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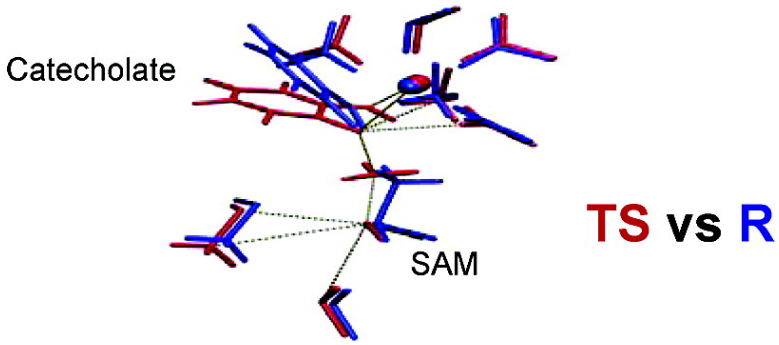

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On the Nature of the Transition State in Catechol *O*-Methyltransferase. A Complementary Study Based on Molecular Dynamics and Potential Energy Surface Explorations

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Abstract: The way in which enzymes influence the rate of chemical processes is still a question of debate. The protein promotes the catalysis of biochemical processes by lowering the free energy barrier in comparison with the reference uncatalyzed reaction in solution. In this article we are reporting static and dynamic aspects of the enzyme catalysis in a bimolecular reaction, namely a methyl transfer from *S*-adenosylmethionine to the hydroxylate oxygen of a substituted catechol catalyzed by catechol *O*-methyltransferase. From QM/MM optimizations, we will first analyze the participation of the environment on the transition vector. The study of molecular dynamics trajectories will allow us to estimate the transmission coefficient from a previously localized transition state as the maximum in the potential of mean force profile. The analysis of the reactive and nonreactive trajectories in the enzyme environment and in solution will also allow studying the geometrical and electronic changes, with special attention to the chemical system movements and the coupling with the environment. The main result, coming from both analyses, is the approximation of the magnesium cation to the nucleophilic and the hydroxyl group of the catecholate as a result of a general movement of the protein, stabilizing in this way the transition state. Consequently, the free energy barrier of the enzyme reaction is dramatically decreased with respect to the reaction in solution.

Introduction

The question of how molecular motions within the protein's structure may influence the enzyme's catalytic properties has been the subject of numerous studies in the past decades.[1−5] Thus, while the attention of most of the scientists was originally focused on the chemical system, along the years the target of understanding enzyme catalysis has moved to the substrate−environment interaction effects and the role of the protein itself. As a first approximation, only the residues in the active site were considered but nowadays the study of the full protein is calling the attention of the scientific community.[6]

According to the fundamental transition state theory assumptions (TST),[7,8] the protein catalyzes chemical reactions by lowering the free energy barrier. When the free energy barrier is computed by means of a potential of mean force (PMF), the fluctuations of the protein are intrinsically included in the calculations assuming an equilibrium distribution.[9] Following the seminal ideas of Pauling proposing a complementarity between the enzyme's active site cavity and the transition structure,[10] recent studies have suggested that the reorganization of the enzyme is less important in the transition state (TS) than in the Michaelis complex (MC), and thus this difference in the protein deformation between the two states would be an important term to the free energy barrier diminution by stabilizing the TS.[7,8,11] Nevertheless, although the effect of enzyme structure in diminishing the barrier is the most important contribution to catalysis, the departures of the rate constant computed from the TST may also have a significant effect. These departures are usually measured by the transmission coefficient, κ. From the dynamical point of view, estimation of

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the transmission coefficient is usually obtained by means of rare events molecular dynamics (MD) trajectory calculations started from the previously localized TS, as the maximum in the PMF profile.[7,12,13]

Changes of the protein from MC to TS can be a response to the changes in geometry and electronic structure of the substrate, but they can also be coupled in such a way that, at some extent, enzyme promoted the changes in the substrate. If this is the case, enzyme and substrate cannot be considered in equilibrium and a separation between substrate chemical system and protein changes is not adequate. Consequently, some of the parameters defining the protein reorganization along the reaction mechanism have to be incorporated into the reaction coordinate.[7]

To check these ideas, a bimolecular reaction was selected, namely methyl transfer from *S*-adenosylmethionine (SAM) to the hydroxylate oxygen of a substituted catechol catalyzed by catechol *O*-methyltransferase (COMT, EC 2.1.1.6).[14] COMT is important in the central nervous system where it metabolizes dopamine, adrenaline, noradrenaline, and various xenobiotic catechols. One important substrate for COMT is levodopa, presently the most effective drug for Parkinson's disease.[15] This reaction involves attack on a methyl group, originally bonded to the sulfur atom of the co-enzyme SAM, by a catecholate O^- in a direct bimolecular S_N2 process. This reaction can be formally considered as an inverse Menschutkin reaction where ionic reactants proceed toward neutral products.[16−18] The enzymatic process also requires the presence of a magnesium cation (Mg^{2+}) in the active site. Due to these features, the effect of the environment must be relevant and changes in the protein or solvent can be strongly coupled to the chemical process. The origin of enzyme catalysis in this particular reaction has been explained by using MC dynamics, through the definition of a near attack conformer (NAC),[19] or by means of free energy profiles in solution and in the enzyme.[20,21] In our recent study, differences of electrostatic effects in the enzyme and in solutions were stressed as an important contribution to catalysis.[21]

In this article, from the free energy profile previously obtained,[21] long QM/MM molecular dynamics are computed in the TS region. This biased trajectory will allow us to select different initial structures that will be refined as TS structures, thus allowing us to obtain the transition vector (TV). Afterward, intrinsic reaction coordinate (IRC) paths can be traced down to their MC. Furthermore, rare event molecular dynamics trajectories are run from many configurations obtained in the TS region, thus allowing us to calculate the transmission coefficients. The analysis of the reactive trajectories can show the dynamic role of the protein along the reaction path.

