Enzymes produce the largest rate enhancements of all catalysts. The accepted definition of the rate enhancement of a given enzyme is \( k_{cat}/k_o \), where \( k_o \) is the rate constant for the reaction in water at pH 7. Wolfenden has drawn attention to the fact that \( k_o \) may vary over as much as \( 10^{16} \)-fold, while values of \( k_{cat} \) are restricted to a range (10\(^2\)–10\(^9\)) that allows the reactions to be of biological relevance.\(^1\) Dependent on the reaction in water, the rate enhancement of the enzyme may be required to be anywhere from \( 10^3 \) to \( 10^2 \). What features of mechanism provide the required rate enhancement of an enzyme reaction? Is there essentially one principal means by which rate enhancements of enzymatic reactions is due to the preferential interaction of enzyme with the transition state (TS) as compared to the substrate (S). Apparently, less obvious is the fact that the values of \( \Delta G_{cat}^o \) – \( \Delta G_{o}^o \) are also dependent upon relative stabilities among ground-state conformers in water and in the enzyme.

Probably the most unobscure comparisons of the importance of transition-state and ground-state structures to enzyme catalysis involve unimolecular reactions in which enzyme covalent intermediates are not formed. We present here results\(^2\) from observations of *Medicago sativa* chalcone isomerase and compare these to studies of *E. coli* chorismate mutase.\(^3\) Both chalcone isomerase (Scheme 1) and chorismate mutase (Scheme 2) reactions are intramolecular rearrangements, which share about the same values of \( \Delta G_{cat}^o \) and \( \Delta G_{o}^o \). The transition states can only be reached through ground-state near attack conformers (NACs) in which atoms involved in bond making are at van der Waals distance (Chart 1).

From molecular dynamics (MD) simulations of chalcone E+S, the mole percentage of the Michaelis complex present as NACs is 32%. When S is present in water solution, we find after a 5000 ps MD simulation that NACs are present 0.45% of the time. Thus, in water, the standard free energy (\( \Delta G_{NAC}^o \)) for NAC formation is 3.2 kcal/mol, while in the enzyme \( \Delta G_{NAC}^o = 0.7 \) kcal/mol (Figure 1A).

The experimentally determined value of \( \Delta G_{cat}^o - \Delta G_{o}^o \) for the isomerization of chalcone is 10 kcal/mol.\(^4\) Thus, in terms of free energy, 25% of the rate enhancement is due to the advantage of the enzyme in NAC formation. Comparisons of molecular dynamic structures of E+S and E-TS show that the TS is significantly more stabilized by bringing the enolate form of O7 in close proximity of the protonated amino function of Lys97 (Figure 2). In addition, the smaller size of the TS relative to S allows for the release of \( \sim3 \) water molecules, trapped in the ground state at the active site. This suggests a possible entropic gain on going from E+S to E-TS. Thus, the transition state is stabilized, more so than in water, by Lys97 general-acid catalysis (Figure 2) and release of water molecules. Such stabilizations are, under present nomenclature, spoken of as transition-state binding. Thus, 75% of the enzyme rate enhancement (\( \Delta G_{cat}^o - \Delta G_{o}^o \)) is due to TS stabilization, and 25% is due to the advantage of enzyme ground-state NAC formation.

Chorismate exists in diaxial and diequatorial conformations in water. From our MD simulations,\(^5\) we have identified seven diaxial conformers. The near attack conformer (NAC) required for the Claisen rearrangement to provide prephenate (Scheme 2) is formed by a thermal fluctuation from diaxial conformer I, which places the side chain carboxylate above the ring bringing the reacting C5 and C16 into close proximity in like-TS geometry (Chart 2).

From a total of 30,000 ps MD simulations, the mole fraction of NAC in water is 0.00007%, while from a 2000 ps MD simulation of E-chorismate, the mole fraction of NAC at the active site is 34%. Thus, in water, the standard free energy (\( \Delta G_{NAC}^o \)) for NAC formation is 8.4 kcal/mol, while in the enzyme \( \Delta G_{NAC}^o = 0.6 \) kcal/mol (Figure 1B). From experimental measurements,\(^6\) \( \Delta G_{cat}^o - \Delta G_{o}^o = 9 \) kcal/mol. It follows that the kinetic advantage of chorismate mutase, as compared to the water reaction, is \( \sim90\% \) due to the ability of the enzyme to support the NAC conformation at the active
site. The problem with making a NAC in water is that the two carboxylate functions have to be brought into close proximity for NAC formation. This is very unfavorable. At the active site of the E. coli chorismate mutase, the guanido functions of Arg11* and Arg28 are rigidly held in place such that when the two carboxylates form strong electrostatic interactions with Arg11* and Arg28, the substrate is properly positioned for NAC formation.4 Other movements of the substrate are discouraged by van der Waals contact with Val35 and Ileu81. The structure of chorismate mutase other movements of the substrate are discouraged by van der Waals NAC formation. This is very unfavorable.7 At the active site of the enzyme, and the similar K_M, k_cat, and k_o values. Chalcone isomerase owes its rate enhancement primarily to features characterized as transition-state binding with some ground-state contribution of NAC formation. E. coli chorismate mutase, on the other hand, owes its rate enhancement overwhelmingly to ground-state NAC formation with little preferential binding of the TS (Figure 1). In anthropomorphic terms, these enzymes have done what one would have expected to exhibit useful rates of reaction in excess of the water reactions. For the rearrangement of chorismate, the population of NACs in water is very small such that the enzyme rate enhancement (ΔG_cat^o − ΔG_S^o) can be greatly promoted by a large population of NACs in the Michaelis complex. Once that was accomplished in the enzyme, there was little else that could be done to increase the rate of the Claisen rearrangement — so the enzyme simply watches the rearrangement occur. Chalcone isomerase has very little advantage in NAC formation, simply because NAC is readily formed even without an enzymatic assistance. However, the intramolecular nucleophilic addition to an α,β-unsaturated ketone function can be greatly promoted by general-acid catalysis, and so the enzyme does so.

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**Supporting Information Available:** Experimental details (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

**References**


(2) Structural investigations were carried out by nanoseconds molecular dynamics simulations of enzyme-ligand complexes (E-S and E-TS) or ligands (S), both in TIP3P (ref 3a) water pools with periodic boundary conditions using CHARMM v27 force fields (ref 3b) with S and TS calibrated by ab initio calculation at the B3LYP/6-31+G(d,p) level of theory. Details are presented in the Supporting Information.


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