Theozymes and compuzymes: theoretical models for biological catalysis

Dean J Tantillo, Jiangang Chen and Kendall N Houk*

A theozyme is a theoretical enzyme constructed by computing the optimal geometry for transition-state stabilization by functional groups. It is created in order to permit quantitative assessment of catalytic function. Theozymes have been used to elucidate the role of transition-state stabilization in the mechanisms underlying enzyme- and antibody-catalyzed hydroxyepoxide cyclizations, eliminations and decarboxylations, peptide and ester hydrolyses, and pericyclic and radical reactions. The enzymes studied include orotodine monophosphate decarboxylase, HIV protease and ribonucleotide reductase.

Addresses
Department of Chemistry and Biochemistry, University of California, 405 Hilgard Avenue, Los Angeles, CA 90095-1569, USA
*e-mail: houk@chem.ucla.edu

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Abbreviations

B3LYP	Becke 3-parameter hybrid DFT–HF method with Lee–Yang–Parr correlation functional
DFT	density functional theory
HF	Hartree–Fock theory
HIV-PR	human immunodeficiency virus type 1 protease
ODCase	orotidine monophosphatase decarboxylase
RNR	ribonucleotide reductase
TSA	transition-state analog

Introduction

The understanding and mimicry of biological catalysis at the atomic level is a 'holy grail' for mechanistic chemists. Much recent progress can be attributed to the discoveries of structural biologists and computational chemists. X-ray crystallography and NMR spectroscopy provide a multitude of structural information about enzymes and their interactions with other molecules in both the solid state and in solution. Theoretical chemistry can provide structural information on transition states and snapshots of molecules in the act of reaction; direct detection of such transient events is not possible by normal physical methods. Information from both of these sources, along with data from biochemical experiments, can be used to construct potential energy surfaces for enzyme-catalyzed reactions. The chemist's goal is to understand the interactions between individual atoms of the enzyme and the substrate, and to determine how these interactions facilitate the reactions of the substrate.

Stabilization of the transition state relative to the ground state lowers the activation energy of a reaction, leading to acceleration of the reaction rate. Quantifying specific catalyst–transition-state interactions is essential for an understanding of the details of biological catalysis. Theory and modern computational methods now make it possible to obtain very accurate information about the energies and geometries of transition states of reactions. This is still only feasible for small systems relative to substrates and catalysts on the biological scale, however. Consequently, model systems are still very valuable approaches to understanding biological catalysis.

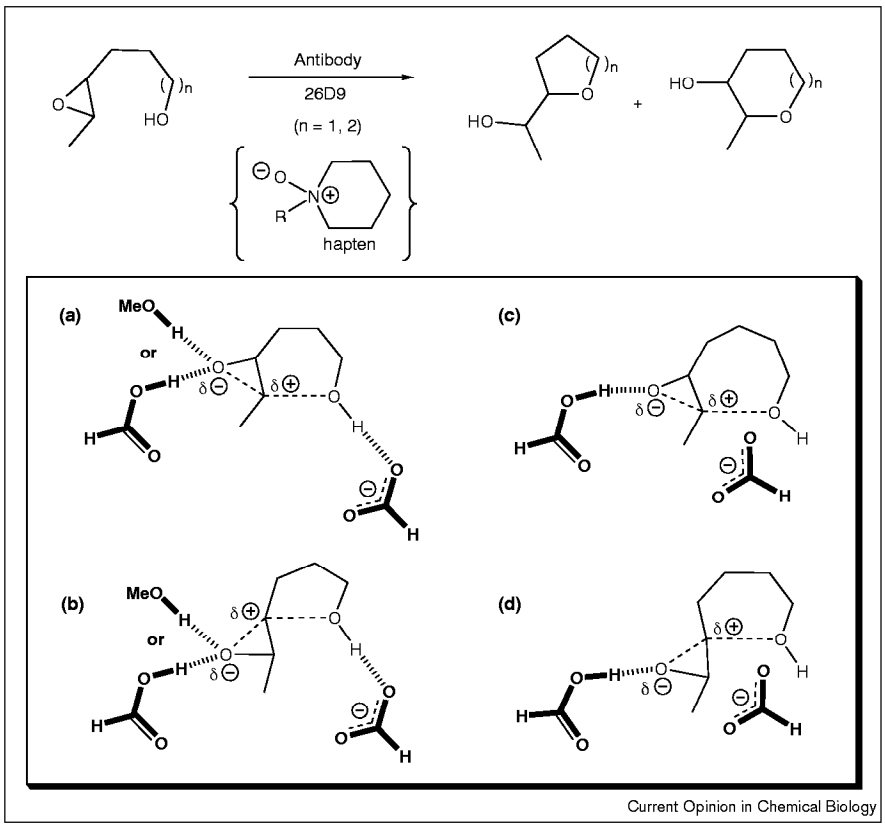
We have defined a specific type of model for biological catalysis, which takes advantage of modern quantum mechanical methods and programs. We define a 'theozyme' (which is short for 'theoretical enzyme') as an array of functional groups in a geometry predicted by theory to provide transition-state stabilization. Such a hypothetical arrangement of functional groups, fixed in space by arbitrary constraints, provides a means for the quantitative assessment of the contribution of individual atomic interactions to catalysis. Theozymes have also been called 'compuzymes' to emphasize their computational origin. Theozymes or compuzymes can be employed to better understand biological catalysts or to design new inhibitors, haptens or synthetic catalysts.

