by the diffusion of enzyme and substrate in solution. That implies that many enzymes tend to be open to substrate access most or all of the time (i.e. that an enzyme's active site tends to remain in an open configuration before substrate binding). The movements just mentioned would seem understandable if the substrate were first bound weakly by an open form of the enzyme, and the active site were then to change in such a way as to surround the altered substrate in the transition state. That would allow maximization of the solid angle of contact, and of attractive forces of attraction, between E and S‡. In that way, alternation of the enzyme between open and closed configurations might allow rapid substrate access to be reconciled with tight binding in the transition state. The only additional formal requirement would seem to be that the motion of the enzyme not be intrinsically costly from an energetic standpoint, i.e. that the enzyme be able to move easily between two structural extremes, as in the opening and closing of a first baseman's glove (sometimes described as a 'hinge-bending' movement) involving two domains of the protein.

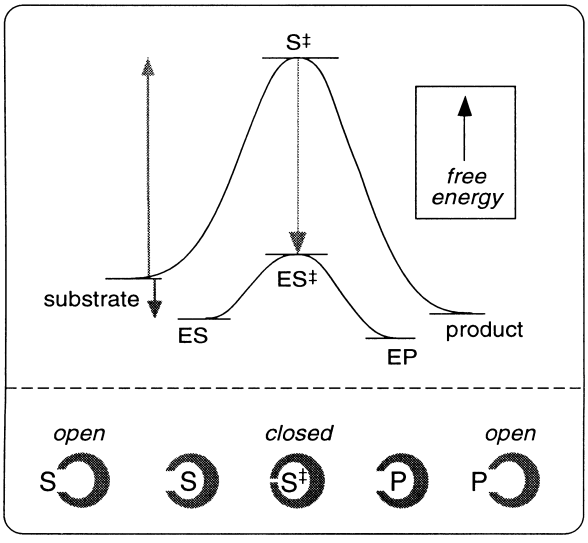


Figure 3. If the free energy benefit of improved contact between E and S‡ exceeds the cost of enzyme distortion from its native (open) structure, then distortion of the enzyme can be expected to benefit catalysis (ref 20).

To the extent that this behavior is general, it might be a mistake to attempt to design an analogue to fit the native, or open, configuration of the enzyme. Much higher affinity is achieved in the transition state complex, in its closed configuration. Accordingly, that structure, rather than the native structure in its 'open' configuration, appears to offer an ideal 'template' for further improvements in inhibitor design. It is most directly investigated experimentally by determining the crystal structures of transition state analogues of high affinity. This should help to reveal how the potential energy of the active site's conformation changes with changing circumstances, allowing optimization of inhibitor design by successive approximation.

Does transition state affinity depend on a few, or on many, interactions?

Differences in structure between S and S‡ are apparently even more obvious to an enzyme's active site than they are to a chemist, viewing their structures on paper. The remarkable fact remains that nearly all the structural features of S are usually present in S‡, and the differences between them seem to be so few in number that an enzyme's ability to maintain such a sharp quantitative distinction between these structures, in terms of binding affinity, is baffling. Confronted with the relative binding affinities shown in Figure 4, a reductionist might conjecture that the distinguishing structural feature of the transition state should, by itself, tend to confer very high binding affinity on a potential inhibitor. Does the ability of an enzyme's active site to distinguish between S and S‡ depend on a few local interactions at the sites of difference, or on an ensemble of interactions that involves every part of a substrate's structure?

