Point of VIEW

ACS chemical biology

Rational Design of Transition-State Analogues as Potent Enzyme Inhibitors with Therapeutic Applications

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ABSTRACT The structures of the transition states for a variety of enzyme-catalyzed ribosyl group transfer reactions, determined by computational evaluation of multiple tritium and heavy atom kinetic isotope effects on these enzymatic reactions, have been found to show a considerable variation in the extent of bond cleavage at the ribosyl anomeric carbon. The calculated transition-state structures have been used to guide the design of high-affinity transition-state analogue inhibitors for 5′-methylthioadenosine nucleosidases with potential as therapeutic agents.

The high-resolution X-ray crystal structure of the Michaelis complex between an enzyme and its substrate can be sufficient to provide a description, using curly arrows, of the movement of electrons during the enzyme-catalyzed reaction. However, such crystal structures cannot provide a full description of the mechanism by which an enzyme achieves its enormous catalytic rate acceleration. This determination requires, minimally, a knowledge of the structure of the transition state for the enzyme-catalyzed reaction, along with evaluation of the interactions at the Michaelis enzyme–substrate complex and of the much stronger interactions of the enzyme with the transition state (Figure 1, panel a) (*1*). The important and difficult problem of determination of the structures of transition states for enzymatic reactions is often considered separately from the role of enzymes in biology. On page 725 of this issue, Schramm and co-workers describe experiments that show that these determinations have broad consequences for the design of therapeutically useful tight-binding transition-state analogue inhibitors of enzyme-catalyzed reactions (*2*).

Transition-state structures for enzyme-catalyzed reactions can be evaluated by purely computational methods, but many will argue that these methods are still too primitive for this task. The effect of changing substrate and catalyst structure on transition-state stability can be determined from the effects of these changes on the kinetic parameters for enzyme catalysis (*3, 4*). Such experimental studies provide unmatched insight into the structures of transition states for enzyme-catalyzed reactions, but on their own they are not sufficient to define a unique structure. Finally, insight into transition-state structure can be obtained by evaluating the binding affinities of stable molecules that incorporate structural features of the particular transition state (*5, 6*). These transition-state analogues bind much more tightly to enzymes than do their substrates, with affinities potentially greater than femtomolar (Figure 1, panel a). There are a number of practical applications for such tight-binding ligands to enzyme catalysts (Figure 1, panel b).

A more realistic and feasible approach to the determination of the structure of the transition state for an enzyme-catalyzed reaction is the iterative evaluation of the results of experimental and computational studies (Figure 1, panel b). There is often sufficient flexibility in computational approaches to obtain good agreement between calculations and any single experimental result. The real problem is obtaining agreement between calculations and a broad range of determinations of kinetic isotope effects and/or the effects of site-directed mutagenesis and changing substrate structure on the activation barriers for enzyme-catalyzed reactions. Schramm has developed the following protocol for the

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Published online November 16, 2007
10.1021/cb700228t CCC: $37.00

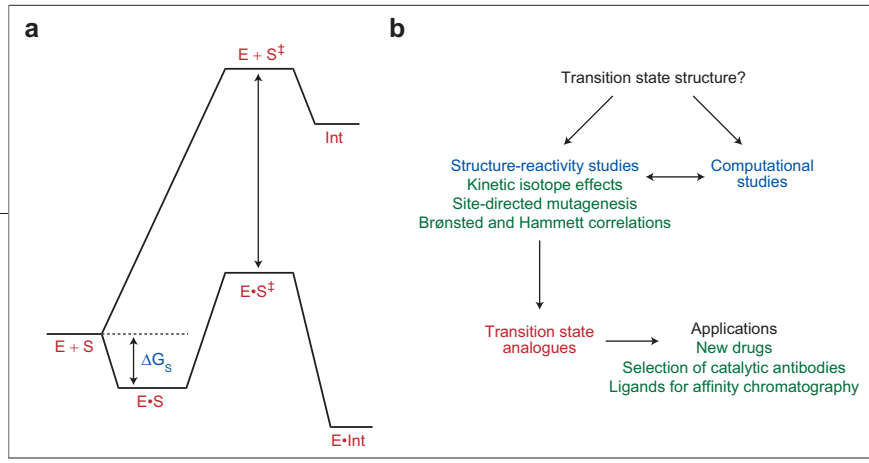


Figure 1. Transition-state analogues, which mimic the tight binding of transition states to enzyme catalysts, have been designed in combined experimental/computational studies. a) Free energy profile that shows how enzymatic catalysis of the formation of an unstable reaction intermediate, such as a carbanion or carbocation, can result from strong specific molecular recognition of the transition state by the enzyme catalyst. A significant fraction of this transition-state binding energy can be recovered in the binding of transition-state analogues. b) Transition-state structure may be elucidated by iterative experimental and computational studies. These studies can guide the design of high-affinity transition-state analogues with many potential applications.

