Preorganization and Reorganization as Related Factors in Enzyme Catalysis: The Chorismate Mutase Case

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Abstract: In this paper a deeper insight into the chorismate-to prephenate-rearrangement, catalyzed by *Bacillus subtilis* chorismate mutase, is provided by means of a combination of statistical quantum mechanics/molecular mechanics simulation methods and hybrid potential energy surface exploration techniques. The main aim of this work is to present an estimation of the preorganization and reorganization terms of the enzyme catalytic rate enhancement. To analyze the first of these, we have studied different conformational equilibria of chorismate in aqueous solution and in the enzyme active site. Our conclusion is that chorismate mutase preferentially binds the reactive conformer of the substrate—that presenting a structure similar to the transition state of the reaction to be catalyzed—with shorter distances between the carbon atoms to be bonded and more diaxial character. With respect to the reorganization effect, an energy decomposition analysis of the potential energies of the reactive reactant and of the reaction transition state in aqueous solution and in the enzyme shows that the enzyme structure is better adapted to the transition structure. This means not only a more negative electrostatic interaction energy with the transition state but also a low enzyme deformation contribution to the energy barrier. Our calculations reveal that the structure of the enzyme is responsible for stabilizing the transition state structure of the reaction, with concomitant selection of the reactive form of the reactants. This is, the same enzymatic pattern that stabilizes the transition structure also promotes those reactant structures closer to the transition structure (i.e., the reactive reactants). In fact, both reorganization and preorganization effects have to be considered as the two faces of the same coin, having a common origin in the effect of the enzyme structure on the energy surface of the substrate.

Keywords: chorismate mutase · enzyme catalysis · molecular dynamics · pericyclic reaction · QM/MM methods

Introduction

Enzymes are biological catalysts capable of speeding up chemical reactions by many orders of magnitude.[1] The pioneering idea of Pauling[2] is that the famous "lock and key" analogy relating to enzyme and substrate[3] should be applied to enzyme and substrate in its transition state (TS). This means that enzymes would stabilize the TS preferentially to the ground state. Practical applications of this bright idea are the design of TS analogues[4] that can act as efficient inhibitors and, on the other hand, the synthesis of catalytic antibodies from the TS analogues. Nevertheless, the main question of how enzymes achieve their catalytic rate enhancements relative to the corresponding uncatalyzed reactions remains open.[5] This question is particularly timely in the era of protein engineering, due to the fact that this powerful experimental technique allows the role of each residue to be explored and its catalytic function analyzed. Modern methods of theoretical chemistry are widely recognized and complementary tools to be used.[5] These new techniques, such as molecular dynamics[6] and stationary point location on the potential energy surfaces of large systems,[7] not only allow interpretations of experimental data to be obtained but also enable new results to be predicted in order to design more effective and powerful inhibitors or catalytic antibodies.

A suitable reference point for investigation of enzyme activity is the reaction in solution. Warshel introduced the concept of the "solvent cage" (see Figure 1) to compare calculations regarding a specific reaction in an enzyme and in

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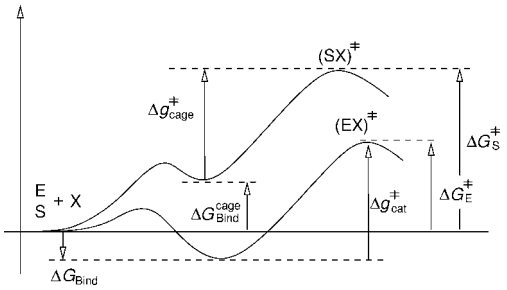

Figure 1. Free energy terms involved in enzyme catalysis (see text). Labeling is as follows: E is the free enzyme in solution, S describes the solvent molecules, and X corresponds to the substrate/solute.

solution.[8] The activation free energy of a reaction in solution is divided into two components: the free energy, ΔG_{bind}^{cage}, which involves the assembling of the reacting fragments into a single solvent cage, and $\Delta g_{cage}^{\ddagger}$, which represents the activation free energy contribution. According to Warshel's approach, the first component is the entropic factor associated with bringing the reacting fragments into the same cage. In the enzyme, the total activation free energy can be divided into the binding free energy of the Michaelis complex (ΔG_{bind}, related to the dissociation constant K_M) and the free energy needed to reach the TS from this complex, $\Delta g_{cat}^{\ddagger}$, which corresponds to the rate constant k_{cat}. Direct comparison between $\Delta g_{cage}^{\ddagger}$ and $\Delta g_{cat}^{\ddagger}$ can then be made. Warshel's conclusion would be that enzymes attain a large k_{cat} by providing more stabilization of the charges in the TS than the corresponding stabilization in water, due mainly to a preorganized polar environment in the enzyme. Consequently, a minor energy cost is required for the *reorganization* of the enzymatic environment when the reaction proceeds. In solution, linear response models predict that this term amounts to up to one half of the interaction energy and so it supposes an important energy penalty.[9] Here we use the term *reorganization* energy in a general sense to refer to the most important contribution to the relative electrostatic stabilization of the TS in the enzyme.