The answer to that question, so far as it has been learned from experiments with transition state analogue inhibitors, seems to be that enzymatic transition states

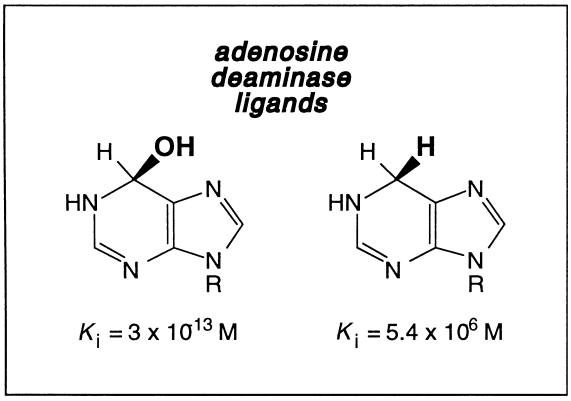


Figure 4. The contribution to binding affinity made by a single hydroxyl group in the 1,6-covalent hydrate of inosine, a transition state analogue inhibitor of adenosine deaminase (ref 13).

are designed to exploit multiple interactions. Weak individually, these interactions gain collective strength when they occur together, as in an enclosed transition state analogue complex. Experiments involving individual mutations of either the protein or the ligand show that elimination of any single binding interaction, in one of these tight complexes, can result in catastrophic losses of binding affinity *even if they are distant from the site of chemical transformation of the substrate*. Conversely, the gain of binding strength that individual interactions derive from the fact that they are connected, in adenosine[21] (Fig. 5) and cytidine[22] deaminases approaches the very large increments ($\sim 10^8$ in terms of effective molarity) that were estimated in theory by Page and Jencks.[23] The finding that catalysis is intensely dependent on the structural context of the substrate group that is being transformed is familiar from the long-recognized substrate specificity of enzymes, which often fail to act on substrates smaller than their natural substrate, even though these 'mini-substrates' would be expected to be capable of entering the active site. What is surprising is that seemingly irrelevant substituents (such as a ribose hydroxyl group in the case of cytidine deaminase),[22] sometimes play an overwhelmingly important role in enhancing k_{cat}, rather than K_m. In rigid substrates like these, an unchanging scaffold of nonparticipating groups in a substrate appears to provide a setting that is essential for ideal expression of an enzyme's catalytic action. That behavior seems understandable by analogy with binding phenomena such as the 'chelate effect',[23] in which one binding interaction introduces structural constraints that greatly increase the probability of a second binding interaction. Individually, these interactions are so weak as to be unobservable in simple model systems in water. Together, they achieve great strength. Observations of this kind suggest a means by which extremely high affinities could be generated from ordinary forces (H-bonds, electrostatic interactions, and nonpolar interactions), almost without limit.

How large affinities are expected of ideal transition state analogue inhibitors?

Figure 1 indicates that the rate enhancement that an enzyme produces determines its susceptibility to inhibition by an ideal transition state analogue. By varying the structure of the substrate, that possibility has been confirmed by comparison with the K_i values for several protease inhibitors.[24–26] Similarly, if the structure of the enzyme is altered by mutagenesis, the enzyme variant with the greatest catalytic power should be most sensitive to inhibition by an ideal transition state analogue inhibitor. This latter expectation has been tested and exploited as a means of separating enzymes, using a transition state analogue inhibitor to elute enzymes in

