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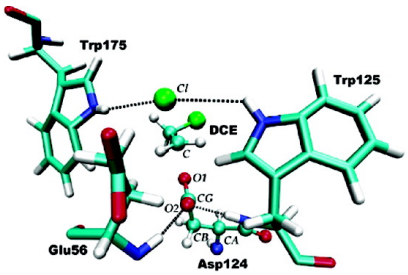

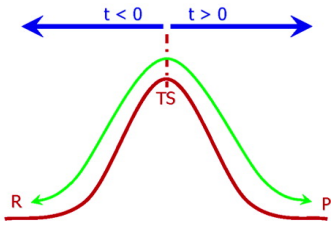

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Dynamic and Electrostatic Effects in Enzymatic Processes. An Analysis of the Nucleophilic Substitution Reaction in Haloalkane Dehalogenase

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Abstract: We present an analysis of rare event trajectories for the nucleophilic displacement of a chloride anion of 1,2-dichloroethane by a carboxylate group in haloalkane dehalogenase from *Xanthobacter autotrophicus* (DhlA) and in aqueous solution. Differences in the transmission coefficient are rationalized on the basis of the electrostatic coupling between the chemical system and the environment. Detailed analysis of the reactive trajectories reveals that the evolution of the hydrogen bond interactions established between the substrate and the environment present significant differences in aqueous solution and in the enzyme. The structure of the enzymatic active site provides a more adequate interaction pattern for the reaction progress.

1. Introduction

Enzymes are biological catalysts able to speed up chemical reactions by several orders of magnitude, making these processes compatible with life. For example, the hydrolysis of glycosidic linkages in cellulose would require several million years to reach its half-time in the absence of the appropriate catalyst, while it is easily done by some living organisms.[1] In fact, enzymes may increase the rate constants of chemical reactions in an order of magnitude of 10^6 to 10^{20}, which represent an amazing enhancement of chemical kinetics with respect to the counterpart reaction in solution. A convenient way to study the different factors contributing to the increase of the rate constant is provided by transition state theory and its variational extension.[2−4] According to this, the rate constant of a reaction, k, is related to the activation free energy $\Delta G^{\ddagger}$ obtained from the free energy difference between the reactant state and a constrained transition state defined as the maximum along a reaction coordinate:

$$k = \kappa \frac{k_B T}{h} \exp\left(-\frac{\Delta G^{\ddagger}}{RT}\right) \quad (1)$$

where T is the temperature, h is Planck's constant, k_B Boltzmann's constant, and κ is the transmission coefficient. This last factor accounts for trajectories recrossing the transition state dividing surface. In the variational version of the transition state theory, this transmission coefficient is substituted by a generalized transmission coefficient accounting not only for recrossings but also for tunneling and nonequilibrium effects.[5]

At room temperature, an increase of 10^6 in the rate constant can be attained with an activation free energy reduction of about 8 kcal/mol. In principle, this activation free energy reduction can be obtained either from transition state stabilization or from reactant state destabilization, and up to now a consensus has not been reached about the origin of the enzymatic reduction of the activation free energy.[5−9] The reduction of the activation free energy is the more important factor responsible for the rate acceleration of chemical reactions in enzymes, and hence most computational studies of enzymatic processes have been devoted to obtain and analyze the potential of mean force associated to the corresponding reaction coordinate.

Enzymatic effects on the rate constant through the transmission coefficient are much more moderate.[10] Departures from transition state theory due to recrossings can be interpreted on the basis of the generalized Langevin equation.[11] According to this, the movement of the system along the reaction coordinate is governed by the potential of mean force and a frictional random force due to the environment and the nonreactive coordinates of the reactant system.[12] The first term represents

