Transition States: Substrate-induced Conformational Transitions

Secondary article

Article Contents

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Substrate binding produces a variety of conformational changes in an enzyme that result in favourable substrate–protein interactions and influence catalysis in different ways. The altered form of the enzyme often appears to be more active in catalysis. A specific conformational change that continues into the transition state complex is the basis for substrate specificity.

Introduction

The extensive and ever-expanding database of known structures for enzymes and enzyme complexes has revealed that a conformational change in the enzyme often accompanies binding of substrates or cofactors.

$$E + S \rightleftharpoons E_{act}S \rightleftharpoons E + P \qquad [1]$$

In eqn [1], E is the stable form of the enzyme in the free state and E_{act} is the conformation of the enzyme stabilized by interaction with the substrate S; P represents the reaction product. The structure E_{act} is more complementary to the substrate than is E and thus is the more catalytically active form. Changes in the conformation of an enzyme as a result of substrate binding might be anticipated to reduce catalysis by the enzyme, since the free energy expended to change the enzyme to a less stable structure diminishes the free energy of binding and lowers substrate affinity. A rigid-template enzyme, as proposed by Emil Fischer, with optimal transition-state binding would be maximally efficient. Clearly there is more to enzymatic catalysis and the evolution of enzymes than simply maximizing affinity for a single transition state. The idea of achieving maximal catalytic efficiency with a rigid template enzyme that ideally complements the transition state is admittedly simplistic. It discounts the natural conformational flexibility of enzyme molecules and ignores the variation in physical requirements for maintaining functional stability throughout the life cycle of the enyzme. For example, many enzymes function in multiple-step reactions, so that more than one transition state must be stabilized and there must be transitions between them. One easily recognized role for substrate-induced conformational change is promotion of substrate binding and product dissociation. Conformational change provides a mechanism by which substrates can enter and dissociate from a relatively open active site, while rearrangements in the enzyme structure to close down and surround the substrate would maximize favourable interactions over the course of the reaction. Another possible role is related to substrate specificity. Koshland introduced the concept of structural flexibility and the idea that an induced fit might be the basis for substrate specificity (Koshland, 1958). He offered the induced-fit model in comparison to the rigid-template hypothesis for enzymatic activity when little was known about protein structure, just prior to the time the first three-dimensional structure of an enzyme was obtained in 1965 by using X-ray crystallography by Colin Blake, David Phillips and co-workers. Since that time, considerable knowledge of the physical properties of proteins and enzymes has been accumulated. The conformational flexibility of these molecules is now well documented by direct structural methods of crystallography and NMR spectroscopy as well as by neutron diffraction and other spectroscopies. Molecular dynamics simulation has helped to visualize and to understand the range and time scales of protein motions, and the statistical nature of protein conformation. Nevertheless, characterization of an enzymatic reaction coordinate is not yet detailed enough to account for the catalytic rate enhancement of any enzyme.

Given the current knowledge of protein conformational flexibility and intermolecular forces, a change in the average structure upon substrate binding might be considered the common result, rather than the exception. The surface that binds the substrate, and eventually interacts intimately with the transition state, is either solvated by water molecules or forms protein–protein contacts in the apoenzyme. Substrate binding results in the substitution of water and intramolecular interactions with disparate contacts from the substrate. Likewise, the substrate becomes solvated by the enzyme through defined contacts with strong chemical and steric complementarity. This suggests that in general a change in conformational equilibrium of the enzyme and substrate is anticipated in response to the altered intermolecular interactions. The recognition of ligand binding effects on conformation even extends to induced folding of intrinsically unstructured

domains. Of interest in regard to enzymes is how the observed conformational changes relate to catalysis. The requirement that the enzyme structure vary to allow access to an active site that then can close down on the substrate and eventually surround the transition state is noted above. The alternative conformation stabilized by substrate binding has also been observed to complete part of the active site by bringing into position one or more catalytic residues involved directly either in binding the substrate or in the chemical transformation. The conformational change of the enzyme may also provide a better environment for catalysis by arranging the surrounding enzymatic groups to appropriately solvate the transition state. In addition to these factors that can be readily visualized from crystallographic results or modelling, the substrate may also induce more subtle effects on the enzyme; interactions between enzyme and substrate can alter the motions of the enzyme in a way that enhances progress along the reaction coordinate.