Methods

The initial coordinates for the enzyme calculations were taken from the X-ray crystal structure of a COMT-inhibitor complex with 3,5-dinitrocatechol and the cofactor SAM;[22] the nitro groups were removed and one of the hydroxyl groups of catechol was ionized by proton transfer to Lys144. Then, the QM subsystem consisted of the cofactor SAM and the substrate catecholate (63 atoms), while the MM subsystem contained the remainder of the enzyme, the magnesium cation, and waters of crystallization inside a cubic box of 55.8 Å side of TIP3P water molecules. The final enzymatic system contains 3365 enzyme atoms and 4614 water molecules.

To compare the results obtained in the enzyme with the reference reaction in solution, similar QM/MM calculations were also performed for a model comprising SAM and catecholate, which constituted the QM subsystem, placed in a cavity deleted from a 31.4 Å side box of TIP3P water molecules. This aqueous-phase model contained 1001 water molecules.

The PMF for the methyl transfer was previously obtained along the antisymmetric combination of the distances describing the breaking and forming bonds ($d_{S-C}-d_{C-O}$)[21] using the DYNAMO[23] program. The umbrella-sampling approach[24] was used to constrain the system. The probability distributions were put together by means of the weighted histogram analysis method (WHAM)[25] to obtain the full probability distribution along the reaction coordinate. The semiempirical Hamiltonian AM1[26] was used to represent the QM subsystem, and the MM region was described by means of the OPLS-AA potential.[27] Periodic boundary conditions and a temperature of 300 K were used all around the simulation. A switched cutoff radius of 12 Å was applied for all kinds of interactions, and the canonical thermodynamical ensemble (NVT) was used for each simulation. The results of the PMF reproduced pretty well the catalytic effect of the enzyme in reducing the activation barrier, if compared with the reference reaction in solution.

Once the PMFs were obtained in the enzyme environment and in aqueous solution, we carried out 600 ps long QM/MM MD simulation with the system restrained to remain in the TS region. The value of the force constant used to restrain the distinguished reaction coordinate (10 000 kJ mol^{-1} $Å^{-2}$ on the reaction coordinate) was determined to allow a small fluctuation around the value of the reaction coordinate at the one obtained in the TS (±0.08 Å). In these simulations, we used the same conditions applied to build the PMFs. From the restrained dynamic simulation carried out in the TS region, structures were selected at 50 ps intervals; each of these structures was then refined and characterized as saddle points of order one by means of QM/MM methods using a microiterative approach implemented in a modified DYNAMO program. This code was written to implement the same methodology as employed by GRACE[28] to locate first-order saddle points (corresponding to transition structures of chemical reactions). The program divides the total coordinate space into two subsets of atoms: a control space in which the Hessian matrix is calculated to drive the location of stationary points on the potential energy surface, and a complementary space. At each Hessian-guided step of a QM/MM search in the control space, all the coordinates belonging to the

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Table 1. Weights of the Coordinates (Together with Their Standard Deviations) of Different Atoms and Groups in the Transition Vectors Obtained Using Two Different Sizes of Hessians (See Text)[a]

	S	CH_3	O	SAM–(CH_3)	catechol–(O)	Mg^{2+}	rest of core
small Hessian	0.0084 ± 0.0008	0.837 ± 0.012	0.116 ± 0.013	0.0098 ± 0.0012	0.0280 ± 0.0023	−	−
large Hessian	0.0082 ± 0.0006	0.836 ± 0.008	0.115 ± 0.010	0.0092 ± 0.007	0.026 ± 0.003	0.0020 ± 0.0004	0.0031 ± 0.0004

[a] Values have been averaged over 12 different transition structures.

complementary space were optimized by energy minimizations using gradients. From the refined transition structures, we diagonalized the Hessian matrix and the negative value defined the transition vector (TV) of the reaction. We calculated the coefficients of this TV on each atom using two different definitions of the control space and thus two different Hessian matrices. The smallest one contained all the QM subsystem (63 atoms), while the largest Hessian matrix consisted of the QM subsystem, the magnesium cation, Met 40, and some atoms (the closest atoms to the chemical system) of Tyr68, Asp141, and Lys144 (99 atoms in total).

Once the transition structures were located and characterized, the DYNAMO program is also capable of tracing the IRC paths down to the reactant and product valleys. Then, we obtained the MC corresponding to each TS and we compared the structures to observe geometrical changes in the chemical system going from MC to the TS.

Rare Event Trajectories from Transition State. During the 600-ps QM/MM MD simulation restrained in the TS region, the atomic coordinate and velocities were saved at 5-ps intervals, resulting in a total of 120 configurations that we used as starting points to compute the free downhill trajectories, either in the enzyme or in aqueous solution.