This review defines the concept of theozymes and describes recent progress towards understanding biological catalysis of organic chemical reactions resulting from the use of theozymes. Related applications of theory in examining discrete supramolecular interactions, such as theozyme models for nonbiological processes [1], various related 'supermolecule' approaches to solvation and receptor binding [2•,3], the construction of 'minireceptors' for quantitative/structure activity relationships for drug design [4•], and the many other applications of theory to biological catalysis, are beyond the scope of this review.

How can an ideal catalytic array be constructed? Given nearly infinite computer resources, a combinatorial library of every possible arrangement of biologically available functionalities could be created and computationally assayed for stabilization energy of a particular transition state. Such a process would be a theoretical analog of the evolutionary process that has led to supremely efficient enzyme catalysts. It would be extremely interesting to compare such a theozyme to nature's solutions to such problems — the enzymes resulting from centuries of evolution. This strategy is currently impractical, and has not yet been tested.

More typically, the optimal arrangement of a limited number of 'residues' about a transition state (or transition-state model such as a transition-state analog [TSA] inhibitor or hapten) is computed. Experimental information and

Figure 1



Theozymes for general acid/general base catalysis of hydroxyepoxide cyclizations. The general reaction is shown at the top, where the hydroxyepoxide is cyclized by antibody 26D9, which is elicited in response to the *N*-oxide hapten shown. In the box, groups in boldface constitute the theozyme, while regular lines and type represent the transition state of each cyclization. **(a)** and **(b)** show the transition states of cyclizations leading to the six- or the five-membered product, respectively, when n = 1. **(c)** and **(d)** show transition states for the formation of the 7-endo and 6-exo cyclizations when n = 2. Me, methyl; R, alkyl substituent to tether to the carrier protein.

chemical insights into how the transition state might be stabilized tend to govern the choice of residues. In all cases, quantum mechanical (Hartree–Fock theory [HF], density functional theory [DFT] or semi-empirical) calculations are first used to obtain a transition state for the reaction of interest in the absence of external catalytic groups. This transition-state structure is then decorated with residues with which it can interact noncovalently, and the geometry of the resulting theozyme is optimized in the presence of the transition state. Usually, these residues consist of portions of amino acid sidechains or peptide backbone functionalities.

Sometimes the goal of theozyme construction is to refine the orientations of known active-site residues, or to assess their effects on transition-state stabilization. In such cases, initial coordinates for the residues are obtained from X-ray or NMR structures of enzymes. In the absence of such structural information, the identity of active-site residues can sometimes be obtained from biochemical experiments. Theozyme optimization then leads to predictions of the (theoretically ideal) positions of active-site residues. Additionally, biological motifs can be utilized without specific experimental information about the structure of the active site in order to predict ideal catalytic arrays and to design novel synthetic catalysts. This hierarchy of methods represents a progression towards the purest of theozymes, that which is created by theory alone in the absence of the biases imposed by structural and biochemical information or human intuition.

Theozymes for hydroxyepoxide cyclizations

Catalytic antibodies have been used to catalyze difficult chemical transformations that are kinetically unfavorable in solution. Lerner and co-workers [5] reported the generation of an antibody, 26D9, that catalyzes the cyclization reaction shown in Figure 1 (n = 1) to form a six-membered ring tetrahydropyran product, a reaction that is disfavored in solution with respect to the competing cyclization that forms a five-membered ring tetrahydrofuran product. The *N*-oxide hapten (Figure 1) used to elicit this antibody was assumed to be an analog of the higher energy transition state leading to the six-membered ring product.

The transition states of both reaction pathways were obtained by HF/6-31G* calculations (6-31G*, 6-31+G* and 3-21G are designations of mathematical function that represent the atomic orbitals used in the calculations.) The six-membered transition state is 1.8 kcal/mol higher in energy than the five-membered transition state in the gas phase. Since the six-membered transition state has more S_N1 character (i.e. the carbon being attacked bears a larger partial positive charge), stabilization of the developing positive charge was proposed as a mechanism for catalysis [6].