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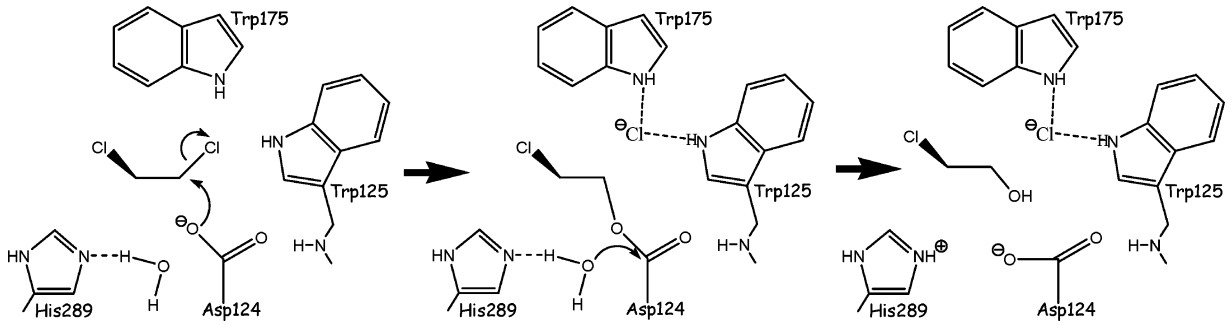
Scheme 1



the equilibrium situation between the reactant system and the environment, and the second represents the effect that enters when the environment does not have enough time to follow the reactant system during the barrier passage. This time-dependent friction $\zeta(t)$ can be obtained from the autocorrelation of the forces acting on the transition state. The net effect of this friction is to reduce the frequency of passage across the barrier region and thus the rate constant. The transmission coefficient is then determined by the ratio between the effective frequency (ω_{ef}) and the equilibrium value (ω_{eq}), determined from the potential of mean force, and it is obviously less than unity. In the nonadiabatic limit, the environment can be considered as frozen, so that the friction is completely determined by the value at the top of the barrier ($\omega_\zeta = [\zeta(\text{at } t = 0)]^{1/2}$). Transmission coefficients have been evaluated for enzymatic reactions,[13,14] and in one case it has been compared to the counterpart process in solution finding modest differences.[14] Probably for this reason, the study of reaction trajectories in enzymes is much less popular than the computation of the potential of mean force. However, this kind of studies provides valuable information not only about the dynamic effects but also about the key factors governing the change of interaction patterns along the reaction progress. Therefore, these studies can then be useful also to clarify the origin of the activation free energy reduction for the catalyzed process.

The nucleophilic substitution reaction between dichloroethane (DCE) and the carboxylate group of Asp124 in a haloalkane dehalogenase offers an excellent example to analyze dynamical effects. Haloalkane dehalogenases are a class of enzymes that catalyzes the cleavage of carbon−halogen bonds yielding the corresponding halide anion and an alcohol.[15,16] These enzymes thus provide a practical way to remove contaminants from the environment. In particular, haloalkane dehalogenase from *Xanthobacter autotrophicus* GJ10 (DhlA) catalyzes the conversion of dichloroethane to 2-chloroethanol and chloride.[17] The complete reaction takes place in two steps.[18] In the first one, dichloroethane undergoes an S_N2 displacement of a chloride anion by means of the carboxylate group of Asp124, resulting in an ester covalently bound to the enzyme. In a second process, a crystal water molecule hydrolyses the ester (see Scheme 1).

Several computational studies have been devoted to the study of the first reaction step,[19−26] and different explanations about the origin of the catalytic power of DhlA have been given. In water, the counterpart reaction between DCE and acetate takes place with a free energy barrier estimated to range between 26 and 29.9 kcal/mol,[27] while, in the enzyme, this is reduced up to 15.3 kcal/mol.[28] This means a catalytic effect between 10.7 and 14.6 kcal/mol. Following Bruice[23] the enzyme reduces the activation barrier pushing up the reactants along the reaction path. However, Warshel and co-workers[24] have quantified this effect and found that its contribution to the total catalytic effect is small. According to these authors[24,25] and our own study[26] enzyme catalysis is the result of TS stabilization relative to water solution caused mainly by electrostatic contributions. Other authors have attributed catalysis also to reactant state desolvation effects.[22] In addition, Gao and co-workers[14] have presented a dynamical study by means of downhill trajectories starting at the transition state, concluding that the transmission coefficient in the enzyme was about twice the value in solution. In this work the importance of the coupling of intramolecular vibrations with the reaction coordinate in the enzyme is stressed very clearly, while no strong electrostatic coupling in the enzyme was found. In a very recent work Warshel and co-workers[25] did not find significant differences between the dynamics of the environment in solution and in the enzyme. In addition, dynamical effects on S_N2 reactions in aqueous solutions have been thoroughly studied using different strategies[12,29−32] and then

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(27) These two values are based on extrapolations from other temperatures (Okamoto, K.; Kita, T.; Araki, K.; Shingu, H. *Bull. Chem. Soc. Jpn.* **1967**, *40*, 1912−1916) or on the use of nucleophilics parameters (see refs 22 and 24).
(28) Obtained from ref 17 using transition state theory applied to k_{cat}.