As the reaction coordinate was restrained during the QM/MM MD simulation, the velocity associated with this coordinate was not correctly thermalized in the characteristic configurations. Several strategies were possible to obtain suitable velocities for the starting points.[29] We selected an approximation of velocity randomization because the system may be represented by different fragments, A−B−C−D (A = methyl group, B = cofactor SAM except methyl group, C = catecholate, D = the rest of the system). The translation velocities of the centers of mass of the fragments were assigned to a random value from a Maxwell−Boltzmann distribution at 300 K, thus preserving the velocities of the other degrees of freedom.[30] Once the randomized velocities were obtained for each selected TS configuration, the downhill trajectories were computed, releasing the restraint previously imposed on the reaction coordinate that kept the structure in the region of the TS. The equations of motion were integrated forward and backward in time until the system reached the reactants and product states. In practice, this was done using the same integration algorithm in both cases, but multiplying the velocities by one, for forward integration, and by minus one, for backward integration.[31]

The downhill trajectories were propagated from −8 to +2 ps, in the enzyme environment, and from −2 to +2 ps, in aqueous solution. The microcanonical thermodynamical ensemble (NVE) was used for these simulations with a small time step of 0.5 fs. All the trajectories obtained in both enzyme environment and aqueous solution can be classified as reactive trajectories, where reactants are connected to products, or nonreactive trajectories, where reactants are connected to reactants or products to products.

Results and Discussion

Transition Structures and Transition Vectors. The analysis of the TV obtained with the small Hessian matrix (first row of Table 1) shows that the sum of the coefficients of the sulfur atom (S) of the SAM, the carbon atom (C) of the transferring methyl group together with the three hydrogen atoms, and the nucleophilic oxygen atom of the catecholate (O) represents 96% of the TV, thus justifying the distinguished coordinate used to obtain the PMF as the antisymmetric combination of the breaking and forming bonds ($d_{S-C}-d_{C-O}$). From these transition structures we have traced down the IRC path, followed by a full optimization, obtaining the corresponding MC.

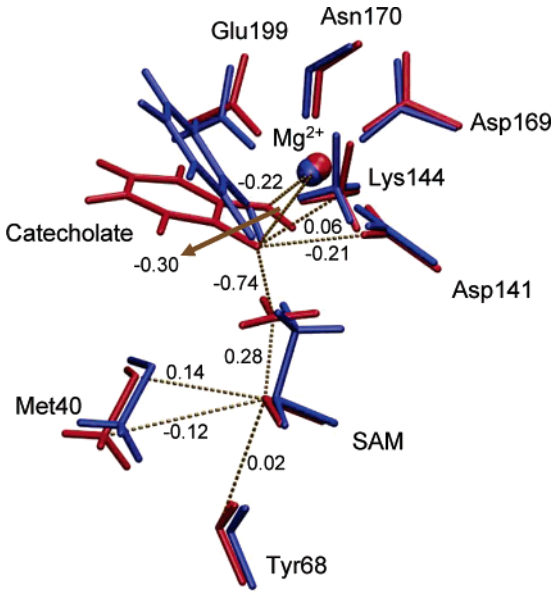

Figure 1. Overlap of representative structures of TS (red) and MC (blue) structures of the reaction catalyzed in the COMT active site. The values of the distances that present the largest average variations between the TS and the MC ($d_{TS}-d_{MC}$) are shown in the figure. These values have been averaged over the 12 reaction paths. For clarity proposes, only the chemical regions Met40, Tyr68, Lys144, and Mg^{2+} and their coordination sphere are depicted.

In Figure 1, an overlap of representative structures of TS and MC is presented where the chemical regions Met40, Tyr68, Lys144, and Mg^{2+} and their coordination spheres are depicted. The values of the distances that present the largest average variations between the TS and the MC ($d_{TS}-d_{MC}$) are also indicated. These values have been averaged over the 12 reaction paths. The increase in the S−C distance and the coupled decrease in the C−O distance, which define the progress of the reaction, can be observed. The relative distances between the S atom and the Met40 and Tyr68 do not change by a large amount and present, in the Met40 case, positive and negative differences indicating a slight rotation. More important changes are observed for the distances involved in the relative position of the two oxygen atoms of the catecholate with respect to the environment. From the analysis of the changes observed in the Figure 1, it seems that Lys144 (the residue that accepted the proton from the catechol, thus forming the anion) moves away from the catecholate while the Mg^{2+} cation and Asp141 approach it. The

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variations presented for the Asp141 and the Lys144 are reasonable from electrostatic arguments, as a negative charge on catecholate is being partially canceled as the system evolves from MC to TS. Nevertheless, the Mg cation, which has a positive charge, shows the opposite behavior as it approaches the catecholate. It is also important to emphasize how the S−C−O angle is approaching linearity as the reaction proceeds and the change in the relative orientation of the catecholate ring from the MC to TS. This last movement maximizes the overlap between the lone pair orbitals of the oxygen atom and the vacant 2p orbital of the carbon atom of the transferring methyl group.

There are important changes between the MC and TS structures, not only for the atoms involved in the chemical reaction itself, but also for the distances and orientations between the depicted amino acids and the Mg^{2+} cation, and the chemical system (see Figure 1). These atoms belonging to the MM region have been incorporated into the enlarged control space (row 2 of Table 1). The resulting transition structures are almost equal to the ones obtained with the smallest Hessian (see Supporting Information). The analysis of the TV allows us to conclude that the environment participates only at 0.4%. An inspection of this 0.4% coming from the environment atoms reveals that the most important participation comes from the Mg^{2+} cation (ca. 40%). While the participation of the entire environment is small in the TV, except for the Mg cation, it could be suggested that the participation of the enzyme in the reaction coordinate takes place before arriving at the TS, not once the TS is reached. We must keep in mind that the TV defines the reaction coordinate just on the TS, not along the whole reaction path.