While most of the concepts regarding enzymatic catalysis were introduced many years ago, current efforts must exploit the growing base of structural and other biophysical data to learn how these concepts are executed in structural terms and to gain evidence for the exact contribution of various effects. To understand how substrate interactions and other physical factors lead to the enormous rate enhancement of enzymatic reactions over solution-state reactions, enzymatic catalysis must be explored using the detailed information of molecular dynamics and explained with physical theory.

Conformational Changes of Induced Fit

Many examples of conformational changes associated with ligand binding have been observed through crystallographic studies. Comparison of the free enzyme structure with the structure of the enzyme complex has documented a wide range of spatial rearrangements. The effect of favourable substrate interactions with the enzyme can alter the enzyme conformation in two ways. The substrate can invert the relative stability of two well-ordered conformations of the enzyme through interactions with the substrate. For this type of response, binding stabilizes an enzyme conformation that is essentially unpopulated in the absence of substrate. A second type of response to binding is that which occurs when ligand association induces structure in weakly ordered regions of the active site. Here, a distribution of multiple conformations in the free enzyme is biased by substrate interactions towards a preferred conformer.

Ligand binding occurs with conformational changes ranging from local side-chain isomerization to large domain reorientations. Structural changes for active-site loops in triosephosphate isomerase (TIM), lactate dehydrogenases, protease from human immunodeficiency virus, and protein kinases have been the subject of considerable study. The preponderance of structural changes associated with ligand binding has generated a classification of conformational differences (Gerstein *et al*., 1994). In many instances, the conformational differences that distinguish the apoenzyme and various enzymatic complexes are critical for assisting in recognition for subsequent binding of second substrates, or for suitably aligning substrates and catalytic residues for execution of the chemical reaction. An example is the synergistic effect between substrate and cofactor to induce the proper arrangement of both the enzymatic groups and the substrate and cofactor observed for phosphoglycerate kinase, PGK (Bernstein *et al*., 1997). A dramatic rotation (32°) of the two protein domains, observed with the PGK ternary complex but not the binary complex, brings the substrate and cofactor into close proximity, as well as bringing active-site residues into contact with the ligands. Reorientation of two helices and formation of a β sheet that stabilize a closed-domain structure can be achieved only after substrate and cofactor have bound. This synergistic effect leading to considerable reorganization is thought to prevent unwanted hydrolysis of the substrate or ATP (adenosine triphosphate).

Catalytic Consequences of Induced Fit

There are different potential consequences of an induced-fit mechanism on catalysis and reactivity. In induced fit, the free energy of the activated enzyme–substrate ($E_{act}S$) complex is lower than that of a complex in which the enzyme maintains the structure of the apoenzyme (ES): $[E_{act}S]/[ES] \gg 1.0$. This stabilization is indicated in **Figure 1** by Δ. If the free energy in the transition state is reduced by a similar amount (**Figure 1a**), the equivalent decrease in the free energy of the enzyme–substrate complex and the transition state complex gives no effect on the reactivity of the enzyme–substrate complex, k_{cat}, but the catalytic rate k_{cat}/K_M of the unsaturated enyzme is increased because of the enhanced binding. Another possible result is that the changes due to substrate binding serve to lower the free energy of the transition state more than the free energy of the enzyme–substrate complex (**Figure 1b**). In this case, the induced fit has a direct consequence on the reactivity of the enzyme–substrate complex rather than simply playing a role in substrate binding.

