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Coupling between Protein and Reaction Dynamics in Enzymatic Processes: Application of Grote−Hynes Theory to Catechol *O*-Methyltransferase

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Abstract: The generalized Langevin equation (GLE)-based Grote−Hynes (GH) theory is used to calculate the transmission coefficients, κ, for the methyl transfer from *S*-adenosylmethionine to catecholate both in aqueous solution and in the catechol *O*-methyltransferase active site. Values of κ, which measures the deviation of the rate constants from the Transition State Theory (TST) predictions, are obtained by means of rare event molecular dynamics simulations. The results are 0.62 ± 0.04 and 0.83 ± 0.03 for the aqueous and enzymatic environments, respectively, while the Grote−Hynes predictions are 0.58 ± 0.09 and 0.89 ± 0.03, respectively. The Kramers theory estimates are much smaller, about 0.01 and 0.1, respectively. Thus, the enzymatic transmission coefficient is closer to TST predictions than the value obtained in solution. In addition, our results show that the enzymatic coefficient is also closer to its nonadiabatic (or frozen environment) limit than is the solution coefficient. These findings can be understood considering that, during the passage over the barrier top, there is a smaller coupling between the reactive system and the environment in the enzyme than in solution, as well as a smaller reorganization suffered by the enzyme. Analysis of the transition state friction kernel leads to the identification of some key vibrational modes governing the coupling between the two different environments and the reacting solute in the transition state region and insights on their relevance for the reaction dynamics' influence on the transmission coefficient.

1. Introduction

The origin of the rate acceleration achieved by enzymes is one of the fundamental questions in molecular biology.[1] In recent years increasing interest is being focused on the link between protein structure and dynamics and its implications for catalysis.[2−6] Protein and solvent dynamics may be involved in many aspects of the enzymatic process, from substrate binding and release to the alteration of reaction rates.

A widely used theoretical approach for studying the rate of chemical reactions, including enzymatic processes, is the Transition State Theory (TST),[7−9] which gives an upper bound to the true classical rate constant:

$$k^{\mathrm{TST}}(T) = \frac{k_{\mathrm{B}}T}{h}(C^0)^{1-n}\, e^{-\Delta G^{\ddagger}/k_{\mathrm{B}}T} \tag{1}$$

where T is the temperature, k_{B} is Boltzmann's constant, h is Planck's constant, C^0 is the standard state concentration, n is the order of the reaction, and $\Delta G^{\ddagger}$ is the equilibrium free energy of activation. The true rate constant of a reaction can be better approximated by introducing the generalized transmission coefficient, $\kappa(T)$, which measures the departure of the rate constant, $k(T)$ from the TST prediction, $k^{\mathrm{TST}}(T)$[10−12]

$$k(T) = \kappa(T)\, k^{\mathrm{TST}}(T) \tag{2}$$

This generalized transmission coefficient may be viewed as composed by different contributions.[10] For reactions not involving hydrogen atom transfers the transmission coefficient is essentially due to the barrier recrossings of the system, and then

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it can be estimated from the fraction of trajectories that cross the transition state in the direction of products but recross the dividing surface to return to the reactant region. The dynamics of the environment is obviously a decisive factor in the determination of the fate of a trajectory and thus in the value of this term.

According to eq 2, the rate enhancement in reactions catalyzed by enzymes can be achieved by lowering the free energy of activation and/or by increasing the transmission coefficient in comparison with the equivalent uncatalyzed reaction in aqueous solution. The reduction of the activation free energy is by far the most decisive effect in catalysis, and it has been the subject of numerous studies.[10,13−21] Here we are interested in the coupling between environment and reaction dynamics, and accordingly we focus on the transmission coefficient for both the catalyzed and the uncatalyzed reaction. The analysis of the different behaviors of the transmission coefficient in aqueous solution and in the enzyme's active site can be useful to understand the origin of this coupling and the consequences for catalysis.

In principle, the recrossing factor for enzyme reactions can be larger or smaller than that for its counterpart process in aqueous solution. One reason for it being smaller is that the use of a single reaction coordinate to identify the transition structure can be a worse approximation in enzymes than in solution, since specific residues can participate in a more complex way in the reaction coordinate.[10] A reason for it being larger is that the water environment can suffer a more important reorganization than the active site environment in the enzyme[13] when the reactants are converted into products, and thus the water molecules are more coupled to the evolution of the reaction system in the transition state region.