Rare Events Trajectories from Transition State. As explained in the Methods section, from each one of the 120 configurations selected on the constrained molecular dynamics, both in the enzyme and in solution, we ran downhill trajectories releasing the restraint previously imposed on the reaction coordinate. The equations of motion were integrated forward and backward in time until the system eventually reached the reactant and product states. As a result, from the total number of calculations ran in the enzyme and in solution, two different types of trajectories were observed: (a) reactant−product (RP) transitions, which are called the reactive trajectories and (b) recrossing trajectories leading from reactants to reactants (RR) or products to products (PP); in both cases they will be considered nonreactive trajectories. The calculations reveal that 65% of the total number of trajectories were reactive in the enzyme and 45% in solution. Regarding the nonreactive trajectories, in the enzyme we have discovered that 33% were R−R type and 2% P−P type, while 46 and 9% were obtained in aqueous solution for R−R and P−P, respectively.

Because of the existence of recrossing, the TST rate constant needs to be corrected by a transmission coefficient κ, which will necessarily be less than unity. We have computed κ using the "positive flux"[30] formulation that assumes that the trajectories are initiated at the barrier top with forward momentum along the reaction coordinate. Then for a given reaction time t, the time-dependent transmission coefficient is defined as:

$$\kappa(t) = \frac{\langle j_+ \theta[q(+t)]\rangle - \langle j_+ \theta[q(-t)]\rangle}{\langle j_+ \rangle}$$

where q is the reaction coordinate, j_+ represents the initially positive flux at $t = 0$, given by $q(t = 0)$, and $\theta(q)$ is a step function equal to 1 in the product side of the reaction coordinate and zero on the reactant side. The averages are taken over all the trajectories.

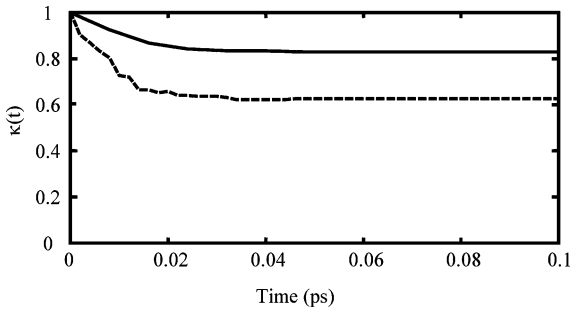

Figure 2. Transmission coefficients, $\kappa(t)$, obtained in the enzyme (solid line) and in solution (dashed line).

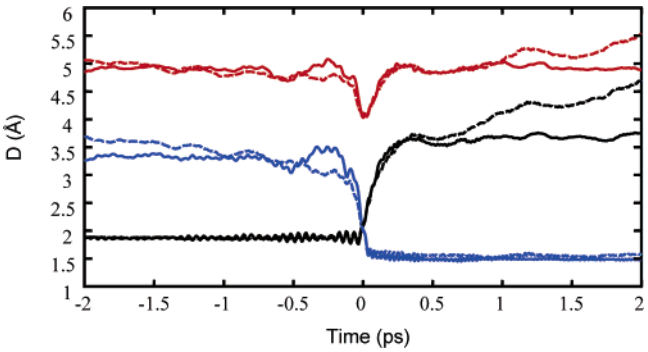

Figure 3. Time evolution of selected averaged distances for reactive trajectories for the enzymatic reaction (solid lines) and in solution (dashed lines). S···C distances are depicted in black, C···O distances in blue, and S···O in red.

As shown in Figure 2, the behavior of $\kappa(t)$ shows a fast decay in both media through the first 30−40 fs. After this period of time, the transmission coefficient reaches a plateau from which the values of the transmission coefficient in solution and in the enzyme can be obtained. The resulting values of κ are 0.83 ± 0.03 in the enzyme and 0.62 ± 0.04 in solution.[32] The larger value obtained in the enzyme compared with that in the solution environment shows that the dynamic effects also favor the catalysis with respect to the reference reaction in solution. Obviously this contribution to catalysis is much smaller than the effect coming from the diminution of the activation free energy. The increase of the transmission coefficient can be translated to a free energy contribution of only 0.17 ± 0.02 kcal·mol^{-1} (at 300 K) to be compared with the computed activation free energy lowering estimated to be around 9.9 kcal·mol^{-1}.[21]

To get a deeper insight into the dynamical behavior of the chemical reaction in both media, we are analyzing the different reactive trajectories in solution and in the enzyme environments. From the geometrical point of view, the distances that define the reaction coordinate, d_{S-C}, d_{C-O}, and d_{S-O}, present the expected behavior for reactive trajectories: d_{S-C} is increasing, while d_{C-O} is decreasing and d_{S-O} reaches a minimum value in the TS. The evolution of these geometrical parameters, averaged over reactive trajectories, is displayed in Figure 3, where the time defines the evolution of the reaction from R to

(32) For details of the error estimation procedure, see: Bradley, J.; Gertner, K. R.; Wilson, K. R.; Hynes, J. T. *J. Chem. Phys.* **1989**, *90*, 3537−3558.