To test this concept, a model was developed based upon general acid protonation of the epoxide and general base stabilization of the partial positive charge (δ+, see Figure 1). In this first application of the theozyme concept, formic acid (or methanol) and formate groups were placed around the hapten, and their relative positions were optimized computationally. The resulting theozyme was used to test quantitatively the predicted catalytic mechanism. When the two transition states were inserted into the theozyme (Figure 1a,b), a 4 kcal/mol preference for cyclization to form the six-membered ring product was predicted, consistent with the experimental results. The theozyme model suggests that a strengthened hydrogen bond to the epoxide oxygen atom and a better positioned formate group are responsible for this selective stabilization; electrostatic stabilization, rather than preferential hydrophobic complementarity, accounts for the observed selectivity.

These results suggested that the antibody should be capable of selective catalysis of endo epoxide openings for systems with different ring sizes. Thus, Na and Houk [7] asked whether this arrangement of functional groups would also favor cyclization to form a seven-membered ring product in a related system (Figure 1, n = 2). Analogous calculations on the interaction of the theozymes with the transition states for 6-exo and 7-endo cyclization (Figure 1c,d) led to the prediction that the seven-membered ring product should be formed preferentially by the antibody (a 5 kcal/mol difference in activation energies was computed) [7]. This prediction was subsequently verified by experiment [8].

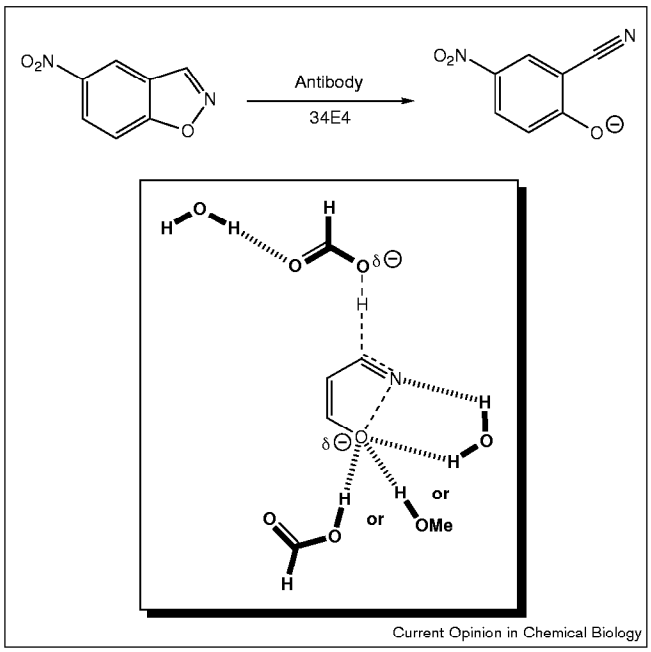
Theozymes for eliminations and decarboxylations

The rate of the base-promoted elimination reaction shown in Figure 2 is subject to large accelerations in nonpolar solvents. Hilvert and co-workers [9] developed an antibody (34E4) that catalyzes the decomposition of a nitrobenzisoxazole with a rate greater than 10^8-fold faster than the acetate-catalyzed reaction in water. This is one of the largest rate accelerations yet observed with catalytic antibodies. Theoretical calculations were carried out in order to quantitate the importance of the catalytic base and various hydrogen bond donor residues [10].

The formate anion and several hydrogen bond donors were chosen as components for the theozymes. Calculations with these models indicated that desolvation of the catalytic base could account for as much as a factor of 10^4 in rate acceleration, that the orientation of the carboxylate group has a significant effect on the rate, and that hydrogen bonding to the forming oxide could potentially lead to greater rate acceleration. The theozymes were also used to design new haptens by calculating the energy of interaction of different potential haptens with the fixed theozyme.

The decarboxylation of α amino acids by pyruvoyl-dependent decarboxylases was examined by DFT calculations utilizing a theozyme model composed of a formic acid residue and a simple peptide fragment (Figure 3), representing glutamate and phenylalanine residues present in the active site of pyruvoyl-dependent histidine decarboxylase [2•]. These calculations predict that the enzyme active site provides a network of hydrogen bonding functionalities that can selectively stabilize a zwitterionic intermediate and transition state. This hydrogen bonding network is predicted to provide greater stabilization than multiple but weaker hydrogen bonds to water. An alternative role for the backbone amide functionality, that of providing an electron sink for the putative oxyanion that forms on the pyruvoyl carbonyl, is predicted to have no effect on the activation barrier.