While there are numerous structural examples of induced conformational changes in enzymes from crystallographic studies, a review of the literature finds fewer kinetic studies that discriminate the steps of the enzymatic reaction that are effected by the conformational change. Nevertheless, an induced fit has been reported for some enzymes to be associated not only with enhanced affinity

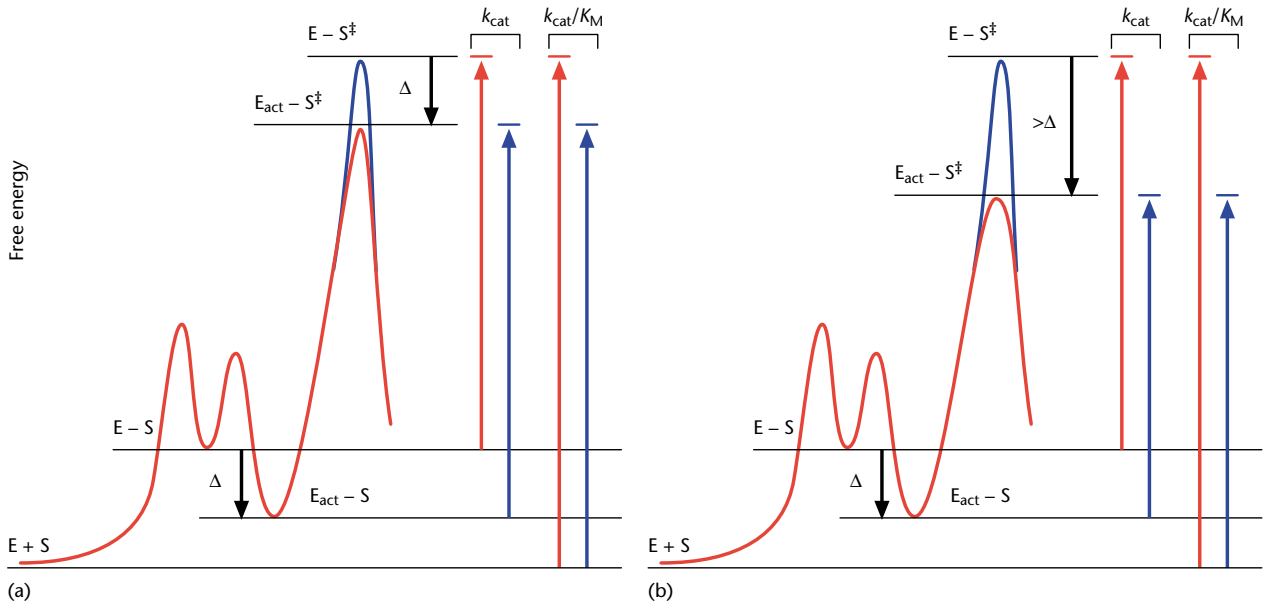

Figure 1 The effect on free energy of substrate-induced conformational change leading to enzymatic activation. The free energy of the enzyme–substrate ground state and transition state, and the free energy of the catalytic rate constant k_{cat} and k_{cat}/K_M are indicated. Features of the low probability state E–S‡ are shown in blue. (a) The substrate-induced changes stabilize the ground state and transition state by an equal amount Δ. The free energy of k_{cat} is uneffected, while that of k_{cat}/K_M is lowered and this rate increases. (b) The transition state is stabilized more than the ground state. The free energy of both k_{cat} and k_{cat}/K_M is lowered, and both rates increase.

but with the progress from the ground state to the transition state. As an example, the loop known from crystallographic studies in TIM to vary in structure has also been shown by mutagenesis studies to play a role in reactivity (Pompliano *et al*., 1990). Substitution of residues in the loop reduced the catalytic rate by as much as 10^5 times that of the wild-type enzyme. Thus, in this case, the changes in conformational equilibrium that result from substrate binding preferentially lower the transition-state free energy over that of the enzyme–substrate complex.