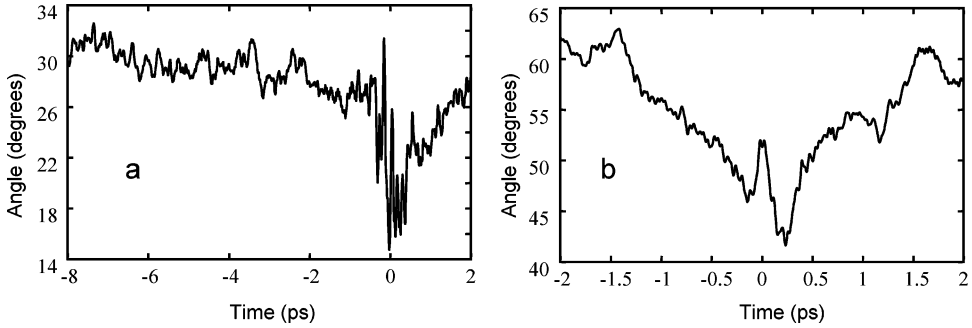

Figure 4. Time evolution of the angle formed between the S···O vector and the one perpendicular to the catecholate ring averaged for the reactive trajectories in the enzyme (a) and in water (b).

P, passing through the TS at $t = 0$. The angle that defines the relative position of the methyl carbon atom with respect to the sulfur and the oxygen atoms (S···C···O, not shown here) approaches 180° in the TS, as shown in Figure 1. Thus, the reaction in both environments can be described as an approach and correct alignment of the two reaction fragments (SAM plus catecholate) together with a fast transfer of the methyl group from the sulfur atom of SAM to the deprotonated oxygen atom of catecholate. This transfer takes places in about 100 fs. Despite the high degree of similarity, an important difference between the enzyme and the solution trajectories is observed. The changes in the d_{S-C}, d_{C-O}, and d_{S-O} distances in water are smoother and more progressive than those in the enzyme. This is due to the diffusion of the reactive moieties in solution, while in the enzyme the system is trapped in the active site during the time of simulation.

Another important geometrical change of the chemical system can be analyzed in Figure 4, where the angle formed between the S···O vector and the one perpendicular to the catecholate ring is displayed. This angle defines the relative orientation of the ring with respect to the attacking direction of the methyl group.

The evolution of this angle, averaged over the reactive trajectories, is presented in Figure 4 for the enzyme (4a) and the solution (4b). In the enzyme it is possible to confirm the results presented previously from the analysis of the representative structures coming from the potential energy profile, that is, the change in the catecholate orientation from the MC to TS maximizing the overlap between the lone pair orbitals of the oxygen atom and the vacant 2p orbital of the carbon atom of the transferring methyl group. The value of this angle changes from around 30° at reactant state (−8 ps) to 15° in the TS ($t = 0$), which corresponds to an optimal angle for the nucleophilic attack. This change is more pronounced in the vicinity of the TS region, but it is already evident, in a smooth way, several picoseconds before reaching the TS. In solution (Figure 4b) it is also possible to observe the same evolution for this angle, reaching smaller values as the system evolves toward the TS. However, the values now range from 60 (at $t = -2$ ps) to 45 (at $t = 0$), far from the optimal orientation between the reaction fragments reached in the enzyme. This different behavior is probably because the chemical system is less constrained in solution than in the enzyme active site. In fact, analysis of the individual trajectories shows deviations around the mean value in solution larger than those in the enzyme. This result also suggests that the protein induces an optimal orientation of the chemical system.

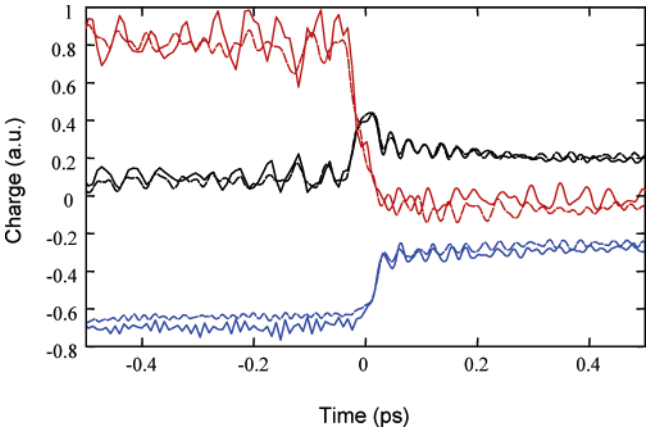

Figure 5. Time evolution of Mulliken charges averaged for reactive trajectories in the enzyme (solid lines) and in solution (dashed lines). The methyl group is depicted in black color, oxygen in blue, and sulfur in red.

From the electronic point of view, Figure 5 presents the averaged charges on sulfur atom of SAM, the methyl group, and the nucleophilic oxygen atom of catecholate along the reactive trajectories both in the enzyme and in solution. It can be observed that the charge of all atoms is almost invariant all along the trajectories, except for a very short time corresponding to the period of time during which the C−S and C−O bonds are being broken and formed, respectively. In this interval, the sulfur atom loses its initial positive charge, while the oxygen atom of catechol loses part of its negative charge, and the methyl group, which in reactants is almost neutral, becomes slightly positive but through a maximum that is reached in the TS region. Then, combining the geometrical and the electronic descriptions of our reaction, this can be described as a three-step process. In the first one, the SAM approaches the negatively charged oxygen atom up to a distance of about 4.5 Å. In the second one, a positively charged methyl group is transferred from the sulfur to the oxygen atom. This process can be also seen as an electron transfer from the oxygen to the sulfur atom. Finally, once the transfer has finished, the neutral product fragments are separated. We have also monitored the variation of the charge in the hydroxyl oxygen of catecholate, not depicted in the figure, and it also presents a similar behavior to the nucleophilic oxygen. The negative charge of this oxygen atom also diminishes, although the variation is more modest than that for the nucleophilic oxygen.