There are to date only a few studies devoted to recrossings in enzymatic reactions. Up to now these studies have employed two strategies. The first one is to carry out molecular dynamic simulations starting from the transition state and following them forward and backward in time. This procedure has been employed before to calculate the recrossings in, for instance, triosephosphate isomerase,[22,23] haloalkane dehalogenase,[11,24] and cathecol *O*-methyltransferase.[25] For the last two cases, comparison to the reaction in aqueous solution always led to a larger value of the transmission coefficient in the enzymatic reaction. In a recent work a similar strategy was used to analyze the effect of particular protein vibrations on the ratio of productive trajectories in cyclophilin A.[3] Another procedure, based on quantum mechanical approaches, has been employed to obtain the recrossing coefficient in other enzymes where hydrogen transfers are involved.[26−31]

Here we explore another approach to estimating transmission coefficients due to recrossing of nonquantum particles: the Grote−Hynes (GH) theory.[32−34] This theory, based on the generalized Langevin equation (GLE), incorporates the important feature that for many reactions the relevant solvent forces affecting the transmission coefficient are only those that act on the chemical system during the short time scale of the barrier passage.[35] This theory has been successfully employed to calculate the transmission coefficients of different reactions in solution.[34,36−38] The most relevant of these studies for the present work are those for S_N2 reactions,[34,38] such as the methyl transfer studied here. Catechol *O*-methyltransferase (COMT, EC 2.1.1.6)[39] catalyzes the methyl transfer from *S*-adenosylmethionine (SAM) to the hydroxylate oxygen of a substituted catechol. COMT is important in the central nervous system where it metabolizes dopamine, adrenaline, noradrenaline, and various xenobiotic catechols.[40] This reaction involves an attack on a methyl group, originally bonded to the sulfur atom of the coenzyme SAM, by a catecholate nucleophilic oxygen atom in a direct bimolecular S_N2 process (Scheme 1). This reaction can be formally considered as an inverse Menschutkin reaction where ionic reactants proceed toward neutral products. 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.[25] We stress that the influence of the environment examined in this paper is not on an entire reaction path (as was explored in refs 2, 31, 41, 42). It is instead on the environment's influence on the transmission coefficient correcting the Transition State Theory rate constant as defined within (which itself contains environmental effects in the activation free energy), which according to the GH theory is determined in the neighborhood of the transition state, as discussed in section 2.2.

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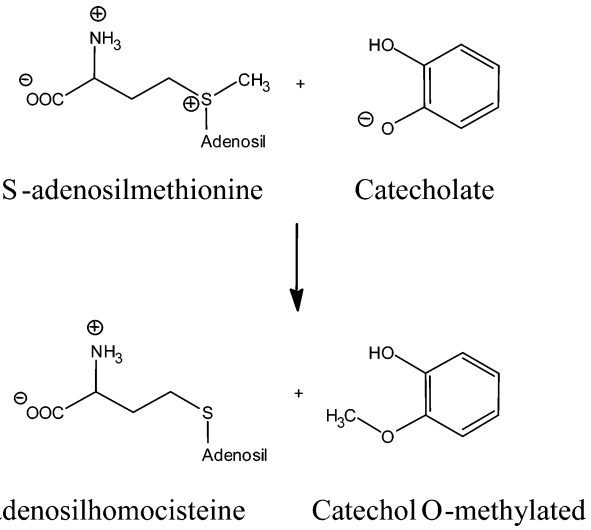
Scheme 1



This work is organized as follows. In the methodological section, we summarize the simulations and calculations carried out to compute the transmission coefficients using GH theory. For the sake of clarity we also briefly summarize our previous calculations of the Potential of Mean Force (PMF)[42] and rare event simulations.[25] We then discuss the results of GH theory and compare them with the Molecular Dynamics estimations of the transmission coefficient. Due to the good agreement found, the GH theory is then used to analyze, in the transition state region, the coupling between the environment and the reactive system for the reaction in solution and in the active site of the enzyme. Some key movements in the active site are identified, and their relevance for the chemical reaction transmission coefficient is discussed.

2. Methodology

2.1. Quantum Mechanical/Molecular Mechanical Evaluation of the PMF and Rare Event Simulations. 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;[43] the nitro groups were removed, and one of the hydroxyl groups of catechol was ionized by proton transfer to Lys144.[44] The Potential of Mean Force (PMF) for the methyl transfer was obtained[42] using the DYNAMO program.[45] The quantum mechanical (QM) subsystem consisted of the cofactor SAM and the substrate catecholate (63 atoms), while the molecular mechanical (MM) subsystem contained the reminding atoms of the enzyme, the magnesium cation, and waters of crystallization inside a cubic box with 55.8 Å sides of TIP3P water molecules. For the reaction in water, we built the PMF for the reaction in aqueous solution that consisted of SAM and catecholate solvated in a cubic box of TIP3P water molecules with 31.4 Å sides. The system was divided into the 63 QM atoms of the solute and 1001 MM water molecules. The free energy barriers obtained from these AM1/MM PMFs were 10.4 kcal/mol for the enzymatic reaction and 19.5 kcal/mol for the reaction in solution.[42]

To evaluate transmission coefficients, free downhill trajectories were started from 120 configurations of the TS region (the top of the PMF) and followed both forward and backward in time until the system reached the product and reactant states.[25] Then we computed the transmission coefficients (κ) using the "positive flux" formulation.[46] The resulting values of κ were 0.83 ± 0.03 in the enzyme and 0.62 ± 0.04 in solution.[25] The larger value obtained in the enzyme compared with 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.