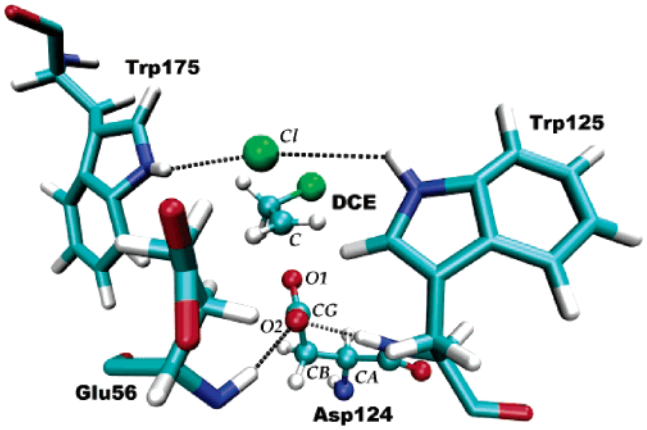

Figure 1. Representation of the enzymatic active site with the substrate (DCE) and some significant residues of the active site.

provide a good comparative framework for the enzymatic reaction.

In the present work we have made a further analysis of protein dynamic effects on the enzymatic reaction in haloalkane dehalogenase. We focus on the role played by the interactions of the chemical system with its environment, and we make a detailed comparison for the reaction in solution and in the enzyme. Within this aim a number of rare-event trajectories (12 ps) have been computed, allowing us not only to compute the transmission coefficient but also to follow the coupled dynamics of the chemical system and the environment. In agreement with Gao and co-workers study,[14] we predict a larger transmission coefficient in the enzyme than in solution, although the effect on the rate constant is very modest when compared to the reduction of the activation free energy. However, in contrast to this previous paper, the importance of electrostatic effects arising from different hydrogen bonds patterns is stressed in our work.

2. Methodology

Constructing the Systems. To perform the dynamical study of the reaction that takes place in the active site of DhlA, we need to use a realistic potential energy surface of the studied system. Because of the size of our system, we have used a quantum mechanics/molecular mechanics (QM/MM) computational scheme.[33−37] The 1,2-dichloroethane (DCE) and part of the residue Asp124 were chosen to be the QM subsystem, while the rest of the enzyme and the crystallization water molecules are in the MM subsystem. A link atom[38] was added to the QM subsystem in order to complete its valence. This link atom was placed between CA and CB atoms of Asp124 (see Figure 1). In this case, we have used the semiempirical Hamiltonian PM3 to represent the QM subsystem.[39] This Hamiltonian has been checked for this system, giving reasonable results, though systematic overestimation of the activation energy is obtained when compared to higher level estimations.[26] The MM subsystem was described using the OPLS-AA[40] potential for the enzyme and the flexible TIP3P potential for the water molecules.[41] The Lennard−Jones parameters for the QM/MM interaction are also taken from the OPLS potential, except for the QM chlorine atoms, for which we used those of ref 42. Although these Lennard−Jones parameters were originally developed for AM1/MM calculations, we tested them with the PM3 Hamiltonian by means of hybrid optimizations of chloride anion−water clusters obtaining results in better agreement with ab initio calculations than with the standard OPLS parameters. A switched cutoff radius of 12 Å was used for all kinds of interactions.