Figure 2



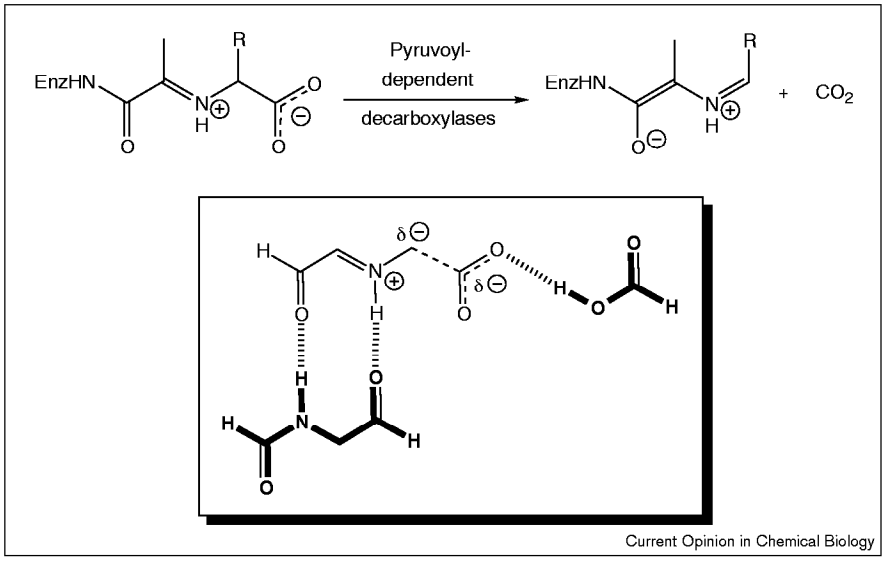
Theozymes for base-promoted ring-opening of isoxazoles. Me, methyl. Theozyme groups are shown in bold and the transition state in regular type. (See text for full details.)

A very simple theozyme was derived to account for the 'prodigious proficiency' of the biosynthetically important enzyme, orotidine monophosphate decarboxylase (ODCase) [11••]. Second order Møller–Plesset pertubation theory (MP2//HF/6-31+G* computations on this decarboxylation in the presence of an ammonium cation near O4 (Figure 4), to represent the lysine residue in a nonpolar cavity known to be essential for catalysis, provided a novel predicted mechanism involving a stabilized carbene intermediate. The theozyme led to the conclusion that protonation at O4 would be much more effective than protonation at O2. Nothing is presently known about the binding site of ODCase.

Theozymes for peptide and ester hydrolysis

Hydrolyses of peptide bonds are involved in many fundamental biological processes, and inhibitors of such

Figure 3

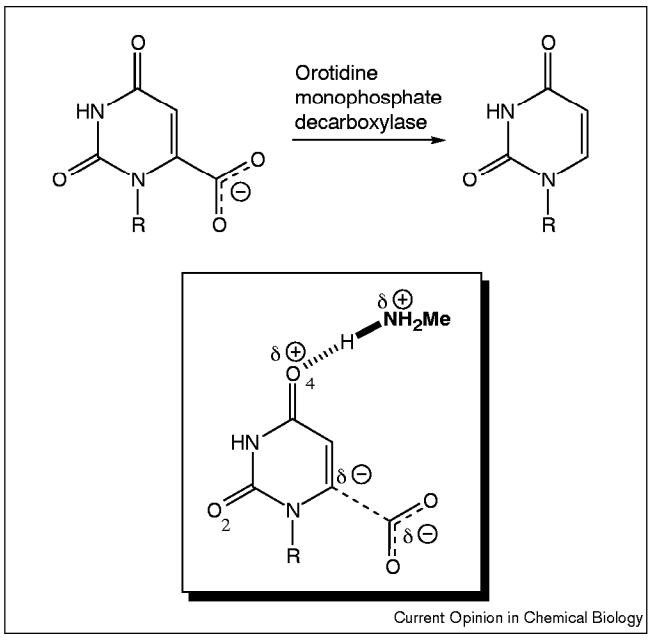


A theozyme for enzymatic decarboxylation of α amino acids. Theozyme groups are shown in bold (formic acid and a simple peptide fragment), and transition state structures in regular type. Enz, enzyme; R, substituent groups. (See text for full details.)

processes are potential therapeutics for preventing particular diseases. Recently, theozymes have been used to gain insight into the nature of catalysis by two of the most common classes of hydrolytic enzymes, the aspartyl and serine hydrolases.

Human immunodeficiency virus type 1 protease (HIV-PR) is a homodimeric aspartyl protease, whose critical role in maturation of the HIV-1 virus involves the catalysis of peptide hydrolysis. Since inhibition of this enzyme leads to a noninfectious virus, details of the mechanism by which HIV-PR catalyzes the hydrolysis of peptide bonds are of considerable interest. Two general mechanisms have been suggested for this transformation: concerted attack of water with loss of an amine group [12], and stepwise addition of water to form an amide hydrate intermediate, followed by elimination of an amine (Figure 5) [13]. A theozyme was created to test each of these pathways, consisting of a formic acid/formate ion pair corresponding to the two aspartyl residues at the active site of HIV-PR (Figure 5). HF/6-31G* optimizations were used to characterize the concerted pathway [14], and HF/6-31G* and MP2/6-31+G* calculations were used to map out the stepwise pathway [15•]. For the stepwise process, elimination was found to be rate-determining. Both pathways exhibit extensive transition-state stabilization through hydrogen bonding and other electrostatic interactions as shown in Figure 5. Considerable movement of the carboxylate moieties is observed along each reaction coordinate. These computations show that both mechanistic pathways are reasonable; further experimental and/or computational experiments are necessary to conclusively differentiate between them. Nonetheless, the geometric details of the transition states obtained from these studies are being used to design TSA inhibitors of HIV-PR.