Other authors have explained enzyme catalysis by focusing on the different contribution terms in the formation of the Michaelis complex (MC) and its possible destabilization in solution. In this view of enzyme catalysis, the emphasis is on the *preorganization* of the substrate in a particular conformation necessary to progress to the corresponding transition structure. The *preorganization effect* term is then used to refer to the enzyme effect on the reactants. Page and Jencks have emphasized entropic factors, not only restrictions in translations as discussed by Warshel, but also restrictions in rotations and vibrational contributions.[10] Contrarily, Menger,[11] Bruice,[12] and Koshland[13] consider that bringing the reactant fragments to a suitable separation and orientation is mainly an enthalpic term. Finally, following this approach, Kollman[14] and Hermans and Wang[15] define ΔG_{cratic} as the free energy required to bring the reactant molecules together and to orient them properly. The difference between reactions in solution and in an enzyme environment is that the enzyme has already done the work in forming its spatial structure and binding the substrate, thus favoring reaction of the chemical system. In the enzyme, the substrate binding energy associated with the formation of the MC (and thus its dissociation equilibrium constant K_M) includes the free energy component for entropic and desolvation contributions. In solution, however, a free energy price must be paid in bringing the substrate to form a reactant complex.

The conversion of (−)-chorismate into prephenate by *Bacillus subtilis* chorismate mutase (BsCM) is formally a Claisen rearrangement, and thus a rare example of an enzyme-catalyzed pericyclic process.[16] We selected this system to perform our study for several reasons: i) the rearrangement of chorismate to prephenate catalyzed by the enzyme BsCM has its counterpart reaction in solution, with experimental data available in the literature, ii) no covalent bonds are formed between the substrate and the protein, avoiding technical problems of frontier treatments between QM and MM regions, and iii) since it is an unimolecular reaction, the contribution of bringing the reactant fragments together is simplified into a conformational problem: the work required to change a non-reactive chorismate conformer structure into a new one ready to undergo the rearrangement to prephenate (see Figure 2).

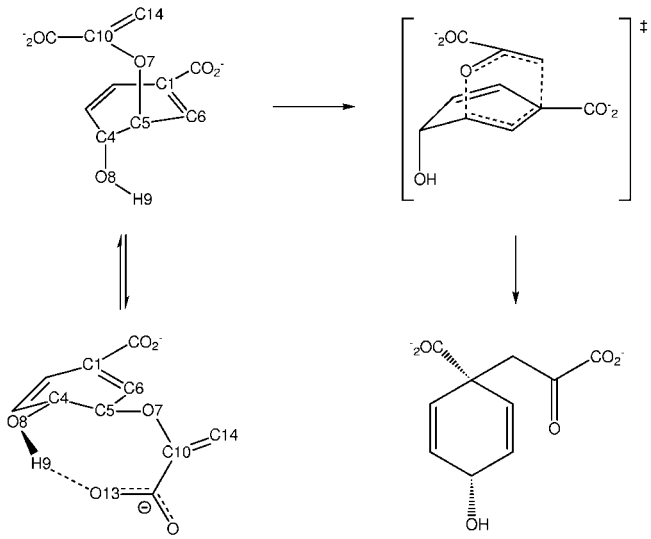

Figure 2. The conformational preequilibrium of chorismate and the subsequent rearrangement into prephenate. Atom labeling is also shown.

For a Claisen rearrangement, Menger et al. examined the relationship between the interatomic distance between the atoms to be bound (C1 and C14 carbon atoms in our system) and the barrier energy, concluding that an important contribution to catalysis could come from confining the reactive centers to contact distances.[11b] The definition of the Near Attack Conformation (NAC) introduced by Bruice et al.[12] is very close to this concept. In the particular case of chorismate, the different conformers can be classified according to two different criteria as shown in Figure 3: the distance between the carbon atoms that will become joined and the pseudodiaxial or pseudodiequatorial character of the ring substituents. Both criteria for classifying the chorismate conformers were used in a previous paper,[17] in which we showed that the substrate with a pseudodiaxial conformation and a short C1−C14 interatomic distance is the closest to the transition

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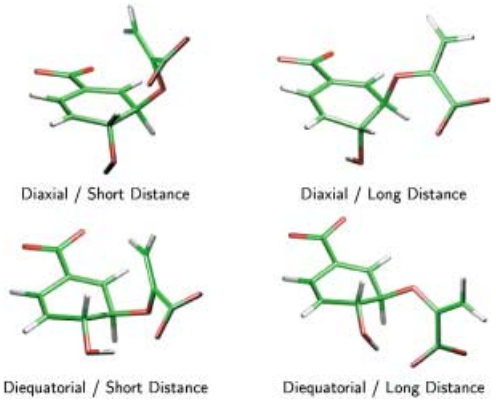

Figure 3. Different possible conformations available to chorismate.

structure for the subsequent rearrangement to prephenate. In the gas phase the absolute energy minimum is a pseudodiequatorial/long-distance conformer with an intramolecular hydrogen bond between the hydroxyl group and the carboxylate of the ether bridge: this conformer is not able to give rise to a transition structure for the rearrangement directly. It has been proposed[18] that polar solvents and enzyme environments could displace this preequilibrium to favor the reactive forms of the chorismate. In this sense, preorganization of the substrate by the enzyme could be a decisive contribution to catalysis.