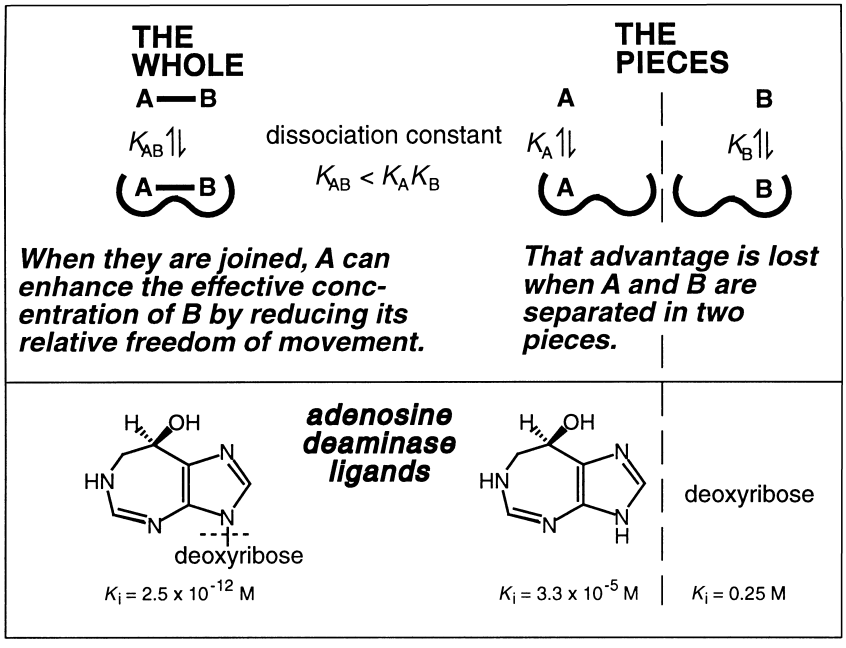


Figure 5. The advantage of connectivity in ligand binding, as proposed by Page and Jencks (ref 23) and as measured for 2′-deoxycoformycin, a transition state analogue inhibitor of adenosine deaminase (ref 21).

order of decreasing turnover number from a conventional substrate affinity column.[27]

Figure 1 also suggests that the slow progress of biological reactions in the absence of catalysts offers a standard by which to judge the catalytic power of existing enzymes, and their potential susceptibility to inhibition by ideal transition state analogue inhibitors. By comparing various enzymes with respect to the rate enhancements that they produce, it should be possible to identify those enzymes that offer the most sensitive targets for inhibitor design. With the exception of a few relatively fast reactions such as the hydration of CO_2, most biological reactions proceed so slowly that their rates have often been regarded as beyond the possibility of measurement in the absence of enzymes, even using Arrhenius plots. Many spontaneous reactions would indeed be far too slow to follow at temperatures below the critical point of water if they doubled in rate as the temperature increases by 10 °C. That property, first described by Harcourt in 1870,[28] has since been imputed to other reactions in aqueous solution by most textbooks that have anything to say about the matter.

Recent experiments have shown that, instead, reactions proceeding spontaneously in solution vary greatly in their kinetic response to temperature, tending to become more temperature-dependent as their rates decrease. Instead of doubling in rate ($\Delta H^{\ddagger} = 12$ kcal/mol), the very slow decarboxylation of orotidine 5′-phosphate increases by a factor of 12.5 as the temperature increased from 20 to 30 °C ($\Delta H^{\ddagger} = 44$ kcal/mol).[29] That tendency renders it possible to follow even very slow reactions in neutral solution in sealed tubes at high temperature, using Arrhenius plots to obtain an extrapolated rate at room temperature. These experiments reveal that the progress of some uncatalyzed reactions is slow even on a geological time scale. At pH 7 and 25 °C, half-times for hydrolysis are 400–500 years for peptide bonds,[30] 180,000 years for phosphodiesters,[31] and 5–8 million years for *O*-glycosides.[32] For the decarboxylation or orotidine 5′-phosphate, the last step in pyrimidine biosynthesis, the half-time is 78 million years, implying a rate enhancement of 10^{17}-fold and a dissociation constant of less than 10^{-23} M for the enzyme–substrate complex in the transition state.[29] Table 1 shows rate enhancements and transition state affinities that have been determined recently for enzyme reactions of several kinds.

Table 1 Dissociation constants (K_{tx}) of substrates from enzymes in the transition state

Uncatalyzed reaction	$k_{25\,°C}$ sec^{-1}	k_{cat}/k_{non}	K_{tx} M
OMP decarboxylation[29]	3×10^{-6}	1.4×10^{17}	5×10^{-24}
Phosphomeonoester hydrolysis[32]	5×10^{-14}	1×10^{15}	$<3\times10^{-20}$
Mandelate racemization[31]	3×10^{-13}	1.7×10^{15}	2×10^{-19}
β-Methyl glucoside hydrolysis[33]	5×10^{-15}	$>10^{17}$	$>10^{-22}$
Fumarate hydration[34]	3×10^{-14}	3.5×10^{15}	1×10^{-21}
Phosphodiester hydrolysis[32]	2×10^{-13}	1×10^{17}	$>10^{-22}$
Peptide hydrolysis (average)[30]	5×10^{-11}	1.3×10^{13}	3×10^{-18}
Chorismate mutation[35]	3×10^{-5}	1.9×10^{6}	4×10^{-10}
Carbonic anhydrase[36]	2×10^{-3}	7.7×10^{6}	9.2×10^{-8}

Are such magnitudes attainable in real inhibitors?