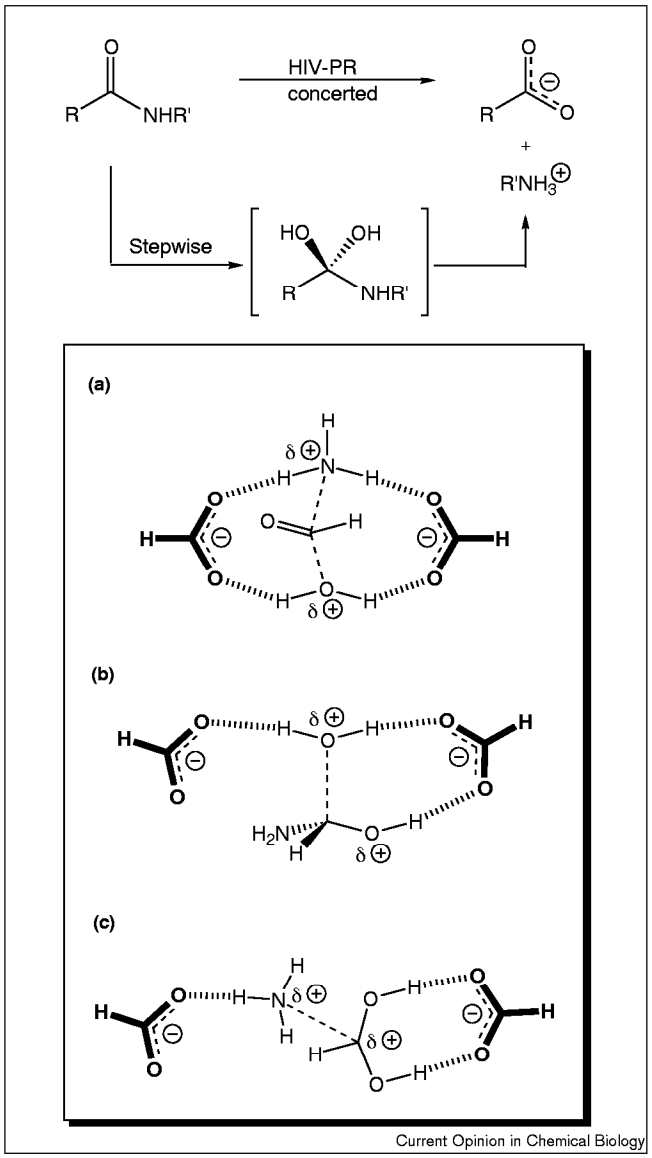
Figure 4



A theozyme for decarboxylation of orotidine monophosphate by an ammonium ion in a nonpolar environment. Again, the theozyme (the ammonium cation) is shown in bold, and the transition state as regular text (see box). A novel predicted mechanism for ODCase catalysis was elucidated using this method. Me, methyl.

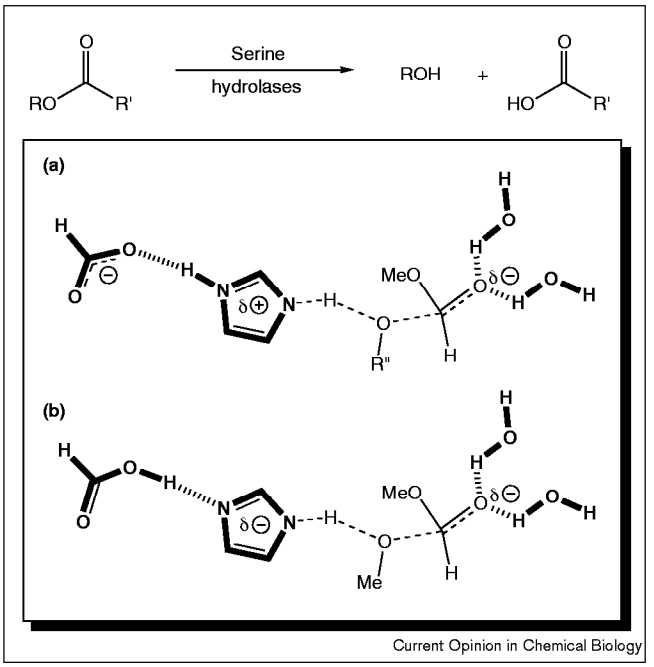
Serine hydrolases are enzymes which hydrolyze amides and esters by utilizing the Asp-His-Ser catalytic triad to activate the hydroxyl group of an active site serine residue for nucleophilic attack. The exact function of the catalytic triad has been the subject of many biochemical and theoretical studies. Theozymes consisting of formic acid and imidazole, corresponding to the aspartic acid and histidine