The first aim of this work is to present an analysis of the substrate preorganization in chorismate mutase. As we show, this preorganization is closely related to the reorganization term, and both contributions to the enzyme catalytic rate enhancement arise from the enzyme structure, which is already adapted to optimize its interactions with the TS. Our calculations show how both contributions have to be considered as two faces of the same coin. This paper develops a computer simulation approach that allows the determination of these key factors.

Computational Methods

The full theoretical treatment of complex molecules such as enzyme catalysts and their chemical reactivity involves the initial calculation and construction of a multidimensional potential energy surface (PES) with a quantum mechanical treatment of the bond-breaking/forming processes associated with the chemical reaction that takes place in the active site of the enzyme. Afterwards, since a PES exploration is not usually able to describe the huge number of structures contributing to each state (reactants, transition and product states), statistical simulations have to be carried out to average all the single structures of these large systems that contribute to the full catalytic phenomenon. Different computational strategies by which to obtain the energy and/or free energy profiles of chemical processes taking place in condensed media have been proposed. From the point of view of the flexibility of the system we can consider two main categories. In the first one, only the dynamics of the environment (solvent or non-reacting residues) are considered.[14] In the second one, the relaxation and/or dynamics of the reacting fragment are also included. Since this part must be described at a quantum mechanics (QM) level, in order to avoid excessive computational cost, simple QM treatments such as empirical valence bond[5, 8, 19] or semiempirical Hamiltonians must be used. In this work, since we are interested in changes taking place in the structure of the substrate, the QM subsystem (the chorismate molecule) must be flexible and here is described by AM1 Hamiltonians.[20]

From a technical point of view, in order to obtain the free energy change associated with the conformational equilibrium between reactive and non-reactive conformers in the gas phase, in aqueous solution, and in the enzymatic environment, we have traced the corresponding potentials of mean force (PMFs).[21] These PMFs have been calculated by the umbrella-sampling approach[22] implemented in the DYNAMO program.[23] The starting geometry was a pseudodiaxial/short-distance conformer optimized in each different medium[17] with the AM1 semiempirical Hamiltonian by means of the Gaussian98[24] and CHARMM25[25] packages. Since a distinguished internal reaction coordinate is needed to obtain the PMF, in order to explore the equilibrium between long- and short-distance conformers, the C6-C5-O7-C10 dihedral angle (which defines the relative position of the ether bridge with respect to the ring; see Figure 2) is used instead of the C1−C14 distance. We have demonstrated that both geometrical parameters can be used as distinguished coordinates in PMF calculations relating non-reactive and reactive conformers in the gas phase.[26] In solution and in enzyme environments, however, the latter coordinate presents important hysteresis problems, the results being dependent on the starting structure of the protocol. For this reason we selected the C6-C5-O7-C10 dihedral angle as the biased coordinate to follow the PMF. Large values of this dihedral angle correspond to long C1−C14 distances or open conformers, while small values of this dihedral angle correspond to short C1−C14 distances or closed conformers. To obtain the PMF of the transformation between pseudodiaxial and pseudodiequatorial conformers we used the O7-C5-C4-O8 dihedral angle. This internal coordinate takes values of approximately 240 and 290° for pseudodiaxial and pseudodiequatorial conformers, respectively.

PMF calculations require series of molecular dynamics simulations, in which the distinguished coordinate is constrained to particular values. The probability distributions obtained for each simulation window are afterwards all combined together to obtain the full probability distribution along the selected reaction coordinate. The chorismate molecule (24 atoms) was treated by the AM1 semiempirical approximation during the simulations, while the rest of the system (water molecules and/or enzyme residues) was described by using the OPLS-AA molecular mechanics potential[27] as implemented in the DYNAMO program.[23] For simulations in water the chorismate molecule was embedded in a box of 31.4 Å sides with a total of 1016 water molecules described by the TIP3P empirical potentials.[28] For the enzymatic process, the substrate plus the enzyme were centered in a box of 55.8 Å sides with 3835 water molecules. The total number of classical atoms in this case was 17159. To make the calculations

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feasible, atoms located more than 20.0 Å away from the substrate were kept frozen. The values of the force constant used for the harmonic umbrella sampling (0.5 kJ mol^{-1} degree^{-2}) were determined to allow full overlap of the different windows traced in the PMF evaluation (105 in solution and 94 in BsCM), but without losing control over the selected coordinate. The length of each window (30 ps) was shown to be long enough to sample a wide range of structures at a reference temperature of 300 K. The canonical ensemble was employed throughout.