Conformational changes observed crystallographically are a rearrangement of catalytic residues in the active site, an enthalpic effect from the energy of interactions. In addition to an enthalpic effect, entropic factors could be effected by substrates. It has been suggested that the active-site environment of an enzyme could enhance catalysis by entropic destabilization of the enzyme–substrate complex in a way that funnels the population of the substrate conformers to the transition state and reduces the entropic barrier to activation (Young and Post, 1996). This suggestion of entropic guidance from enzymes derives from the observation that binding of the cofactor NADH (nicotinamide–adenine dinucleotide, reduced form) to lactate dehydrogenase alters the fluctuations of NADH in a manner that promotes a pyramidalization of the glycosidic N required for the transition state of hydride transfer. The reactive-state pyramidalization is a high-energy conformation not sampled in the free state of NADH. The concept is that the cofactor fits the active site without affecting the average conformation but by specifically altering the population of NADH conformations about the free energy minimum in a manner that favours the reaction. The entropy of NADH is reduced, with the advantage of eliminating nonreactive conformations otherwise populated by thermal fluctuations in free NADH while enhancing NADH conformations thought to be the reactive form. The proposal for entropic guidance should not be confused with the influence on conformational preferences of the substrate that result from intermolecular interactions. Entropic funnelling is a ground-state destabilization and differs from biasing the distribution of substrate conformations towards its reactive form, which is a change in the average conformation. Entropic guidance is a change in the shape of the free energy surface that need not change the minimum-energy conformation but alters the thermal fluctuations that 'guide' the ground state to the reactive one.

Substrate-binding effects may also be manifest in the enzymatic reaction through an influence on protein dynamics. Thermal motions are inherent to chemical reactivity. The way in which protein dynamics couple to catalysis and the reaction coordinate is just beginning to be probed experimentally and assessed theoretically. It has been suggested that protein motion may couple directly to bond breaking or that energy from fluctuations could transfer to other vibrational frequencies of the reaction coordinate. However, this particular role of dynamics would not appear likely on the basis of the 10^3–10^6-fold

difference in time scales for bond breaking and protein motions that might facilitate this process. In addition, vibrational relaxation of the protein molecule would diminish the likelihood of such coupling (Villa and Warshel, 2001). On the other hand, dynamics could serve a role in catalysis through conformational fluctuations that alter the reaction barrier. In this instance, the appropriate organization of the protein environment surrounding the site of chemical reaction occurs transiently to solvate the bond breaking/bond making process. Such a process could occur with a time-dependent rate constant, giving a complex behaviour to the enzymatic reaction (Karplus, 2000). One feature of substrate binding and formation of the active enzyme complex may be to induce the concerted fluctuations in the protein conformation to produce the collective motion of many atoms that is the source of a lower reaction barrier. Some support for this role of substrate-induced effects comes from molecular dynamics simulation of dihydrofolate reductase (DHFR) (Radkiewicz and Brooks, 2000). The studies revealed an unanticipated correlation in fluctuations that involved the loop with an induced-fit conformation in the case of the reactive ternary complex. The correlations were not observed in two-product complexes. Moreover, these correlated motions involve residues that are implicated by mutagenesis data as being significant to particular chemical steps of the catalysed reaction. Given that ligand binding alters protein fluctuations and correlated motions, it is reasonable that these effects could couple to the reaction coordinate to play a role in reactivity.

Substrate Specificity by Induced Fit

Substrate-induced fit would appear to be a natural mechanism for substrate specificity. Substrates that cannot activate the enzyme by stabilizing the conformational change required for efficient catalysis are poor substrates. While the lock-and-key model of Fischer explains certain aspects of substrate specificity, it fails to resolve some other issues of relative catalytic efficiency. The lack of reactivity of large substrates or those that do not otherwise bind the active site is explained trivially. Inefficient catalysis is also easily rationalized for a substrate with inherent chemical reactivity that differs from that of the optimal substrate. Less obvious is how to address the question of specificity related to a molecule smaller than the natural substrate, or even a fragment of the natural substrate, that has the same chemical reactivity but that is a poor substrate or nonsubstrate. Koshland proposed the induced-fit model for enzymatic activity (Koshland, 1958) and suggested that poor substrates fail to react because of inadequate orientation of catalytic groups of the enzyme. This theory was motivated in part by the observation that phosphoglucomutase, an enzyme that transfers phosphate to a sugar molecule at a rate of $1000\,s^{-1}$, does not catalyse phosphate transfer to water. In fact, the rate of transfer to water is only 3×10^{-10} that of transfer to sugar (Ray and Long, 1976). Other examples cited by Koshland (1958) include the selectivity by 5′-nucleotidase for hydrolysis of adenylic acid over ribose 5-phosphate, a fragment of adenylic acid, and by amylomaltase for maltose over the smaller fragment α-methylglucoside.