2.2. Application of Grote−Hynes Theory. The Grote−Hynes theory for calculating transmission coefficients makes use of the Generalized Langevin Equation (GLE) to describe the movement of the reactive system along the reaction coordinate in the transition state region.[32,33] This GLE can be analytically solved assuming that the free energy barrier is parabolic in the region close to the transition state and that the force exerted there by the environment on the reaction coordinate (the friction kernel) does not depend explicitly on the reaction coordinate.[34] The transmission coefficient can be conveniently expressed as the ratio between the actual reactive frequency and the frequency obtained under the assumption of equilibrium between the reaction coordinate and the remaining degrees of freedom of the system:[32]

$$\kappa = \frac{\omega_r}{\omega_{eq}} \tag{3}$$

The equilibrium frequency has been obtained from the PMF of the reaction in solution and in the enzyme. For this purpose we traced again the PMFs using the same procedure as that in our previous work,[42] but only along a small range of the reaction coordinate values (approximately 0.2 Å) centered on the TS region, and fitted them to a parabolic function.

Once the equilibrium frequency is known, the reactive frequency can be easily obtained from the following relationship (GH equation):[32,34]

$$\omega_r^2 - \omega_{eq}^2 + \omega_r \int_0^\infty e^{-\omega_r t}\, \zeta(t)\, dt = 0 \tag{4}$$

where $\zeta(t)$ is the friction kernel, obtained from the autocorrelation of the forces (ACF) exerted on the reaction coordinate (F_{RC}) when the system is constrained at the transition state:[34]

$$\zeta(t) = \frac{\langle F_{RC}(0)\, F_{RC}(t)\rangle_{TS}}{\mu_{RC} k_B T} \tag{5}$$

k_B is the Boltzmann constant, T, the temperature, and μ_{RC}, the reduced mass of the reaction coordinate.

From the previous GH equation two interesting limiting behaviors can be derived. The first one is the nonadiabatic limit,[47−49] where the environment remains essentially frozen compared to the motion of the reaction coordinate in the passage through the TS region (note that this assumption is most definitely not applied for the entire reaction path leading to the transition state region from the reactants, for which assorted rearrangements are required).[2,31,41,42] In that case the friction kernel is simply substituted by its zero-time value ($\zeta(t = 0)$) and eq 4 reduces to

$$\omega_{na}^2 - \omega_{eq}^2 + \zeta(t = 0) = 0 \tag{6}$$

The other interesting limit is the Kramers regime,[50] where the reaction dynamics is very slow and the environment exerts its full frictional influence during barrier crossing. In such a case, the GH equation can be written as

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$$\omega_{Kr}^2 - \omega_{eq}^2 + \omega_{Kr}\int_0^\infty \zeta(t)\,dt = 0 \qquad (7)$$

To obtain the friction kernel we ran 50 ps of MD simulation with the system constrained at the top of the PMF. For this purpose, we used a RATTLE-like algorithm[51] adapted to work with a combination of internal coordinates through the use of Wilson's matrix. MD simulations were carried out with a very small time step (0.1 fs) to ensure the good convergence of the constraint under NVE conditions at 300 K using the Velocity−Verlet algorithm.[52,53] Other details of the simulation are the same as those used to obtain the PMFs.[42] Forces on the reaction coordinate were saved at each simulation step, and we verified that the averaged values in both media were close to zero, indicating that the simulations were effectively carried out in the respective transition states.

3. Results

3.1. Evaluation of Transmission Coefficients. The PMFs obtained in the vicinity of the transition state in solution and in the enzyme's active site are shown in Figure 1. To obtain the equilibrium barrier frequency these PMFs are fitted to a parabolic function of the form

$$\Delta PMF = -\frac{1}{2}K_{eq}(RC - RC^{\ddagger})^2 \qquad (8)$$

where ΔPMF is the potential of mean force difference with respect to the maximum and RC‡ is the value of the reaction coordinate at the maximum of the profile. From the associated equilibrium force constant we obtained the equilibrium frequency as

$$\omega_{eq} = \frac{1}{2\pi c}\sqrt{\frac{K_{eq}}{\mu_{RC}}} \qquad (9)$$

where μ_{RC} is the reduced mass associated to the reaction coordinate, and c, the light's speed. The equilibrium frequencies obtained in this way were 1200 ± 20 cm^{-1} for the enzymatic reaction and 900 ± 50 cm^{-1} for the reaction in aqueous solution. The smaller value of the frequency associated with the equilibrium barrier in solution is due to the fact that the nearly neutral transition state is destabilized, with respect to the charged reactants, in solution.[42] The addition of such a destabilization energy term, as a function of the reaction coordinate, leads to an increased energy barrier and to a reduction of the curvature around the energy maximum. This reduction is larger in solution than in the enzyme, reflecting the larger relative destabilization of the transition state. The comparison of the standard deviations of the frequencies reflects the fact that the parabolic fit works somewhat better for the enzyme reaction than in solution. The solvent clearly favors the charged reactants and thus introduces an important asymmetric contribution to the free energy profile. In any case, we have verified that the assumption of a parabolic free energy barrier is reasonable for both cases. From inspection of the recrossings described in a previous work,[25] we have estimated the maximum averaged distance of the reaction coordinate away from the barrier top attained before returning to it. The obtained values are 0.017 and 0.019 Å for the enzymatic and in solution reaction, respectively. These maxima