Focusing attention on the interactions between the chemical system and the environment, Figure 6 shows the variation of distances associated with the octahedral coordination sphere of the Mg^{2+} cation. In Figure 6a, changes in the distance between

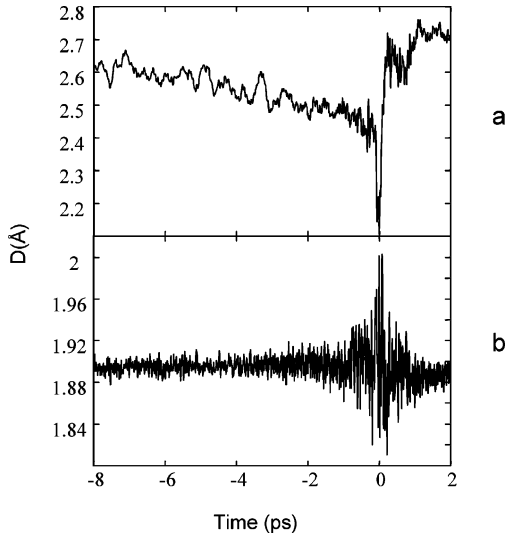

Figure 6. Time evolution of the averaged distance between the hydroxyl oxygen of the catecholate and the Mg^{2+} (a) and between the Mg^{2+} and carboxylate oxygen atom of Glu199 (b).

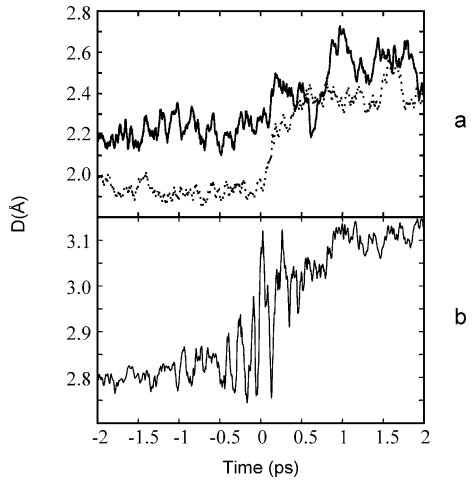

Figure 7. (a) Time evolution of the averaged shortest hydrogen bond distances established between water molecules and the two oxygen atoms of catecholate for the reactive trajectories in aqueous solution. Nucleophilic oxygen is depicted by the dashed line and the hydroxyl oxygen by the solid line. (b) Time evolution of averaged distance between protonated Lys144 and the nucleophilic oxygen of the catecholate.

the hydroxyl oxygen of the catecholate and the Mg^{2+} cation show how the cation is approaching this oxygen atom when the system evolves from the reactant state to TS (the Mg−O distance diminishes from 2.6 to 2.1 Å), and once the methyl is transferred to the oxygen and the catecholate loses part of its negative charge, the Mg^{2+} cation goes away from the chemical system. A similar behavior has been observed for the distance between the Mg^{2+} cation and the nucleophilic oxygen, although this movement takes place at longer distances (from 5.1 to 4.5 Å). This time evolution of the Mg^{2+} cation approaching the catecholate is similar to the rotation of catecholate ring observed before reaching the TS, and thus these movements are probably coupled. On the other hand, the behavior of these distances along the reactive trajectories is different from that for the rest of the coordination shell of the Mg^{2+} cation. In Figure 6b, we present the time evolution of the Mg···Glu199 distance. This distance shows an oscillatory behavior around a mean value, but the amplitude of the oscillations is clearly amplified as the system evolves from the reactant state to the TS. Once the products are formed, this amplitude is quickly reduced. The same kind of picture is obtained when the other distances of the coordination shell (with residues Asp141, Asp169, Asn170, or a crystal water molecule) are represented. This observation suggests that in the TS region the vibrational motions associated to the sphere of coordination of the Mg^{2+} cation are excited.

The movement of the Mg^{2+} cation toward the oxygen atoms of catecholate when going from the reactants to the TS may be amazing considering that the methyl transfer reaction implies a diminution of the negative charges of the two oxygen atoms of catecholate (see above). As a matter of fact, the opposite trend is observed from the analysis of the reaction in solution. Figure 7a shows the averaged time evolution of the shortest hydrogen bond distances established between water molecules and the two oxygen atoms of catecholate for the reactive trajectories in aqueous solution. Obviously, as the negative charge of the oxygen atom diminishes, the hydrogen bond interaction with water molecules is weakened. This is clearly reflected by the lengthening of the averaged hydrogen bond distance in both oxygen atoms. The effect is stronger in the case of the nucleophilic oxygen because the change in the charge is also more intense. This intuitive evolution of the interactions established between the chemical subsystem and the environment as the reaction proceeds is also observed in some interactions of the enzyme not mentioned above. Figure 7b shows the distance between Lys144 (a positively charged residue that received a proton from the catechol, thus forming the reactive catecholate anion) and the nucleophilic oxygen of the catecholate. This distance, which corresponds to a hydrogen bond interaction in the reactants, is increased when the system evolves toward the TS (moving from 2.8 to 3.1 Å). The weakening of this interaction, which is equivalent to the lengthening of the hydrogen bond distances in aqueous solution, is also related to the diminution of the negative charge of the nucleophilic oxygen when the TS is reached. The weakening of this interaction does not favor the diminution of the barrier.