With the plethora of information now characterizing the conformational flexibility of proteins, an induced-fit hypothesis to explain specificity would seem appropriate. Nevertheless, this notion was challenged (Fersht, 1985; Herschlag, 1988) by the claim that when the chemical steps of the reaction are rate-limiting, the relative ability of a substrate to stabilize the active form of the enzyme does not form the basis for specificity. The argument is that the process of activating the enzyme reduces the catalytic efficiency equally towards all substrates and therefore activation does not confer specificity. For reaction mechanisms in which the rate-determining step is binding or conformational activation rather than the chemical step, how specificity can derive from an induced-fit mechanism has been lucidly described (Herschlag, 1988). The high specificity of Factor D protease for a high-molecular-weight substrate over a short peptide substrate with identical sequence at the hydrolysis site may be an example of this latter case for an induced-fit basis for specificity (Taylor *et al*., 1999).

The conclusion that induced fit does not confer specificity when the chemical step is rate-limiting is based on an assumption that there is only one reactive state of the enzyme. A contrary view (Post and Ray, 1995) argues that it is possible for an induced-fit mechanism to confer specificity if the substrate-specific activation of the enzyme persists in the transition state. That is, when the structure of the enzyme in the transition state differs because of interactions with the substrate, then differences in catalytic efficiencies for those substrates are attributed to the induced-fit mechanism. Generalization of the description of induced-fit activation, by removing the assumption of an invariant form of the enzyme of the transition state, allows one to define what is necessary for an induced-fit basis for specificity.

Thermodynamic model of induced-fit specificity

The thermodynamic cycle shown in **Figure 2** (Fersht, 1985) isolates the conformational activation step of the enzyme in what can be described as a virtual step: conversion of the stable but inactive form of the enzyme, E, to an active form, E_{act}, in the absence of substrate with the equilibrium constant K_{act}. We consider here only schemes where the chemical step is rate-limiting. In **Figure 2**, the actual physical process involves the substrate binding the

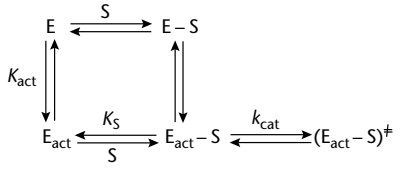

Figure 2 Thermodynamic binding cycle to represent an induced-fit process. In the physical process, the substrate S binds E and induces the change to the catalytically active form E_{act}. The energetically equivalent, albeit hypothetical, process factors out the conformational activation of the inactive enzyme E to the active form E_{act} with the equilibrium $K_{act} \ll 1.0$. This step is followed by S binding E_{act} with the dissociation constant K_S.

unreactive form of the enzyme, E. Binding promotes the conformational transition of the enzyme since the substrate stabilizes the activated form, E_{act}, that exists in the transition state. The cycle in **Figure 2** separates the conformational activation component of the enzymatic reaction from the chemical process by introducing the nonphysical but energetically equivalent pathway to get from (E + S) to $E_{act}S$. The conformational change is factored out in an independent step $K_{act} = [E_{act}]/[E] \ll 1$. Binding and activation captured in the experimental $1/K_M$ are factored using the nonphysical steps of the cycle into the product of the activation step K_{act} and substrate binding to E_{act}, $1/K_s = [E_{act}S]/[E_{act}][S]$. The catalytic efficiency of an induced-fit enzyme becomes that given by eqn [2].