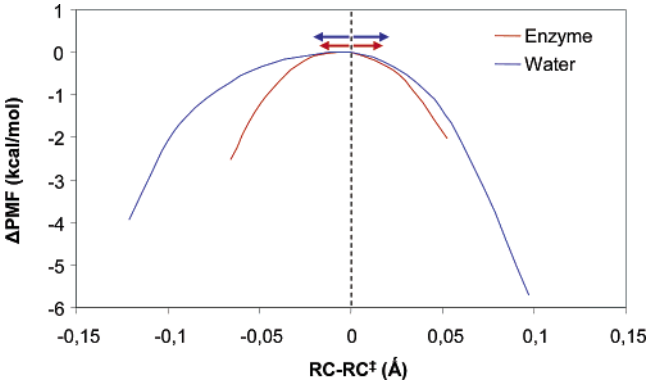

Figure 1. PMFs traced in the vicinity of the transition state for the reaction in solution and in the enzyme. Arrows indicate the magnitude of the maximum averaged distance attained before recrossings in both media.

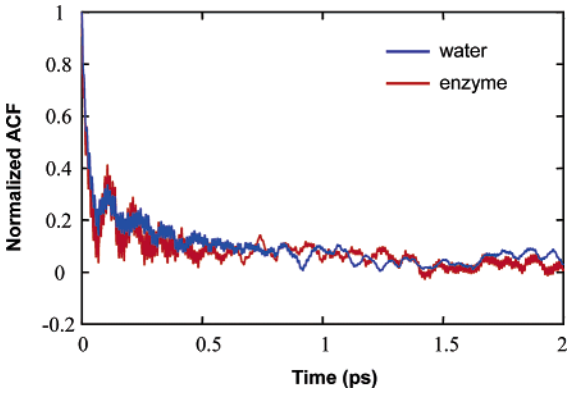

Figure 2. Normalized autocorrelation functions of the forces on the reaction coordinate calculated at the transition states in aqueous solution and in the enzyme.

displacements are schematically shown in Figure 1 by means of two arrows. For displacements within these ranges, the PMF can be quite safely considered as parabolic.

The other term appearing in GH theory (eq 4) is the friction kernel eq 5, which is derived from the autocorrelation of the forces on the reaction coordinate obtained for a constrained transition state. The normalized autocorrelation functions (ACFs) of the forces on the reaction coordinate are presented in Figure 2. The zero-time value of the ACF is very different in both media, 1348 and 766 kJ^2 mol^{-2} Å^{-2} in solution and in the enzyme, respectively. These values clearly reflect a stronger coupling of the reaction with the environment in aqueous solution at the transition state (in fact, this zero-time value is very similar to the one obtained for the symmetric [Cl−CH$_3$−Cl]$^-$ reaction in solution).[34] Despite the different initial values, the dynamical evolution of the ACFs is very similar in both media. The two normalized ACFs at the transition state display a very rapid relaxation at a time scale of about 60 fs and fast oscillations which are due (see below) to the strong coupling of the reaction coordinate with the stretching of the hydrogen atoms in the transferred methyl group (with a characteristic frequency of about 3100 cm^{-1}) and the bending movements of these atoms (about 300 cm^{-1}).

Using these ACFs to obtain the friction kernels, we employed the GH equation to calculate the transmission coefficient and also their nonadiabatic and Kramers limits. The equilibrium and reactive frequencies (expressed as wavenumbers) are given in

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Table 1. Characteristic Frequencies of the Reaction (in cm^{-1}), Chemical System Time Scale, and Environment Time Scale (in ps)[a]

	ω_{eq}	ω_r	ω_{na}	w_ζ	chemical system time scale	environment time scale
water	900	520	395	805	0.010	0.77
enzyme	1200	1070	1035	600	0.005	0.17

[a] See text for definitions.

Table 1, together with the reactive frequency obtained under the nonadiabatic regime and the initial friction expressed as a wavenumber:

$$\omega_\zeta = \frac{1}{2\pi c}\sqrt{\zeta(t=0)} \tag{10}$$

To compare the different dynamical behaviors of the chemical system and the environment we also provide the reaction time scale (the inverse of the reactive frequency) and the environment time scale obtained as the 1/*e* correlation time of the friction kernel.[34]

From the reactive frequency results in Table 1, we can see that the passage through the TS region is about twice as fast in the enzyme than in solution. This is a consequence of the larger curvature presented by the PMF around the transition state in the enzyme active site and the lower value of the friction exerted by the environment on the reaction coordinate at the transition state.