Results and Discussion

Preorganization of the substrate: First of all, we present the PMFs obtained in gas phase, aqueous, and enzyme environments for the diaxial/diequatorial conformational equilibrium (Figure 4) and for the transformation between the long- and short-distance chorismate conformers (Figure 5) by use of the O7-C5-C4-O8 and the C6-C5-O7-C10 dihedral angles, respectively. For analysis of Figure 4 it must be remembered that a small value of the O7-C5-C4-O8 dihedral angle represents a diaxial conformer, while large values of the dihedral angle correspond to diequatorial conformers. In the gas phase we only found free energy minima corresponding to psuedodiequatorial forms (with an O7-C5-C4-O8 dihedral angle of about 285°), which are the only ones able to establish intramolecular hydrogen bonds between the hydroxyl and carboxylate groups.[17] In solution, the pseudodiequatorial conformers are still the most stable, but pseudodiaxial forms are clearly more favored than in the gas phase, in agreement with the results previously reported by Jorgensen et al.[18b] In the enzyme we only found free energy minima corresponding to pseudodiaxial structures (with an O7-C5-C4-O8 dihedral angle of about 245°). Pseudodiaxial structures allow better interaction between the carboxylate group of the ether bridge and the charged arginine residues of the active site.[17] Moreover, we can observe that in the enzyme the pseudodiaxial conformers (left side of the profiles) present a smaller value of the O7-C5-C4-O8 dihedral angle than in aqueous solution. This means that the enzymatic medium pushes the reactant's structure closer to the geometry of the transition structure of the chorismate-to-prephenate rearrangement, which clearly has a pseudodiaxial structure.

The PMF plots of Figure 5 correspond to the transformation between long- and short-distance conformers. A small value of the C6-C5-O7-C10 dihedral angle describes closed or short carbon–carbon distance conformers, while large values of the dihedral angle correspond to open or long carbon–carbon distance conformers. It can be observed that this chorismate conformer equilibrium is almost thermoneutral in the gas phase. As we have previously shown,[17] there are two nearly degenerate pseudodiequatorial chorismate conformers presenting very different carbon–carbon distances but both conserving an intramolecular H9···O13 hydrogen bond. In contrast, the short-distance chorismate configuration is slightly favored in aqueous solution by about 1.7 kcal mol^{-1}. This is not unexpected, as in solution the intramolecular hydrogen bond can be substituted by intermolecular interactions and so other conformers can be explored during the dynamics. In the enzyme's active site the reactive conformers, with short carbon–carbon distances, are strongly favored. In the enzyme we were unable to locate a clearly defined free energy minimum when the ether bridge is displaced away from the ring. Only a very shallow minimum is found around 180°. This means that the enzyme clearly favors a short-distance chorismate conformer, closer to the TS of the chemical reaction catalyzed by the enzyme: the rearrangement of chorismate to prephenate. In this sense our PMFs are in agreement with the recent work of Karplus et al.,[29] in which free molecular dynamics simulations started from different chorismate conformers complexed with yeast chorismate mutase always evolved towards the same reactive conformer. There is also a complementary view of the enzyme activity from our calculated profile: reactant structures that are notably different from the transition structure can also be accommodated into the enzyme's active site at a moderate free energy cost (ca. 7 kcal mol^{-1}). This means that the enzyme structure is quite flexible and thus able to establish different interaction patterns with different reactant struc-

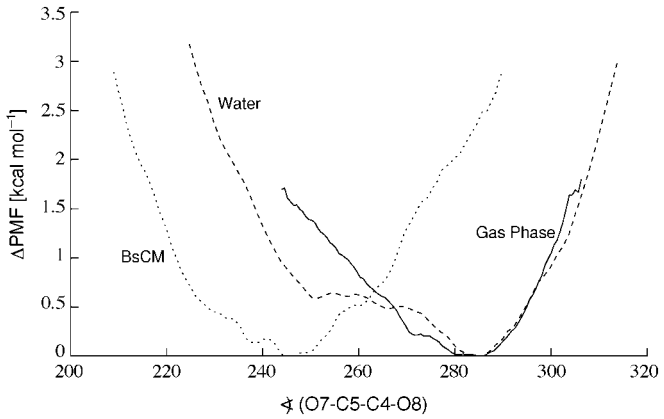

Figure 4. PMFs [kcal mol^{-1}] obtained by using the O7-C5-C4-O8 dihedral angle [°] as distinguished internal coordinate in the gas phase (——), in solution (– – –), and in the enzyme active site (••••).