$$\frac{k_{cat}}{K_M} = K_{act}\left(\frac{k_{cat}}{K_s}\right) \qquad [2]$$

In the challenge to an induced-fit basis for specificity (Fersht, 1985; Herschlag, 1988), the active form of the enzyme is assumed to be unique, so that the only difference between a good substrate and a poor one is the ability to stabilize E_{act}. Stated in this way, K_{act} is by definition equivalent for all substrates and determined only by the enzyme. Any substrate-specific differences are isolated in the steps defined by K_S and k_{cat}, independent of the activation step, and can therefore be explained without regard to the induced-fit process or the ability of the substrate to stabilize E_{act}. The lack of an effect on specificity when K_{act} is constant is illustrated by considering the ratio of k_{cat}/K_M for two substrates: S_G, a good substrate, and S_P, a poor one. Using eqn [2], the specificity, γ, for S_G relative to S_P is given in eqn [3].

$$\gamma \equiv \frac{\left(k_{cat}/K_M\right)_G}{\left(k_{cat}/K_M\right)_P} \equiv \frac{K_{act}\left(k_{cat}/K_S\right)_G}{K_{cat}\left(k_{cat}/K_S\right)_P} = \frac{\left(k_{cat}/K_S\right)}{\left(k_{cat}/K_S\right)} \qquad [3]$$

The value of γ is seen to be independent of K_{act} when there is only one form of E_{act}. Thus, because the value of γ can be explained in terms of a rigid, albeit hypothetical enzyme, E_{act}, specificity does not derive from the activation induced by substrate. This view is reasonable, if E_{act} and K_{act} are independent of substrate.

A more general view of enzymatic activation by substrate-induced fit exists for which the substrate's ability to alter the enzyme conformation is a determinant of specificity (Post and Ray, 1995). In this generalized description of activation, the requirement for a unique form of the enzyme in the transition state is removed, with the result that K_{act} is inextricably linked to differences in the substrate. K_{act} is not constant. The manner in which an enzyme could adopt substrate-specific structural differences in the transition state might involve variations in complementarity between the enzyme and the transition state, such as alternative orientations of side-chains that stabilize or 'solvate' the chemical transformation of the substrate in the transition state. Another possible variation might involve residues with a direct chemical role (as would be detected by differences in the position of bond making and bond breaking along the reaction coordinate). Substrate-specific alterations in the enzyme dynamic processes and entropy of activation could also differentially influence the transition state. Thus specific differences in the active form induced by substrate i in the enzyme, E^i_{act}, could occur in either the binding region or reactive region of the enzyme. In the case of a substrate-dependent form of the activated enzyme in the transition state, $K^G_{act} \neq K^P_{act}$, and the ratio γ does not reduce to terms isolated to the activation step (eqn [4]).

$$\gamma = \frac{K^G_{act}\left(k_{cat}/K_S\right)}{K^P_{act}\left(k_{cat}/K_S\right)} \qquad [4]$$

The specificity of the enzyme for the good substrate over the poor substrate cannot be rationalized in the absence of the conformational change, since there is no single form of the enzyme that gives the value of γ from eqn [4].

Induced-fit changes as specific effects in the transition state complex

A physical or structural description is useful to make clearer the kinetic expressions related to induced-fit specificity when chemistry is rate-limiting. The premise of eqn [4] is that the enzyme molecule differs in the transition state of the reaction in a substrate-dependent fashion. Both activated forms, E^G_{act} and E^P_{act}, catalyse the same chemical transformation, but the environment created by the enzyme molecule to solvate the chemical step differs with alternative substrates. The sequence in **Figure 3** gives a simple example of how E_{act} could vary in the transition state complex with different substrates. (The reader is