A further indication that the coupling between the environment and the reaction coordinate at the transition state is stronger in solution than in the enzyme is the initial value of the friction kernel, reflected in ω_ζ, which is significantly larger in solution than in the enzyme. This stronger coupling in solution reflected in both ω_r and ω_ζ is related to the fact that in the transition state region the aqueous medium is much more perturbed by the advance of the reaction than the enzyme. Effectively, as the reaction advances, the charge separation of the solute is annihilated and then the reaction field created by the solvent diminishes. This means an important reorganization of the solvent coupled to the reaction progress. As the charges of the solute disappear, the solvent molecules reorganize, establishing weaker interactions with the solute. The enzyme is much less perturbed by the reaction advance in the transition state region. In fact we already found in a study of the reaction path that the electrostatic profile created by the enzyme is nearly the same in the Michaelis complex and in the transition state.[42] The protein structure avoids the reorganization of the medium so that it is already prepared to favor the reaction progress relative to the process in solution, and this feature is reflected in the transition state regions we are examining here. Finally, this weaker coupling in solution compared to the enzyme case is also reflected in the fact that the time scale over which the transition state frictional effect from the environment is significant is considerably larger in solution than in the enzyme (more than 4 times).

The transmission coefficient values obtained in solution and in the enzyme from Molecular Dynamic simulations, together with those obtained applying GH theory and its different limiting behaviors (nonadiabatic and Kramers regimes), are given in Table 2. The first important point to be commented upon, in view of these results, is that the agreement between MD results and GH theory is impressive. In both media the values are within one standard deviation. There are some minor defects: GH theory provides a transmission coefficient slightly smaller than the one obtained from MD for the reaction in solution. This is the usual behavior of GH theory described also in other S_N2 reactions in water.[34] However, the value provided for the enzymatic reaction is slightly larger than the MD results. By contrast, the use of Kramers theory leads to very large errors in the estimation of the transmission coefficient. According to the time scales presented in Table 1, the barrier crossing is much faster than the environment time scale characterizing the friction, and thus the assumptions of this regime[50] do not hold at all. Finally, the nonadiabatic transmission coefficients are in reasonable agreement with the values obtained from MD, especially in the case of the enzyme. As discussed above, the barrier crossing is faster in this latter case, as reflected in its higher barrier frequencies in Table 1, so that the frozen environment approximation is expected to work better than that in aqueous solution. Another reason for the better performance of the frozen environment approximation for the enzymatic reaction is that, as discussed above, the aqueous solution reorganizes significantly during the passage from the reactants to the transition state, while the protein environment hardly changes,[42] and this disparity also applies in the transition state region for the present reaction. Anyway, it should be kept in mind that we are discussing the passage over the barrier top. It is in this case that the frozen environment seems to be a good approach to describing the behavior of the system. Obviously, these conclusions cannot be directly extrapolated to analyze the behavior of the environment during the whole reaction path. In relative terms with respect to water, the enzyme clearly makes a case favoring that a particular trajectory started in the reactant state reaches the transition state region. This is reflected in the diminution of the activation free energy.

Table 2. Transmission Coefficients Obtained from MD, According to the GH Theory (Eq 4), the Nonadiabatic Limit (Eq 6), and the Kramers Regime (Eq 7)

	κ_{MD}	κ_{GH}	κ_{na}	κ_{Kr}
water	0.62 ± 0.04	0.58 ± 0.09	0.44 ± 0.09	0.01
enzyme	0.83 ± 0.03	0.89 ± 0.03	0.86 ± 0.02	0.10

3.2. Coupling between Chemical System and Environment Dynamics. To understand in more detail how the environment dynamics influence the reaction we need to identify which motions couple with the reaction coordinate and how they contribute to the friction kernel in both media. With this purpose we have obtained the friction spectra as the Fourier transforms of the friction kernels:[34]

$$\zeta(\omega) = \int_{-\infty}^{+\infty} \zeta(t) e^{i\omega t}\, dt \tag{11}$$

The friction spectrum can be decomposed into two parts, the relaxation spectrum ($\zeta^+(\omega)$) and the rigid spectrum ($\zeta^-(\omega)$):[34]

$$\zeta^+(\omega) = \frac{\zeta(\omega)}{\omega_{eq}}\left(\frac{\omega^2}{\omega_r^2 + \omega^2}\right) \tag{12}$$

$$\zeta^-(\omega) = \frac{\zeta(\omega)}{\omega_{eq}} - \zeta^+(\omega) \tag{13}$$