The values in the last column of Table 1 indicate that extremely high affinities should be attainable in principle, if a perfect analogue of the altered substrate in the transition state could be devised. Is the theory itself correct, in indicating that the binding affinity of E–S‡ must be very high?

Figure 1 assumes that enzymatic and nonenzymatic reactions proceed through transition states in which the altered substrate has a similar structure. That assumption need not be correct. One can easily imagine, for example, an enzyme reaction that could proceed by an associative displacement mechanism while the uncatalyzed reaction proceeds by a dissociative mechanism involving a carbonium ion intermediate. In such a case, there presumably exists in principle a nonenzymatic pathway in solution that follows a displacement mechanism. However, that associative pathway must present a higher free energy barrier to activation than the dissociative pathway, since the latter is followed most of the time in solution. As a result, the enzyme's affinity for (and ability to stabilize) the transition state will have been underestimated, and K_{tx} will have been overestimated, according to the path that it actually followed by the enzyme reaction. The same appears to be true if k_{cat} is limited, as is often the case,[37] by a physical event such as product release or a change of enzyme conformation. The chemical transition state in substrate transformation is then reached more easily than k_{cat} would indicate. In summary, if enzymatic and nonenzymatic reactions proceed through transition states that are different, one is led to conclude that the *binding affinity of E–S‡ must be at least as high, and may be higher, than the observed rate enhancement would indicate*, according to Figure 1.[7]

How is it possible for an active site to generate such extreme affinities in watery surroundings, and to exhibit such acute discrimination between similar molecules, using conventional forces of attraction? That question seems baffling in view of the well-known weakness of noncovalent interactions in water, as indicated by the behavior of model systems. It can be addressed by measuring the binding affinities of ground state and transition state analogues and examining their enzyme complexes by exact structural methods. The chelate effect, discussed in previous section, suggests a means by which extremely high affinities could in principle be generated from ordinary forces (H-bonds, electrostatic interactions and nonpolar interactions), almost without limit. Those observations indicate that any one of the forces involved in stabilizing the transition state, if it is eliminated by chemical alteration or mutagenesis, results in drastic losses in binding affinity. Conversely, the appearance of a new binding interaction in the transition state could in principle result in the very large increase in binding affinity that is needed to explain the rate enhancement that an enzyme produces. To the extent that chemists can satisfy the delicate

conformational requirements of these powerful interactions, major improvements in the design of stable inhibitors and man-made catalysts should be possible.

Acknowledgements

This work was supported by the National Institutes of Health research grant GM-18325.