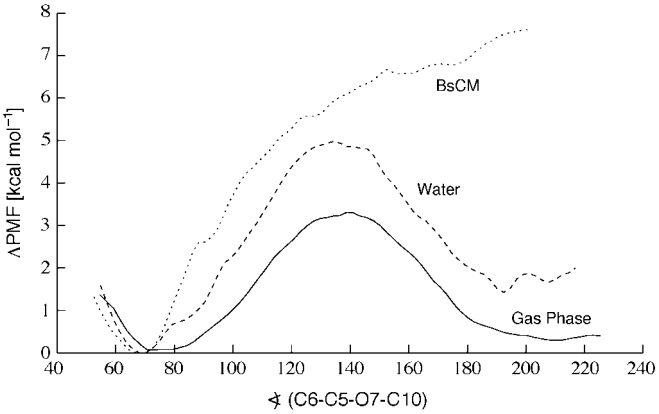

Figure 5. PMFs [kcal mol^{-1}] obtained by using the C6-C5-C7-C10 dihedral angle [°] as distinguished internal coordinate in the gas phase (——), in solution (– – –), and in the enzyme active site (••••).

tures, as discussed below. Finally, if we compare the values of the dihedral angle in the reactive conformer minima (left-hand side of the profiles), the aqueous and enzyme environments force the substrate to present slightly smaller values of the dihedral angle that describes the position of the ether bridge: that is, a smaller distance between the two carbon atoms to be bound during the subsequent reaction.

The pressure effect exerted by the enzyme on the reactant structures can also be deduced by analysis of Figure 6. In this figure, the QM/MM trajectories of the windows corresponding to both PMF minima of Figure 5 (short- and long-distance pseudodiaxial conformers) obtained in different media are superposed and compared with the QM/MM trajectories of the chorismate-to-prephenate TS window. This transition structure of the chorismate rearrangement was previously located (see ref. [30]) as the maximum of a PMF constructed by using the antisymmetric combination of the C14–C1 and C5–O7 distances as distinguished coordinate in the different media. For each structure obtained during the trajectories, the value of the O7-C5-C4-O8 dihedral angle, which defines the diequatorial/diaxial position of the ring substituents and the distance between the carbon atoms to be bound (C14–C1), are plotted in Figure 6a. Analysis of these aqueous and enzymatic QM/MM trajectories clearly shows the effect of the enzyme (as compared with the solvent) on the reactant's structure. In BsCM the pseudodiaxial/short-distance conformer of chorismate is not only the most stable (as demonstrated above) but it is also closer to the transition state geometry: that is, it has shorter C1–C14 distances and smaller values of the O7-C5-C4-O8 dihedral angles than in aqueous solution. Although there is an important overlap between the two media trajectories, there are significant displacements of the average distance and dihedral angle of the chorismate towards the values corresponding to the transition structure of the reaction to be catalyzed. The enzyme is favoring reactive structures of the chorismate molecule better than the solvent does. In Figure 6b we show the averaged values for these two coordinates over the gas-phase, water, and enzymatic media simulations of the transition structure and the two chorismate conformational averages (reactive and non-reactive conformers). The geometrical parameters of the transition states obtained in the three different media are very similar. Thus, the transition structure, which can be viewed as quite invariant, can be taken as the reference structure for analysis of the role played by the environment. With respect to the reactants, during our simulations that used the ether bridge dihedral angle as biased coordinate, we found a larger number of structures with a short C1–C14 distance and more pseudodiaxial character in the enzyme than in solution. In addition, but not so dramatically, water has a similar effect if compared with the gas-phase process, as may be deduced by comparison of the respective averaged values presented in Figure 6b. In other words, the enzyme favors those structures capable of progressing towards the TS. This finding is close to the NAC concept defined by Bruice[12] and recently applied to the chorismate rearrangement catalyzed by *Escherichia coli* chorismate mutase.[31] The main difference is that we do not need an arbitrary choice to define our reactive reactant