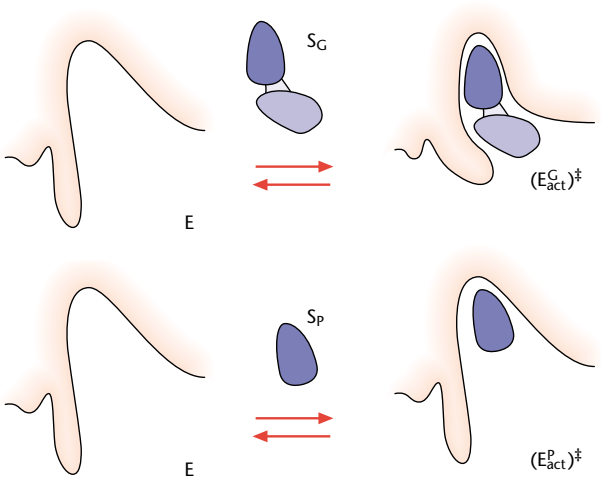

Figure 3 Schematic representation of a substrate-induced fit that confers specificity because the specific changes in the enzyme E exist into the transition state. The enzyme structure in the transition state of the good substrate E^G_{act} produces an optimum environment for catalysing the chemical step and has high catalytic efficiency. The enzyme form in the transition state for the poor substrate E^P_{act} differs and solvates the bond making/bond breaking in the transition state less well. The chemical transformation is rate-limiting for both substrates and occurs in the dark blue moiety of the good substrate. Binding of the poor substrate, which is the reactive fragment of the good substrate, induces a small closing of the enzyme active site. The change in E to form the optimum transition state environment requires the additional binding interactions of the light blue fragment.

reminded that **Figure 3** refers to a reaction for which the chemical step, and not the conformational change, is rate-limiting.) In the free enzyme, a loop is well solvated and in an extended conformation. A good substrate S_G effectively competes with the solvation of the loop by water; binding of S_G results in the substitution of the interaction with water in the extended loop conformation by interactions with the substrate (light blue in **Figure 3**) and a different conformation of the loop. This loop region in the case of S_G is involved in 'solvating' the substrate in the transition state and stabilizing the orientation of the reactive region of the substrate (dark blue in **Figure 3**) to best align with catalytic residues. In contrast, S_P does not have the groups to compete with the water interaction and the enzymatic loop region is not available to stabilize the substrate in the transition state. Hence, the alignment of catalytic groups and the environment that the enzyme generates to solvate the chemical transformation differ in the transition state for S_G and S_P. A smaller k_{cat}/K_M for S_P will arise partly from its failure to induce a conformational change in the enzyme.

This type of change in the ability of the enzyme to solvate the chemical transformation of the substrate is likened to effects observed with mutagenesis of enzymes. Mutations that produce a less efficient enzyme, as opposed to a 'dead' enzyme, likely involve residues that are not directly involved in bond making/bond breaking but otherwise stabilize the transition state. Consider that replacement of a residue by mutation is analogous to different spatial orientations of that residue in the transition state with one substrate versus another substrate. Interactions with one substrate lead to a different conformation E_{act} of the enzyme in the transition state and a change in catalytic efficiency. Such solvation or environmental differences due to alternative substrates could be the basis for the large differences in catalysis observed for substrate analogues that are fragments of the natural substrate.

The dynamics of the $ES^‡$ complex can also be altered specifically. The remarkable specificity of the protease complement Factor 5 for a protein substrate over a peptide substrate was suggested by Taylor *et al*. (1999) to arise from an induced-fit mechanism whereby the good substrate lowered the free energy of the chemical step to the extent that it was no longer the rate-limiting step. An alternative interpretation of their results invokes differences in the ability of the substrates to alter the dynamics of Factor 5. If the appropriate spatial arrangement of multiple catalytic residues of Factor 5 is generated transiently by concerted motions of the enzyme, then the substrate could be a critical component of those concerted motions, as described above for DHFR. The long-range and extensive interactions of the protein substrate may be more effective than those of the peptide substrate at generating the concerted motions of Factor 5 required to achieve the proper environment for the chemical transformation of the reaction.

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