Once we analyze the "normal" evolution of the interactions established between the oxygen atoms of catecholate and their surroundings as the reaction advances, we can emphasize the surprising behavior of the Mg^{2+} cation in the enzyme. Considering just the chemical system and the Mg^{2+} cation, in a process where the environment followed the chemical system, the cation would not approach the oxygen atoms as they are losing negative charge. According to the electrostatic interactions, the distance between the Mg^{2+} cation and the oxygen atoms of catecholate should be increased as the methyl group was transferred. The effect of this "abnormal" evolution of the Mg^{2+}−oxygen distances is that the TS will present shorter distances than expected, and thus we will have an effective larger stabilization of the TS with this approach, lowering the free energy barrier of the reaction as compared with the reference reaction in solution. This movement of the Mg^{2+} cation, which has been shown in the previous section to participate in the reaction coordinate, is dynamically coupled to the movement of the chemical system as the reaction proceeds.

To have a perspective of the global contribution of the environment to the reaction process, we have carried out here a complementary electrostatic analysis along our reactive trajectories in aqueous solution and in the enzyme. Figure 8 displays the averaged projection of the electric field created by

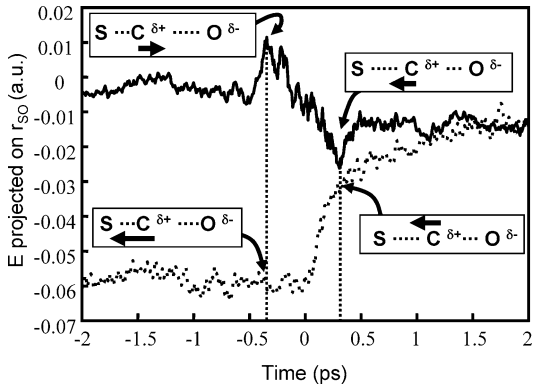

Figure 8. Time evolution of the averaged projection of the electric field (over the total number of reactive trajectories) created by the environment on the transferring methyl group along the S−O vector. The solid line is used for the enzyme, and the dashed line is used for solution. Electric forces due to the environment acting on the methyl group are schematically depicted.

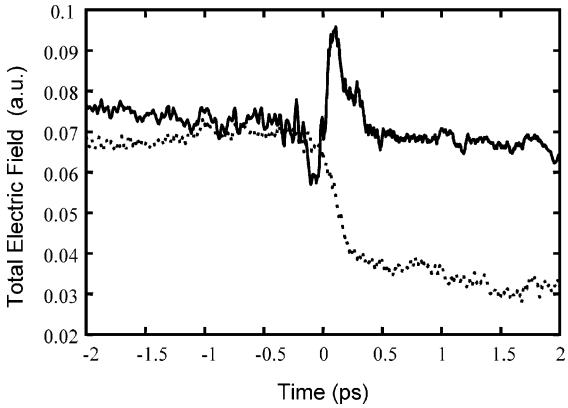

Figure 9. Time evolution of the averaged modulus of the total electric field (over the total number of reactive trajectories) created by the environment in solution (dotted line) and in the enzyme (solid line) on the transferring methyl group.

the environment on the transferring methyl group along the S−O vector, while the averaged modulus of the total electric field created by the environment on the transferring methyl group is presented in Figure 9.

The projection of the electric field can be directly interpreted as the electrostatic force due to the surroundings that favors or hinders the movement of the methyl group from the sulfur atom to the nucleophilic oxygen. According to our criterion, a negative value means electrostatic force acting on the methyl group against the transfer to the nucleophilic oxygen, while a positive value means a force favoring the transfer from sulfur to oxygen. In aqueous solution, at negative times we have found a strong electrostatic field acting against the methyl transfer. This is so because the solvent stabilizes the charge separation existing in the reactants. We must keep in mind that the reaction proceeds from charged species in reactants [S^+−Me···O^-] to neutral species in products [S···Me−O]. Thus, the solvent reaction field clearly acts against the advance of the reaction. At positive times, when the system arrives to the product state, the projection of the field is still negative but much smaller. The products are neutral, and consequently, the projection of the reaction electric field created by water molecules is less intense than that for the charged reactants. A completely different behavior is observed in the enzyme. First, the projection of the electrostatic field does not change as much as that in solution when going from negative to positive times. This is, the projection of the electrostatic field created by the enzyme is much more fixed than that in solution, and it is essentially independent of the charge of the substrate. Second, while the projection of the electric field is slightly negative for $t = -2$ ps, in the vicinity of $t = 0$ (when the system is crossing by the TS) the electrostatic field created by the protein environment favors the stabilization of the TS. Effectively, at $t = -0.3$ ps, the projection of the electric field becomes positive, which means that the enzymatic environment creates an electrostatic force favoring the transfer of the methyl group. When the TS has been crossed, up to $t = +0.3$ ps, the electrostatic force becomes more negative, and thus it now favors the movement of the methyl group backward. These electric forces are also schematically presented in Figure 8. Thus, the enzyme is able to provide an adequate electrostatic environment for the changes taking place in the chemical system. The presence of the protein structure avoids the response of the environment as a mere consequence of the changes produced in the reaction. In this case, where the polarity of the system diminishes as the TS is reached, such a response would mean an increase of the activation energy barrier because the electrostatic interactions are weakened, as observed in aqueous solution. Note that in this discussion we are referring to a particular component of the electric field. The analysis of the averaged modulus of the total electric field created by the environment (Figure 9) and also of the electric field for individual trajectories (shown as Supporting Information) displays a very similar behavior for the reactant state in the enzyme and in solution, as required to have a good binding of these polar reactants. In conclusion, while the aqueous solution clearly favors the stabilization of the reactant state, the protein, even stabilizing reactants, stabilizes the TS much better.