Figure 5



A theozyme for HIV-PR-catalyzed amide hydrolysis. The general reaction is shown above, and can follow either the concerted or stepwise pathway. A theozyme was created in order to test which mechanism was involved in catalysis. Transition states for **(a)** concerted hydrolysis of formamide, **(b)** addition of water to formamide and **(c)** elimination of ammonia from an amide hydrate intermediate are shown in the box. Computations using this theozyme indicated that both pathways are feasible.

Figure 6



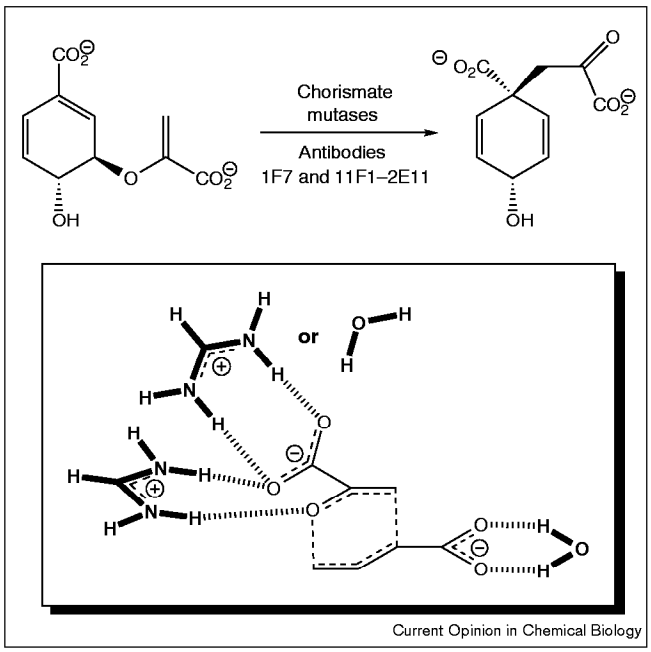
A theozyme for ester hydrolysis. Transition states for **(a)** acylation and deacylation (R'' = Me [methyl], transition state for acylation; R = H, transition state for deacylation), and for **(b)** an alternative 'double-proton transfer' acylation pathway are shown in the box. The general reaction is shown above. Theozyme groups (formic acid, water and imidazole) are shown in bold. (See text for full details.)

residues of the triad, and two water molecules, representing an oxyanion hole (Figure 6), were constructed and optimized in the presence of transition states for attack of methanol (corresponding to the active site serine residue) on the model substrate methyl formate at the HF/6-31+G* and B3LYP (Becket 3–parameter hybrid DFT–HF method with Lee–Yang–Parr correlation functional)/6-31+G* levels of theory, and single point energies for each geometry were also obtained at the MP2/6-31+G* level [16]. The acylation step was found to be rate-determining. Hydroxyl attack with concerted proton transfer was promoted by interaction of the proton-accepting imidazole group with the anionic carboxylate group of the triad, and the developing negative charge on the carbonyl oxygen atom was stabilized by hydrogen bonding to the oxyanion hole. In smaller theozymes without the carboxylate group, one or both water molecules were also examined in order to dissect the stabilization provided by the theoretical binding site. These calculations predict that the oxyanion hole provides approximately 7 kcal/mol of stabilization energy and that the carboxylate moiety is responsible for 5–9 kcal/mol of stabilization energy; these predictions are in reasonable agreement with biochemical data from mutagenesis experiments. A 'double-proton transfer' pathway (which involves alternative protonation states of the aspartic acid and histidine residues; Figure 6) was found to be disfavored with respect to the acylation pathway. The acylation theozyme can be superimposed quite closely on the X-ray crystal structure of chymotrypsin bound to a trifluoromethyl ketone inhibitor. The similarity between the two structures suggests that the serine hydrolases have, in fact, been optimized by evolution for the optimum transition-state binding geometry.