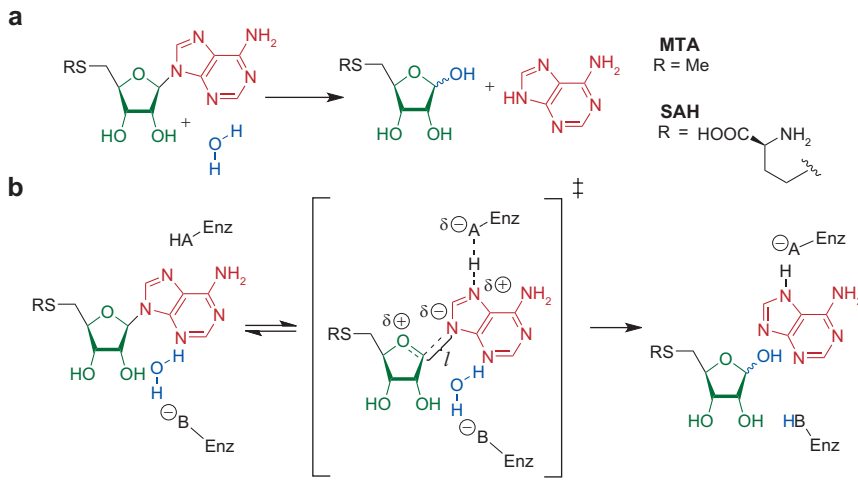
evaluation of transition-state structure and the design of potent transition-state analogues for enzyme-catalyzed ribosyl group transfer reactions (Figure 1, panel b) (*7–9*).

(i) The effects of substitution of tritium at the reacting carbon, of tritium at carbons remote from the reaction site, and of heavy atoms (^{14}C, ^{15}N) at the reaction center on the kinetic parameter *V*/*K* for the enzymatic reaction are first determined.

(ii) Calculations are then carried out to identify a unique structure for the transition state that is consistent with the experimental kinetic isotope effects.

(iii) The calculated transition-state structure is then used to guide the rational design of tight-binding transition-state analogues, which in some cases have applications as therapeutic agents.

This protocol has proven spectacularly successful in studies of 5′-methylthioadenosine nucleosidases (MTANs) and 5′-methylthioadenosine phosphorylase (MTAP) (*10–14*). The nucleosidases are bacterial enzymes that catalyze the irreversible hydrolytic deadenylation reactions of 5′-methylthioadenosine (MTA) and *S*-adenosyl homocysteine (SAH, Scheme 1, panel a). The phosphorylases are present in mammals and catalyze transfer of the 5′-methylthioribosyl group to a phosphate anion. MTANs and MTAP lie on pathways for

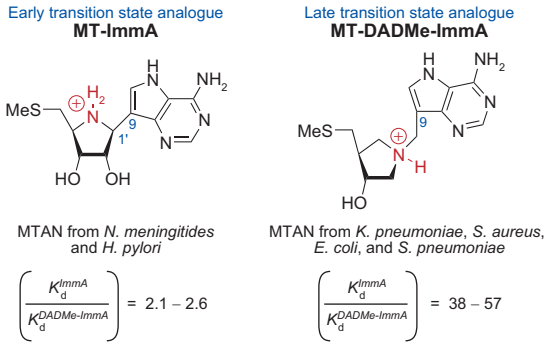


Scheme 1.

the metabolism of *S*-adenosylmethionine that are key to a variety of biological processes such as bacterial quorum sensing (*15*), methylation reactions (*16*), purine salvage (*17*), and polyamine biosynthesis (*18*). The direct roles of MTANs in the biosynthesis of autoinducers that function in quorum sensing pathways make these enzymes good targets for antibiotic agents.