These findings are similar to the conclusions reached by Olsson and Warshel[33] and one of us[34] on the analysis of the solute solvent dynamics in the S_N2 reaction of haloalkane dehalogenase compared to that of solution. In ref 34, the authors demonstrated that almost the entire catalytic effect of enzyme is associated with the reorganization of the protein−substrate coordination, while the effect of the difference in the relaxation time of the environment coordinate in the enzyme and in solution was less important. Our Figure 8 also reveals a very different reorganization of the reaction medium but taking place in a similar time scale.

Conclusions

We have studied the dynamic effects on the bimolecular reaction catalyzed by catechol *O*-methyltransferase. The participation of the environment has been first analyzed by the inspection of the transition vectors of different transition structures and, afterward, studying the molecular dynamics trajectories ran from selected TS configurations previously obtained from the restrained dynamic simulation carried out in the TS region.

The first conclusion that can be derived from our study is that enzyme participates in the real reaction coordinate, although not dramatically. In particular, we have shown that the motion of Mg^{2+} cation is the most important component of the environ-

(33) Olsson, M. H. M.; Warshel, A. *J. Am. Chem. Soc.* **2004**, *126*, 15167−15179.
(34) Soriano, A.; Silla, E.; Tuñón, I.; Ruiz-López, M. F. *J. Am. Chem. Soc.* **2005**, *127*, 1946−1957.

ment atoms to the TV. Moreover, the distances between the Mg and the oxygen atoms of the catecholate are the ones that present the most significant changes from reactants to transition structures. If we included the participation of the Mg into the distinguished reaction coordinate, the free energy profile would be slightly different. This effect is usually named as the nonequilibrium effect.[7,8] Nevertheless, the reaction coordinate used in this study has been demonstrated to be a good choice to monitor the chemical reaction. The free energy profile obtained is thus reasonable. This is also confirmed by the fact that high transmission coefficients have been computed for this reaction. Although a lower value of the transmission coefficients in water could be interpreted as a wrong choice of the distinguished reaction coordinate, as it is less satisfactory in water than in the protein environment, we think that the distinguished reaction coordinate used in this work is enough to capture the different response of the media with the reaction. The larger value obtained in the enzyme would then show that the dynamic effects favor the process with respect to the counterpart reaction in solution. Obviously this contribution to catalysis is much smaller than the effect coming from the diminution of the activation free energy.

The analysis of the reactive trajectories obtained in both environments reveals another very different dynamic behavior between enzyme and solution. On average, the changes of the solvent appear to be a response to the changes of charge of the chemical system. This is shown by the analysis of the evolution of the hydrogen bonds established between the water molecules and the initially negatively charged catecholate. The diminution of the polarity of the chemical system as the reaction proceeds provokes a weakening of the water−solute interactions. On the contrary, not all the protein movements are following the chemical system evolution. This can be observed by the displacement of the Mg^{2+} cation that approaches the catecholate oxygens that are losing charge during the process. This movement partially precedes the charge evolution. The simultaneity of this movement with the change of the relative orientation of the catecholate ring suggests a collective protein motion that favors the chemical process. The origin of this behavior must be due to the structure of the enzyme, which is better prepared to accommodate a transition structure than the reactant structure. The changes described in this work between the reactants and the TS structures (distances, positioning of the catecholate ring, and charge distribution) clearly discriminate between them, allowing a better fit of the protein active site with the TS structures.

The different dynamic behavior of the protein and the solution is reflected in a global property such as the electric field created by both media, which has been computed along the reactive trajectories. The first important difference is that the projection of the electric field created by the full protein on the transferring methyl methyl group along the donor−acceptor vector (S−O) appears to be much more robust than that in solution and independent of the changes of charge distribution of the solute. And second, while the projection of the electric field created by the solution favors the charge separation (i.e., the reactants state), the one created by the protein in the vicinity of the TS stabilizes this structure. This different electrostatic coupling between the reactant system and the two environments makes the most important contribution to the differences in the transmission coefficients. Previous experience on another enzymatic reaction[34] supports this hypothesis.

As a general conclusion, we suggest that the influence of the COMT protein is beyond the view of the traditional separation of the substrate and protein movements. First, by analyzing transition vectors we have shown that the protein participates in the definition of the reaction coordinate. Second, we have observed some substrate−protein-coupled fluctuations from rare event molecular dynamics simulations that, to some extent, can determine the magnitude of the catalytic effect of the enzyme.

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Supporting Information Available: Complete Hessian matrix of a representative transition structure computed by means of the small and large subsets of atoms together with their TV components. Projection of the total electric field and its modulus, created by the environment along the S···O vector on the transferring methyl group for single trajectories starting from structures of the long molecular dynamics simulation at 20, 140, and 360 ps. This material is available free of charge via the Internet at http://pubs.acs.org.

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