Theozymes for pericyclic reactions

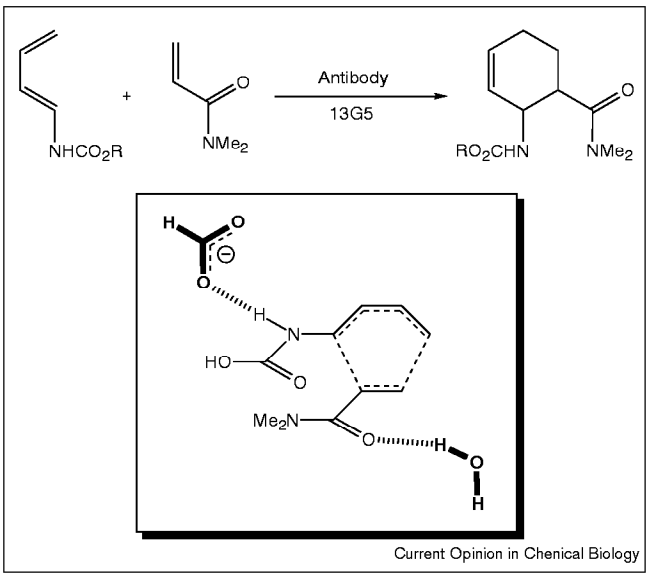
The chorismate–prephenate rearrangement, a biological pericyclic reaction known as a [3,3]-sigmatropic shift, is

Figure 7



Theozymes for catalysis of the [3,3]-sigmatropic shift of a simple chorismate model. Theozymes consisting of different stabilizing residues based on known antibody and enzyme structures are shown in bold.

Figure 8



A theozyme for an antibody-catalyzed Diels–Alder reaction, with the general mechanism shown above. Theozyme water and formate molecules were used to establish details of transition state stabilization and enantiomer specificity in this reaction. Me, methyl; R, a benzylcarboxylate sidechain. (See text for full details.)

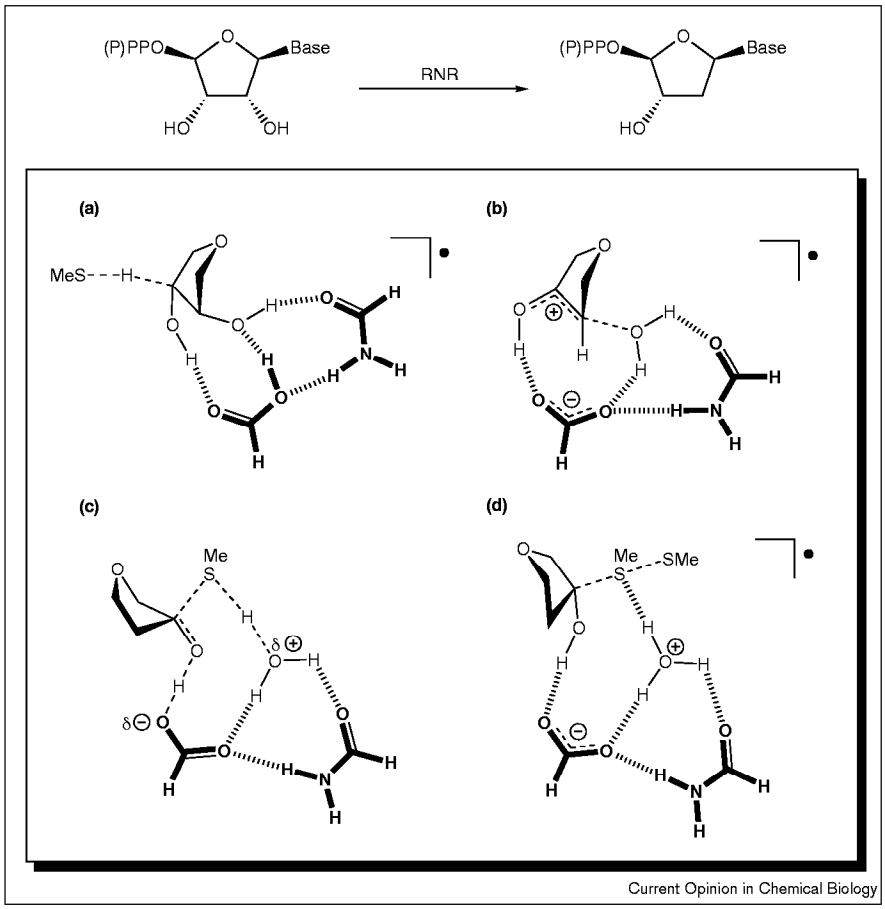
catalyzed by chorismate mutase enzymes [17,18], as well as two catalytic antibodies, IF7 and IIF1–2E11 [19,20]. The specific interactions responsible for catalysis of this reaction were elucidated through sequential application of biochemical methods, X-ray crystallography and one of the earliest-reported theozyme studies ([21] and references therein). Theozymes consisting of different combinations of stabilizing residues based on known enzyme and antibody structures (Figure 7) allowed specific noncovalent interactions that lead to transition-state stabilization to be pinpointed. In particular, appropriately positioned hydrogen bond donor residues were shown to be the most important factor for catalysis, leading to a decrease in activation energy of 6 kcal/mol.