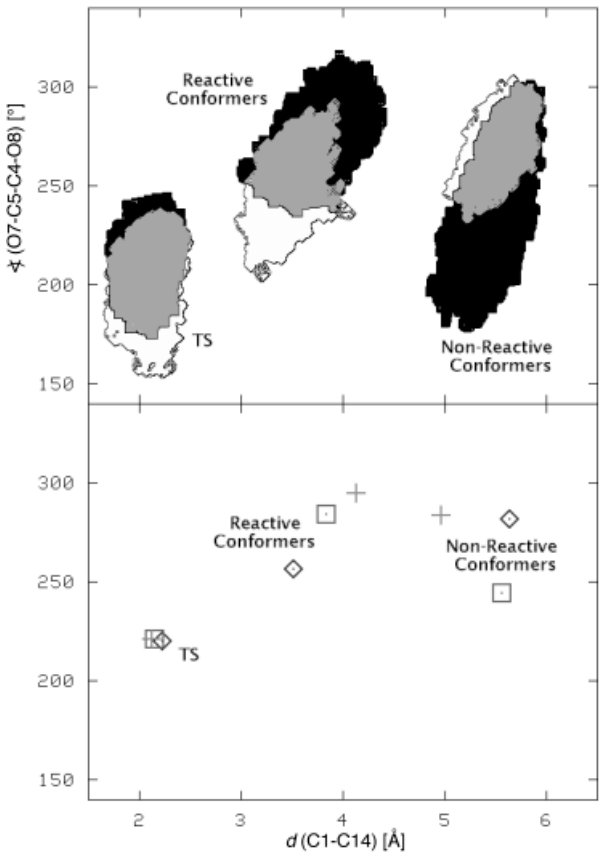

Figure 6. Top: QM/MM trajectories (C1–C14 distance in Å and O7-C5-C4-O8 dihedral angle in degrees) corresponding to reactive and non-reactive chorismate conformers' free energy minima and transition structure of the chorismate-to-prephenate rearrangement obtained in solution (black) and in the BsCM environment (white). Superpositions of both media trajectories are displayed in gray. Bottom: The averaged values of the represented internal coordinates for trajectories in the gas phase (+), in solution (□), and in the BsCM environment (◇) corresponding to the transition structure and the non-reactive and reactive forms of chorismate.

because it appears as a true free energy minimum (the short-distance/pseudodiaxial conformer), while the NAC as defined by Bruice would correspond to some fluctuations around this stationary point on the free energy surface.

Reorganization of the enzyme: From our previous discussion it is clear that the enzyme acts on the reactants, favoring the reactive reactants: those conformers that are geometrically closer to the transition structure. At this point in the discussion, one could ask whether the enzyme is suited to interact better with a particular reactant conformation or with the transition structure of the reaction to be catalyzed, as proposed by Pauling.[2] To answer this crucial question we carried out an energy decomposition analysis of the potential energy barrier of the chorismate rearrangement in aqueous solution and in the enzymatic environment. A preliminary study of the chorismate-to-prephenate reaction step, in which the activation free energy was calculated from the PMF traced from the reaction transition state to the reactive reactants, has been presented elsewhere.[30] The potential energy barrier has been obtained here as the difference between the averaged potential energies calculated from constrained molecular

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dynamics simulations corresponding to the maximum (TS) and minimum (reactive reactant) of this PMF. As the calculation of the energy barrier involves the evaluation of small differences between very large numbers, molecular dynamic simulations were extended to long times (360 ps in water and 1.8 ns in the enzyme) in order to provide converged time averages. The total QM/MM potential energy was then decomposed into three different contributions:

$$E_T = E_X^0 + E_{int} + E_{env} \quad (1)$$

where the first term is the energy of the substrate in the gas phase, the last term is the energy of the MM environment (water or enzyme), and the second term is the interaction energy between the two subsystems including the electron polarization energy of the substrate. According to this decomposition, the energy barrier of the reaction is the sum of three contributions, given in Table 1 for the reaction in water and in BsCM. Free energy barriers,[30] which are known to be more reliable, are also given for comparison. In solution, the solvent interacts better with the transition structure than with the reactant (by about 2.8 kcal mol^{-1}). However, in order to gain this interaction energy the system must pay an energy cost due to the solvent polarization. The solvent energy difference between reactant and transition structure is 1.4 kcal mol^{-1}, half of the change in the interaction energy, as predicted by linear response solvent models. In the enzyme we have a completely different situation. It can be observed how the enzyme interactions are clearly optimized to interact with the transition structure, as the change from the reactant to the transition structure is accompanied by a change in the interaction energy of -16.2 kcal mol^{-1}. We further decomposed this contribution of the interaction energy to the potential energy barrier into electrostatic and non-electrostatic components. We found that the non-electrostatic contribution (due to the Lennard–Jones term of the potential function) is slightly positive (ca. 0.9 kcal mol^{-1}), while the electrostatic part amounts to -17.1 kcal mol^{-1}. This would mean that the preference of the enzyme for the transition structure is electrostatic in nature. In this sense our results disagree with the work of Bruice et al.[31] in which the authors conclude on the basis of analysis of substrate–enzyme distances that electrostatic interactions between the *Escherichia coli* chorismate mutase and the reactant or transition structures are quite similar. However, a direct comparison with our results should be made with caution, as experimental data support the possible existence of differences in the mechanisms of *Escherichia coli* and *Bacillus subtilis* chorismate mutases.[31] In our case, in agreement with Warshel's interpretation of enzymatic activity,[5, 8, 32] this better electrostatic interaction of the enzyme with the transition structure is not accompanied by a substantial energy cost due to enzyme reorganization. The cost in the enzyme self-energy when passing from the chorismate reactive reactant to the transition structure is only 1.2 kcal mol^{-1}, far from half of the change in the interaction value. This means that the enzyme structure is able to decrease the reaction energy barrier by means of optimized electrostatic interaction with the transition structure, while the deformation or reorganization energy needed to reach this interaction is small when compared to the interaction energy. As the enzyme is already organized, it is not necessary to pay an important energy price to optimize the interaction with the transition structure (the *reorganization term*) while in aqueous solution an increase in the solute–solvent interactions means a more broken solvent structure and thus an energy cost of about one half of the interaction energy.