Once the potential energy function is defined we need to explore the chemical process. In the case of the enzyme, we took the X-ray crystal structure of the enzyme−substrate complex (Protein Data Bank code 2DHC)[43,44] and placed it in a cavity deleted from a 55.8 Å side box of TIP3P water molecules. As the resulting system was very large (17 154 atoms in total), we keep frozen all atoms beyond 20 Å from DCE (12317 atoms). As shown in a previous work,[26] the reaction S_N2 attack of Asp124 on dichloroethane can take place through two possible reaction paths, depending on the value of the ClCCCl dihedral angle. In this work we have centered our attention on the path with a lower activation free energy barrier. Due to the nature of the reaction, we have chosen as reaction coordinate (q) the difference between the broken and the formed bonds, that is, the C−Cl distance minus the C−O1 distance (O1 is the attacking oxygen atom of the carboxylate group while the other one is labeled as O2):

$$q = d_{\mathrm{CCl}} - d_{\mathrm{CO1}} \qquad (2)$$

For comparison purposes, we have studied also the counterpart reaction in solution, using the same QM/MM methodology. To do this, we have considered the reaction between DCE and an acetate molecule, as a model of the Asp124 residue, using the PM3 semiempirical Hamiltonian. The QM subsystem was then placed in a cavity deleted from a 31.4 Å side box of TIP3P water molecules, and periodic boundary conditions were employed. The reaction coordinate was chosen as in the enzyme (eq 2).

The potentials of mean force (PMFs) calculated for these systems gave activation free energy barriers of 28.4 and 39.6 kcal/mol in the enzyme and in aqueous solution, respectively.[26] Although these values are clearly overestimated, they correctly predict a substantial catalytic effect (a free energy barrier diminution of about 11.2 kcal/mol compared to the experimental estimation of about 10.7−14.6 kcal/mol).[27,28] Corrections by means of single-point MP2 energy calculations led to a close agreement with experimental data.[26]

Downhill Trajectories. To obtain transition state configurations corresponding to an equilibrium distribution, we ran a total of 750 ps of NVT MD simulations with the system placed at the top of the PMFs discussed above, for the system both in solution and in the enzyme. A temperature of 300 K was used all along the simulations. Details of MD simulations can be found in ref 27. To keep the system at a specific value of the reaction coordinate, we used either a SHAKE-like algorithm[45] adapted to work with internal coordinates or a restraining parabolic potential associated with the reaction coordinate.[46] One complete configuration of the system (atomic coordinates and velocities) was recorded every 5 ps. In this way, we obtained a set of 150

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independent transition state configurations that were used as the starting point for downhill trajectories in solution and in haloalkane dehalogenase.

The velocity associated with the reaction coordinate is not correctly thermalized in the selected configurations obtained during the preparatory NVT MD simulations. In principle, several strategies are possible to take an appropriate set of velocities for the initial configuration.[46] It is possible for example to assign random velocities from a Maxwell−Boltzmann distribution for all the degrees of freedom.[29] However, this approach seems to fail when applied to high-frequency vibration modes. One could in principle randomize the rotational and translational degrees of freedom only, but unfortunately the vibrational modes and the rotational motion cannot be rigorously separated. The approximation of velocity randomization that we have adopted here, already employed in ref 47, is based on the fact that the TS may be schematically represented by a structure A−B−C (i.e., A = the leaving chlorine atom, B = the CH_2CH_2Cl moiety, and C = the rest of the system). The translation velocities of the centers of mass of A, B, and C are assigned to a random value from the Maxwell−Boltzmann distribution corresponding to 300 K, preserving thus the velocities of the other degrees of freedom. In their recent contribution, Gao et al. adopted the strategy of randomizing only the reaction coordinate velocity.[14]

For each one of the selected transition state configurations (with randomized velocities), we ran downhill trajectories releasing the restraint imposed on the reaction coordinate. The equations of motion were integrated forward and backward in time until the system reached the reactant or the product states. In general, backward integration can be simply achieved using a negative time step. In practice, this was done using the same integration algorithm in both cases, but multiplying the velocities by minus one, that has the same effect.[48] We extended our trajectories from −6 ps to +6 ps. A time step of 0.5 fs was used in these simulations because large changes take place in the chemical subsystem and the NVE ensemble was employed for these trajectories. A trajectory file of each simulation is stored to perform further analysis. The trajectories can then be classified as reactive, when reactants are connected to products, or nonreactive otherwise. The latter supposes always a barrier recrossing, although the reactive trajectories can also present recrossings.