The rigid spectrum emphasizes the contribution of the *low*

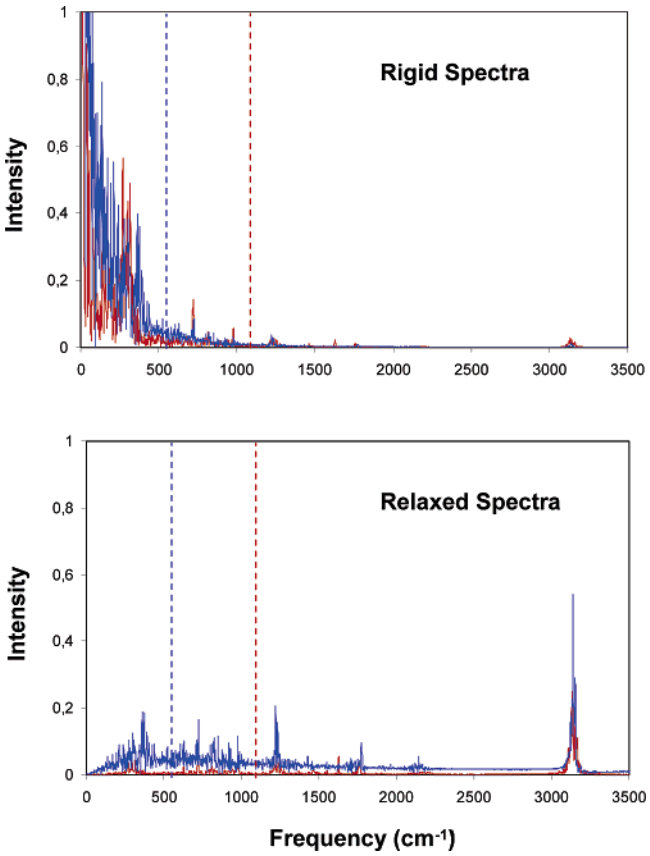

Figure 3. Intensity of the relaxed and rigid transition state friction spectra (in arbitrary units) in water (blue) and in the enzyme (red) versus the frequency (in cm^{-1}). The reactive frequencies are shown as dashed vertical lines, with the enzyme value being higher.

frequency range of the friction spectrum and thus those motions that cannot follow the passage of the system over the barrier top. The rigid spectrum accounts for the deviations of the transmission coefficient from Transition State Theory (this is from $\kappa = 1$).[34] The motions appearing in this spectrum contribute to reaction recrossings because of their *inactivity* during the reaction time scale ω_r^{-1}, as they are essentially static on this time scale. Effectively those movements with characteristic frequencies smaller than the reactive frequency are expected to be unable to respond to the passage of the system over the barrier top causing then nonequilibrium solvation (the TST definition employed in the present work corresponds to equilibrium solvation, as described in ref 34). On the other hand, the relaxation spectrum emphasizes the contribution of the *high* frequency range of the transition state friction spectrum and then corresponds to those motions that dynamically influence the recrossings of the reacting system. The relaxation spectrum accounts for deviations from the nonadiabatic limit.[34] The motions appearing in this spectrum respond most rapidly and become *unfrozen*, allowing the transmission coefficient to be larger than the nonadiabatic value. Of course, in both spectra only those motions coupled to the reaction coordinate at the transition state are reflected. The rigid and relaxation spectra are shown in Figure 3.

To assign the observed signals in the friction spectra we also obtained the frequencies corresponding to the normal modes of the transition state in solution and in the enzyme. These normal modes were obtained after diagonalization of a Hessian matrix corresponding to a stationary structure localized in each medium and characterized as having one and only one imaginary frequency. For the calculation of the Hessian matrix we considered not only the atoms belonging to the QM subsystem (SAM plus catecholate) but also some MM atoms of the environment. In aqueous solution we included all those water molecules hydrogen bonded to SAM or catecholate (a total of 14 TIP3P water molecules). In the enzyme we also included in the Hessian matrix the atoms of the following residues: Mg^{2+}, Met40, Tyr68, Asp141, Trp143, Lys144, Asp169, Asn170, Pro174, Glu199 y HOH51. The sizes of the calculated AM1/MM Hessians were 315 × 315 and 654 × 654 in solution and in the enzyme, respectively. It is important to take into account the limitations of our local normal-mode analysis. First, our analysis is applied only to the transition state region; low frequency motions may differ considerably for different structures, and then our conclusions cannot be directly extrapolated to other states appearing along the reaction path. Second, we are not considering in our analysis the contribution of nonactive-site regions that could couple with the reaction dynamics.[2,31,54]

For the sake of clarity in our analysis the full transition state force spectra appearing in Figure 3 will be decomposed into three different regions:

3.2.1. Below 500 cm^{-1}. This region contains the low-frequency motions. In both media, the motions appearing in this region cannot respond during the time scale of the system recrossings, as their frequencies are below the value of the respective reactive frequencies (520 and 1070 cm^{-1} in water and in the enzyme, respectively). In aqueous solution, this region of the spectrum corresponds to hindered translation, diffusional translation, reorientation, and multimolecular motions of water molecules,[34] while, in proteins, collective motions appear in this frequency region.[55−57] In general, the intensity of the motions in this region is higher in solution than in the enzyme, showing then a stronger coupling with the reaction coordinate at the transition state, which explains the larger departure from unity found for the transmission coefficient in water. This larger coupling in the transition state can be understood on the basis of a more general feature: the different characteristics of the electric field created by the solvent or the enzyme on the solute. The electric field created by the solvent is a reaction field, the magnitude and orientation depending on the electric properties of the solute, while in the enzyme the electric field is essentially permanent.[25] The displacement of water molecules changes this electric field in solution, which is strongly coupled to the variation of the solute's dipole during the reaction progress, along the reaction path and in the transition state region, since they are oriented in opposite directions.[25]

The inspection of the normal modes has allowed us to identify some interesting motions in this region of the spectra. The reorientation of the catecholate ring that allows the alignment between the oxygen lone pair and the vacant orbital of the transferred methyl group is of particular interest.[25] This motion appears at about 30 cm^{-1} in solution and is obviously mixed with displacements of some water molecules. In the enzyme,

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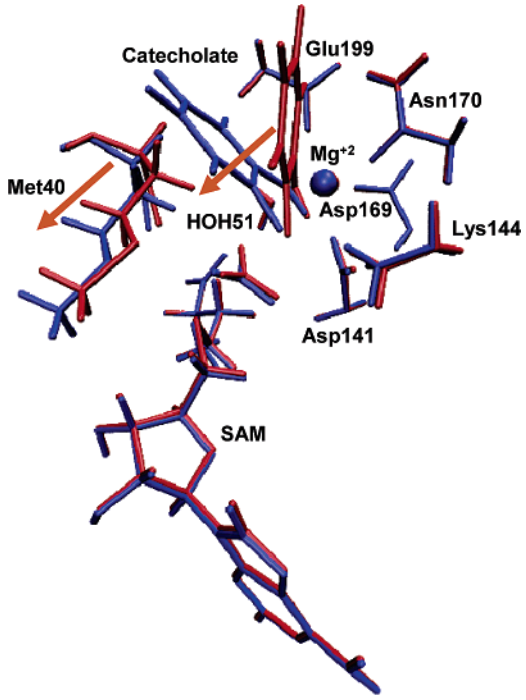

Figure 4. Pictorial representation of one of the low frequency motions (~40 cm^{-1}) strongly coupled to the reaction coordinate in the enzyme. The red and blue structure represents two snapshots of this vibration, which is essentially described as a reorientation of the catecholate ring and a displacement of Met40 (schematically depicted by arrows).

this motion appears as a normal mode with a characteristic frequency of 40 cm^{-1} and, interestingly, is clearly mixed with the displacement of the Met40 residue. Effectively, to move the catecholate ring, room must be made in the active site displacing this residue. Interestingly, the movement of this residue was previously identified as being coupled to the reaction advance.[58] The reason is now clear: the Met40 must be moved away to allow the reorientation of the catecholate ring; then the methyl transfer can take place through the correct positioning of the oxygen lone pair orbital with respect to the vacant orbital of the methyl group (see Figure 4). In both media this motion of catecholate ring is strongly coupled to the reaction coordinate. As the characteristic frequencies of these important vibrational modes are much smaller than the reactive frequencies in both media, the rearrangements involved must take place, at least partly, in advance of the passage over the barrier top. In fact, in our rare event molecular dynamics simulations of reactive trajectories we described that the reorientation of the catecholate ring allowing the correct orientation of the methyl and oxygen orbitals is already observed several picoseconds before the system crosses the TS dividing surface.[25] A similar behavior for some solvent motions was previously described for the S_N2 reaction in aqueous solution.[41]

We have also found a strong coupling in the enzyme with a normal mode with a characteristic frequency of about 50 cm^{-1} that could be described as a breathing motion of the active site. In the range between 50 and 150 cm^{-1} we found other catecholate reorientations due to rotation of the ring around the axis perpendicular to the movement previously described. These movements are coupled to the stretching of the distance between the oxygen atom of the hydroxyl group of catecholate and either a water molecule (in aqueous solution) or the Mg^{2+} ion (in the enzyme). In our previous study,[25] we have shown that, for the reaction in water, the hydrogen bond of the hydroxyl oxygen is weakened as the reaction advances because the negative charge of this oxygen diminishes when the methyl group is being transferred. Consequently the hydrogen bond in the transition state was found to be weaker than in the reactant state in aqueous solution.[25] In the enzyme, while the charge flow follows the same trend, we reported a completely different behavior for the oxygen−Mg^{2+} interaction, which was found to become shorter in the transition state. We then concluded that this interaction makes an important differential stabilization of the transition state in the enzyme relative to the aqueous solution.[25]

The motions appearing in the range 260−330 cm^{-1} are particularly interesting, because the intensity is higher in the enzyme than in solution in this region of the rigid spectrum. Analyzing each of the normal modes obtained from the Hessian matrix in this range we found contributions of the reactive system and some residues of the enzyme. For example, the normal mode with a frequency of 270 cm^{-1} displays the symmetric S−C−O stretching combined with the stretching of the hydrogen bond between the O atom of catecholate with Lys144. Other normal modes of this range correspond to the bending and tilt motions of the transferred methyl group mixed with the stretching of the O−Lys144 hydrogen bond and other hydrogen bonds between protein residues.