Table 1. Averaged values for the free energy (from ref. [30]), the potential energy barrier, and its components [see Eq. (1)] for the chorismate rearrangement in water solution and in BsCM. All values in kcal mol^{-1}.

	Water	BsCM
ΔG^{+}	38.0	29.3
ΔE^{+}	39.0	27.1
ΔE_X^0	40.4	42.1
ΔE_{int}	−2.8	−16.2
ΔE_{env}	1.4	1.2

An integrated view: From our previous comparison between reactive reactants and transition states in the enzyme, it is clear that the enzyme structure is suited to accommodate the transition state by means of favorable electrostatic interactions, and so this is reached without a significant deformation energy cost for the enzyme. However, and this is the important point here, this same enzyme structure has a considerable effect on the reactants. The equilibrium among reactants' substrate conformers is displaced towards reactive conformations geometrically closer to the TS, thus avoiding the energetic penalty associated with the deformation of the full enzyme–substrate system. That is, substrate preorganization and enzyme reorganization have a common origin in the effect of the enzyme structure on the substrate.

These points are analyzed in Figures 7 and 8. Figure 7 shows the reaction TS (6A), the reactive reactant (6B), and a non-reactive reactant (6C; a long-distance pseudodiaxial conformer) in the active site of the BsCM. It is clear that the spatial arrangements of the substrate inside the enzyme are very similar in the case of the TS and the reactive reactant, but completely different for the non-reactive reactant. The interaction pattern established between the enzyme and the substrate changes in this last case, except for the carboxylate oxygens of the ether bridge, which seem to be the anchoring points for the substrate. A quantitative analysis of the substrate–enzyme distances for the three situations described in Figure 7 is given in Figure 8, in which the shortest averaged distances between the substrate and the amino acids of the enzyme pocket are drawn in a bar plot. The results reveal the similarity of the enzyme active site when the chorismate is placed in its reactive reactant conformation and in the TS form. For most of the interatomic distances plotted in Figure 8, the TS is dramatically closer to the reactive chorismate than to the non-reactive conformer. In particular, it is especially significant how the interaction of the reactant hydroxyl group with Cys75, which is not present in the non-reactive conformer since this hydroxyl group is then interact-

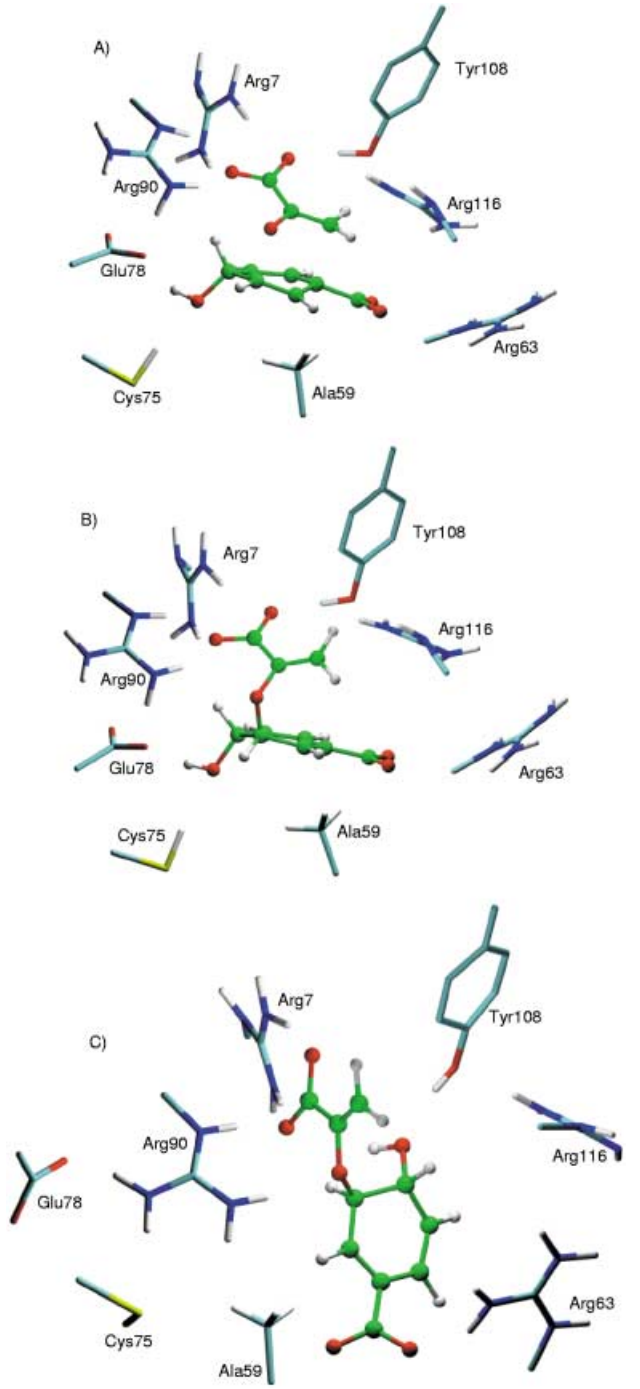

Figure 7. A) TS, B) reactive reactant, and C) non-reactive reactant of the chorismate-to-prephenate rearrangement in the active site of BsCM.