Schramm and his associates determined kinetic isotope effects for the hydrolysis of MTA catalyzed by MTANs from six different organisms: *Neisseria meningitidis*, *Helicobacter pylori*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Escherichia coli*, and *Streptococcus pneumoniae*. The data show that these enzyme-catalyzed ribosyl group transfers proceed through two different types of transition states: (1) The enzymes from *K. pneumoniae*, *S. aureus*, *E. coli*, and *S. pneumoniae* exhibit primary [1′-^{14}C] kinetic isotope effects that are not significantly different from unity and large secondary [1′-^{3}H] kinetic isotope effects of 1.09–1.24. These kinetic isotope effects are consistent with a reaction that proceeds through a *late* transition state in which there is extensive cleavage of the bond between the ribosyl anomeric carbon and the adenine leaving group nitrogen, a long partial bond length *l* (Scheme 1, panel b), and a large development of (delocalized) positive charge at the reaction center. (2) The enzymes from *N. meningitidis* and *H. pylori* exhibit primary [1′-^{14}C] kinetic isotope effects that are significantly greater than unity, as large as 1.039 ± 0.006, and small secondary [1′-^{3}H] kinetic isotope effects of 1.03–1.04. These data are consistent with an *early* transition state for cleavage of the substrate to form an oxocarbenium ion intermediate in a stepwise reaction, a relatively short partial bond length *l*, and a relatively small development of (delocalized) positive charge at the reaction center.

This analysis of transition-state structure provides a simple rationale for the specifi-

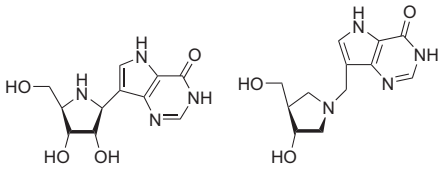


Scheme 2.

city of these different enzymes for the transition-state analogues 5′-methylthio-Immucillin-A (MT-ImmA) and 5′-methylthio-DADMe-Immucillin-A (MT-DADMe-ImmA). The 2.5 Å distance between the cationic 1′-pyrrolidine nitrogen and C9 of 9-deaza-adenine in MT-DADMe-ImmA and the smaller 1.5 Å distance between C1′ and C9 of 9-deazaadenine in MT-ImmA mimic the longer and shorter distances between the ribosyl anomeric carbon and N9 of the adenine ring in the late and early transition states, respectively, for the hydrolysis of MTA catalyzed by MTANs from different organisms. The authors found that MT-ImmA and MT-DADMe-ImmA bind to enzymes that catalyze the cleavage of MTA through early transition states with similar affinity, as measured by K_d. By contrast, MT-DADMe-ImmA, where the separation of charge from the leaving group is large, is bound with a ~50-fold *higher* affinity (smaller K_d) by enzymes that catalyze the cleavage of MTA through a late transition state (Scheme 2). The dissociation constants for the most effective inhibitors are even smaller than 10^{-12} M, which corresponds to a binding energy of >16 kcal/mol for a standard state of 1 M.

Just as impressively, Schramm and co-workers have calculated the structures of the transition states for bovine and human purine nucleoside phosphorylases (PNPs) that are consistent with the experimentally determined kinetic isotope effects (*19, 20*). The calculated transition-state structure for bovine PNP was used to guide the development of Immucillin-H, a first-generation picomolar iminoribitol inhibitor of both bovine PNP and human PNP (*21*). It was noted that the calculated structure of the transition state for human PNP shows a more fully developed ribooxacarbenium ion and greater bond cleavage to the adenine leaving group than does the transition-state structure calculated for bovine PNP (*19*). This led to development of the second-generation transition-state analogue DADMe-Immucillin-H, which shows a higher affinity for human PNP than the first-generation inhibitors and higher specificity for binding to human PNP compared with the bovine enzyme (*22*).

The observation that genetic deficiency of PNP in humans causes a specific T-cell immune deficiency syndrome (*23*) suggests that inhibitors of human PNP might serve as therapeutic agents for conditions that are caused by a proliferation of T-cells, such as T-cell leukemia and T-cell-related autoimmune diseases. The first-generation transition-state analogue inhibitor of PNP, Immucillin-H (Scheme 3), has been reported to be in phase II clinical trials for relapsed/resistant T-cell leukemia and cutaneous T-cell leukemia, and the second-generation inhibitor DADMe-Immucillin-H (Scheme 3) has entered phase Ib clinical trials for T-cell autoimmune disorders (*8*).



Scheme 3.

This exciting work raises a wealth of interesting questions about why there is no evidence from studies of solvolysis in water for the plasticity in transition-state structure that is observed for the MTAN-catalyzed hydrolysis of MTA. It also probes the mechanistic imperatives for enzyme catalysis through early and late transition states, which may lead to lively debate. The focused central conclusion is that transition-state structures obtained by computational analyses of experimental isotope effects have real predictive power in the design of transition-state analogue inhibitors of enzyme-catalyzed reactions. This conclusion is made especially compelling by the resulting rational design of tight-binding transition-state analogue inhibitors that have strong therapeutic potential. These are impressive results, and other academic laboratories and drug companies might decide to work toward their emulation.

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