3.2.2. Between 500 and 1000 cm^{-1}. This region corresponds to motions that can, at least partially, follow the passage of the system over the barrier top in water but that are not able to respond in the reaction time scale of the enzymatic process. Thus, motions appearing here are responsible for departures from the nonadiabatic limit in aqueous solution, a limit that we have already shown to work better for the enzyme.

The librational or hindered rotational motions of water molecules usually appear near 500 cm^{-1} (from 300 to 800 cm^{-1} approximately).[34,59] This region of the spectrum is also more strongly coupled to the reaction coordinate in water than in the enzyme. The exception is the narrow band appearing at about 720 cm^{-1}, which displays a slightly larger intensity in the enzyme. This band has been assigned, through our normal-mode analysis, to the out-of-plane movements of the catecholate hydrogens and is also slightly coupled to the previously described stretching of the intermolecular interaction established between the oxygen of the hydroxyl group of catecholate and either a water molecule (in aqueous solution) or the Mg^{2+} ion (in the enzyme).

3.2.3. Above 1000 cm^{-1}. This region corresponds to motions that dynamically influence the recrossings of the system as they can, at least partially, follow the changes taking place in the system during the crossing of the TS dividing surface. This region is then responsible for the departures from the nonadiabatic value of the transmission coefficient in both media.

One of the most important signals identified in this region is the umbrella motion of the transferring methyl group (about 1220 cm^{-1}). This umbrella motion displays a larger intensity (stronger coupling) in solution than in the enzyme. The decomposition in terms of rigid and relaxed spectra shows that this motion responds to the reaction coordinate motion in

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solution while it is partially frozen in the enzyme. From 1620 to 1640 cm^{-1} we found a signal appearing only in the enzyme. This corresponds to the vibration of the peptide bonds. Finally, the large band appearing between 3100 and 3200 cm^{-1} is assigned to C−H stretching of the methyl group which, in the normal-mode analysis, appears combined to some extent with other X−H stretching motions. These motions, which display an important coupling with the reaction coordinate, cannot be described as frozen either in solution or in the enzyme.

4. Conclusions

We have shown that GH theory successfully predicts the values of the transmission coefficient (κ_{GH}) for a particular S_N2 reaction in solution and in an enzymatic environment. The agreement with the molecular dynamics estimation (κ_{MD}) is impressive, within one standard deviation. We have also estimated the performance of two different limiting behaviors: the nonadiabatic and the Kramers regimes. While the nonadiabatic estimations, in which the environment is regarded as frozen during the barrier passage, are quite reasonable (especially for the enzymatic reaction), the Kramers regime leads to a severe underestimation of the transmission coefficient.

As a general conclusion, after analyzing the contributions to the transition state friction spectra, the reaction in water presents a larger deviation of the transition state predictions because of the stronger coupling in the transition state region with low frequency motions, which remain essentially frozen during the barrier crossing. In the enzyme, analysis of the transition state friction spectra shows a minor coupling in this region, although we have been able to identify some important coupled motions at the transition state: the reorientation of the catecholate ring with the displacement of Met40, or some collective motions, such as the "breathing" of the active site. Moreover, the enzymatic reaction is better described by the nonadiabatic limit than the reaction in solution. Since the reactive frequency in solution is smaller than that in the enzyme, there are more motions able to relax within the barrier passage reaction time scale in aqueous solution. Water librations and hydrogen bond interactions with the catecholate ring are examples of motions in the transition state contributing to increase the transmission coefficient with respect to the nonadiabatic regime.

The present results can be related to the notion of environment reorganization.[13,14,21,60] It has been argued that enzymes reorganize less than aqueous solution during the reaction progress: the protein structure provides a more rigid reaction site which is previously organized to favor the reaction, while solvent molecules, which oppose the annihilation of the solute's dipole, must be strongly reoriented and translated to follow the charge flow taking place in the reaction. This different response of the environment to the reaction has two important consequences for the transmission coefficient, which in GH theory is determined in the more restricted transition state region as opposed to the reaction path. The first is that the transmission coefficient in the enzyme is closer to unity than the value in solution because the coupling in the transition state region between the environment and the reaction coordinate is larger in the latter. The second consequence is that the nonadiabatic approximation (frozen environment) works better for the enzyme than for the reaction in solution since the latter suffers more important changes during the barrier top passage, reflecting in this region the reduced changes in the reaction path transformation of reactants to products.[61] As stressed before this conclusion is drawn from the analysis of the system passage over the barrier top and thus is not directly applicable to discussion about the environment behavior during the whole reaction path. The different role played by the solvent or the enzyme favoring the transition state population is reflected in the activation free energy differences.

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