ing with Tyr108, is reinforced when passing to the transition structure. Thus, it seems that, although the enzyme can be deformed to accommodate reactant structures very different to the TS of the catalyzed reaction, only the reactive reactants, the closest to the transition structure, present a similar interaction pattern. If the reactant is changed to a non-reactive conformation, some new, different interactions can be established but, as demonstrated in our PMFs, an energy cost must be paid. As the reactant geometry becomes more and more different to that of the TS the enzyme structure has to be deformed or reorganized to change the interaction

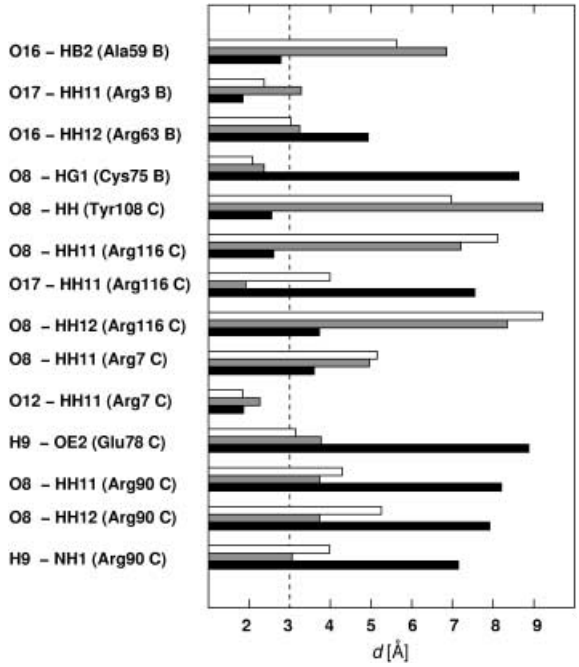

Figure 8. Bar plot of the averaged distances [Å] between the substrate and the amino acids of the enzyme active site. Three different possibilities are considered, corresponding to the substrate in its non-reactive (black) and reactive (gray) minima and to the transition state of the rearrangement (white).

pattern. The result (see the PMFs of Figures 4 and 5) is that the enzyme acts as an attractor on the energy surface of the substrate, stabilizing those reactant structures closer to the TS of the reaction. The evolution from the reactive reactants to the TS can then take place without significant changes in the enzyme active site; this is without an important energy cost due to enzyme deformation.

Conclusion

In this work we have analyzed the reorganization and preorganization effects on the chorismate-to-prephenate rearrangement catalyzed by *Bacillus subtilis* chorismate mutase (BsCM). With this as a goal we first studied the PMFs associated with the conformational equilibria of the chorismate molecules between reactive and non-reactive conformations in BsCM. These equilibria are compared with the same processes in the gas phase and in aqueous solution. Our analysis shows how the enzyme preferentially stabilizes those reactant conformers capable of progressing to the transition structure of the reaction to be catalyzed (with short C1−C14 distances and pseudodiaxial dispositions of the ring substituents). Our calculations reveal that the structure of the enzyme is responsible for stabilizing the transition state structure of the reaction, with concomitant selection of the reactive form of the reactants. The same electrostatic interactions that stabilize the transition structure without significant energy cost (the *reorganization* effect) act as a structure attractor, centered around the transition state location, capable of deforming the reactant geometry towards more reactive structures and/or causing a displacement of the equilibria

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between reactive and non-reactive conformations of the reactants (the substrate *preorganization*).

In this catalyzed reaction the enzyme is optimized to interact with the transition structure, but—and this is our main contribution in this work—these same interactions have important effects on the reactant side. Although the enzyme is able to change or reorganize its structure, accommodating different reactant conformations, the more similar they are to the reaction TS, the less the enzyme structure has to be deformed. In others words, *preorganization* and *reorganization* effects of the protein are terms undoubtedly linked and they can be seen as the consequence of the protein effect on the TS and reactant sides of the substrate energy surface. The key factor, sometimes not stressed enough in the literature, is the importance of the enzyme deformation during the enzymatic process. This study is the first theoretical work in which the flexibility of the enzyme and the energy cost of its deformation have been measured by means of an appropriate protocol. Although these conclusions have been obtained from a particular enzyme, we think that they should be extrapolatable, to some extent, to other systems. This understanding may be decisive for rational development of drug design or new enzyme mutants with particular functions.

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