References

1. Segal, H. I. In *The Enzymes, 2nd edition*; Boyer, P. D.; Lardy, H.; Myrbäck, K., Eds.; Academic: New York, 1959; Vol. 1, pp 1–48.
2. Polanyi, M. *Zeitschrift für Elektrochemie* **1921**, *27*, 143.
3. Schwab, G.-M. *Catalysis* (Transl. from the German by Taylor, H. S. and Spence, R.); van Nostrand: New York, 1937; pp 241–243.
4. Pauling, L. *Chem. Eng. News*. **1946**, *24*, 1375.
5. Jencks, W. P. In *Current Aspects of Biochemical Energetics*; Kaplan, N. O.; Kennedy, E. P., Eds.; Academic: New York, 1966; pp 273–298.
6. Wolfenden, R. *Nature* **1969**, *223*, 704.
7. Campbell, I. D.; Jones, R. B.; Kiener, P. A.; Richards, E.; Waley, S. G.; Wolfenden, R. *Biochem. Biophys. Res. Comm.* **1978**, *83*, 347.
8. (a) Wolfenden, R. *Accts. Chem. Res.* **1972**, *5*, 10. (b) Lienhard, G. E. *Science* **1973**, *180*, 149. (c) Wolfenden, R. *Ann. Rev. Biophys. Bioeng*. **1976**, *5*, 271.
9. Radzicka, A.; Wolfenden, R. In *Contemporary Enzyme Kinetics and Mechanism, 2nd edition*; Purich, D. L., Ed.; Academic: New York, 1996: pp 273–298.
10. Huff, J. R. *J. Med. Chem*. **1991**, *34*, 2305.
11. Maurizi, M. R.; Ginsberg, A. *J. Biol. Chem*. **1982**, *257*, 4271.
12. Schramm, V. L.; Baker, D. C. *Biochemistry* **1985**, *24*, 641.
13. Kati, W. M.; Wolfenden, R. *Biochemistry* **1989**, *28*, 7919.
14. Wilson, D. K.; Rudolph, F. B.; Quiocho, F. A. *Science* **1991**, *252*, 1278.
15. Shih, P.; Wolfenden, R. *Biochemistry* **1996**, *35*, 4697.
16. Webb, J. L. *Enzyme and Metabolic Inhibitors*; Academic: New York, 1966; Vol. 2, pp 245–616.
17. Johnson, L.; Wolfenden, R. *J. Mol. Biol*. **1970**, *47*, 93.
18. Lolis, E.; Petsko, G. A. *Biochemistry* **1990**, *29*, 6619.
19. Betts, L.; Xiang, S.; Short, S. A.; Wolfenden, R.; Carter, C. W., Jr. *J. Mol. Biol*. **1994**, *235*, 635.
20. Wolfenden, R. *Mol. Cell. Biochem*. **1974**, *3*, 207.
21. Kati, W. M.; Acheson, S. A.; Wolfenden, R. *Biochemistry* **1992**, *31*, 7356.
22. Carlow, D. C.; Wolfenden, R. *Biochemistry* **1998**, *37*, 11873.
23. Page, M. I.; Jencks, W. P. *Proc. Natl. Acad. Sci., USA* **1971**, *68*, 1678.
24. Westerik, J. O.; Wolfenden, R. *J. Biol. Chem*. **1972**, *247*, 8195.
25. Thompson, R. C. *J. Biol. Chem*. **1973**, *12*, 47.
26. (a) Bartlett, P. A.; Marlowe, C. K. *Biochemistry* **1983**, *22*, 4618. (b) Mader, M. M.; Bartlett, P. A. **1997**, *97*, 1281.
27. Andersson, L.; Wolfenden, R. *J. Biol. Chem*. **1980**, *255*, 11106.
28. Harcourt, A. V. *J. Chem. Soc*. **1867**, *20*, 460.
29. Radzicka, A.; Wolfenden, R. *Science* **1995**, *267*, 90.
30. Radzicka, A.; Wolfenden, R. *J. Am. Chem. Soc*. **1996**, *118*, 6105.
31. Wolfenden, R.; Ridgway, C.; Young, G. *J. Amer. Chem. Soc*. **1998**, *120*, 833.
32. Wolfenden, R.; Lu, X.; Young, G. *J. Amer. Chem. Soc*. **1998**, *120*, 6814.
33. Bearne, S. L.; Wolfenden, R. *J. Amer. Chem. Soc*. **1995**, *117*, 9588.
34. Bearne, S. L.; Wolfenden, R., *Biochemistry* **1997**, *36*, 1646.
35. Andrews, P. R.; Smith, G. D.; Young, I. G. *Biochemistry* **1973**, *12*, 3492.
36. Ghanem, A.; Tsen, W.; Rowlett, R. S. *J. Biol. Chem*. **1986**, *261*, 1164.
37. Cleland, W. W. In *Contemporary Enzyme Kinetics and Mechanism, 2nd edition*; Purich, D. L., Ed.; Academic: New York, 1996; pp 407–440.