The Diels–Alder reaction is perhaps the best known pericyclic transformation in organic chemistry. Antibodies raised against TSAs for Diels–Alder reactions catalyze this transformation [22–25]. The Diels–Alder abzyme 13G5, an antibody raised by Janda and co-workers [25], catalyzes the reaction shown in Figure 8. Recently, an X-ray structure of this antibody complexed to an inhibitor was obtained at 1.95 Å resolution [26••], clearly delineating the putative active-site surface. Antibody 13G5 catalyzes the ortho–exo process to form a single product, but which ortho–exo enantiomer is formed is unknown. By using the theozyme strategy, the specific interactions leading to catalysis were defined and a prediction was made as to which enantiomer of product is formed preferentially [26••].

The theozyme model used in this case is comprised of a molecule of water and a formate molecule (Figure 8). The theozyme complex with a transition state for a model Diels–Alder reaction (Figure 8) was fully optimized at the HF/3-21G level, and energies were obtained at the B3LYP/6-31G* level. These calculations indicated that hydrogen bonding to the amide carbonyl group of the dienophile can lower the activation energy of the reaction by ~1 kcal/mol. A carboxylate anion hydrogen bonded to the NH proton of the diene can lead to a greater decrease (~6 kcal/mol) in the activation barrier. When both stabilizing residues are present, a synergistic effect is predicted, leading to a lowering of the barrier by 10 kcal/mol. In the antibody combining site, tyrosine and aspartate residues are present and could provide these stabilizing interactions. In order to validate this prediction, the various endo and exo transition states were computationally docked into the antibody combining site. Both enantiomers of the ortho–exo transition states were accomodated in orientations that allow for these stabilizing interactions, but one enantiomer is predicted to be favored; it appears, however, that the combining site is not capable of providing such hydrogen bonds to either ortho–endo transition state. The use of the theozyme concept in this case, in conjunction with biochemical, crystallographic and automated docking experiments, has led to a unified picture of catalysis by 13G5. The specific noncovalent interactions leading to transition-state stabilization were identified and their relative importance assessed, and a testable prediction was made as to the identity of the favored cycloaddition product. Moreover,

Figure 9

A theozyme with transition states for several key steps in RNR-catalyzed reduction of nucleotides to deoxynucleotides. The general mechanism is shown above. Transition states within theozymes are shown in the box. **(a)** The transition state for hydrogen abstraction, **(b)** for water elimination, **(c)** for cysteine attack and **(d)** for disulfide formation. Theozyme groups are indicated in bold. Me, methyl. (See text for full details.)



the similarity between the antibody combining site and the optimized theozyme suggests that the antibody has been selected for optimized transition-state stabilization by a hydrogen bond donor/acceptor pair.

Theozymes for radical reactions

Ribonucleotide reductases (RNRs) catalyze the conversion of ribonucleotides to deoxyribonucleotides (Figure 9). The class I RNRs, in which a tyrosyl radical is generated by an oxo-bridged binuclear iron complex, have been well characterized by X-ray crystallographic [27–29], kinetic, spectroscopic, isotopic labeling and mutagenesis experiments [30,31]. In an effort to clarify the details of the mechanism by which class I RNRs deoxygenate ribonucleotides, Siegbahn [32••] undertook a thorough theoretical study in which a model sugar can react with a model thiol (the active-site radical source) in the presence of several key residues known to be present in the active site of RNRs (in this theozyme, a glutamate residue is modeled by a formate molecule and an asparagine residue is modeled by a formamide molecule; Figure 9). Study of this reaction pathway is a formidable test for theozyme methodology in that the process is multistep, many of the fundamental steps involve radical species, and strict energetic and geometric criteria must be met in order for the computations to be consistent with the extensive body of known experimental data.

Theozymes for the transition states of several key steps in RNR catalysis are shown in Figure 9. Computed activation energetics and geometries (from DFT calculations) were compared for theozymes with different residue permutations and isolated structures in the gas phase and in a continuum with a uniform dielectric constant of four. Significant differences in both geometries and energetics were noted for many of the reaction steps. In some cases, theozyme residues became active participants in catalysis, functioning as general acids or bases; this suggests that the RNR active site has not evolved for noncovalent transition-state binding alone. Consideration of multiple theozymes for each fundamental reaction step allowed for direct comparison of the plausibility of several mechanistic pathways. Overall, a six-step mechanism differing from previous proposals in the details of water elimination and subsequent steps was proposed. This work shows how a complex mutistep process involving several types of reactive intermediates can be explored using multiple theozyme calculations, resulting in a detailed model of enzymatic catalysis that would not be possible from knowing only the location of catalytic residues in the active site of the enzyme.

Conclusions

The theoretical construction of model catalytic sites based on quantum mechanical optimization provides quantitative evaluations of different catalytic mechanisms. Theozymes are quantitative models for biological catalysis, suited for the prediction of mechanisms of action of known catalysts and for the design of new catalytic motifs.

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- •• of outstanding interest

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