3. Results

Calculation and Analysis of the Transmission Coefficient. Trajectories have initially positive velocities in the reaction coordinate which sends them toward products at positive times ($t > 0$) and toward reactants at negative times ($t < 0$). For the total number of trajectories ran in the enzyme and in aqueous solution, a significant fraction presented recrossings of the transition state dividing surface. Few trajectories displayed more than one recrossing (5 in the enzyme and 8 in solution of a total of 150 in each case). For the rest of the trajectories the three different types of crossings observed were (i) a direct reactant−product (RP) transition with no recrossing; (ii) a single recrossing trajectory leading from reactants to reactants (RR); and (iii) a single recrossing trajectory leading from products to products (PP). In the enzyme, 85 (59%) of the total number of trajectories were of the RP type, 32 (22%), of RR type, and 28 (19%), of PP type. In solution the numbers were 60 (42%), 34 (24%), and 48 (34%) for the RP, RR, and PP types, respectively.

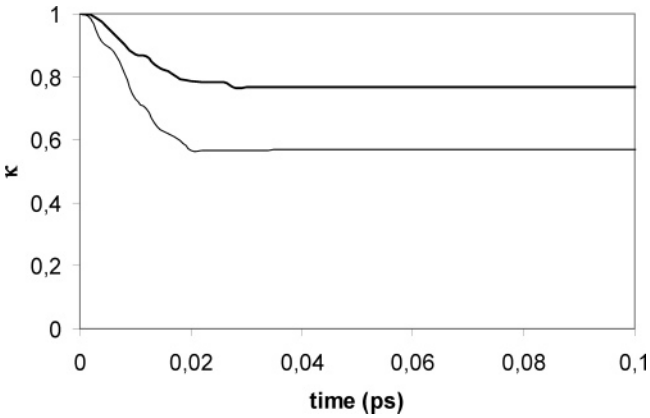

Figure 2. Time dependent transmission coefficient for the reaction in aqueous solution (normal line) and in haloalkane dehalogenase (bold line).

Because of the existence of these recrossings, the transition state theory rate constant needs to be corrected by a transmission coefficient κ, which will necessarily be less than unity. We have computed the transmission coefficient using the "positive flux"[29] 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

$$\kappa(t) = \frac{\langle j_+ \theta[q(+t)]\rangle - \langle j_+ \theta[q(-t)]\rangle}{\langle j_+ \rangle} \qquad (3)$$

where q is the reaction coordinate (the carbon−chlorine distance minus the carbon−oxygen distance), j_+ represents the initially positive flux at $t = 0$, given by $\dot{q}(t = 0)$, and $\theta(q)$ is a step function equal to one on the product side of the reaction coordinate and zero on the reactant side. The averages are taken over all the trajectories. The results obtained for the transmission coefficient in the enzyme and in aqueous solution are presented in Figure 2. The shape of $\kappa(t)$ shows a fast decay in both media. The fate of the reaction is completely defined after the first 20 fs in solution and after 30 fs in the enzyme, in good agreement with other S_N2 reactions.[12,29] After this period of time, the transmission coefficient reaches a plateau from which the values of the transmission coefficients in solution and in the enzyme can be obtained. The computed values of κ are 0.57 and 0.77, respectively. Thus, the enzyme speeds up the chemical process by not only lowering the activation free energy but also increasing the transmission coefficient. The effect on the free energy barrier lowering (around 11−15 kcal/mol) is much more important as this implies a rate constant increase by a factor of 3×10^9 to be compared to the 1.4 factor arising from the transmission coefficients. For the same system Gao and co-workers[14] obtained a κ of 0.53 for the enzymatic process and 0.26 for the uncatalyzed one. Although the absolute values differ, tendencies are the same. Part of the discrepancy with our results can be due to the different potential energy function used. In their work Gao and co-workers used a specifically reparametrized AM1 Hamiltonian and obtained significantly smaller free energy barriers (31.4 and 20.5 kcal/mol in aqueous solution and in the enzyme, to be compared, respectively, with

(46) We employed a parabolic energy penalty centered on the maximum of the PMF with different values of the force constant allowing a maximum fluctuation of the reaction coordinate of about kT, in terms of the PMF. Sets of initial configurations obtained with different values of the force constant led to the same transmission coefficient. In addition we also used constrained reaction coordinate dynamics and tested that averaged properties were the same that those obtained previously. For example, the averaged absolute gradient with respect to the reaction coordinate differed by less than 2% in the different simulations.

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39.6 and 28.4 kcal/mol in our work). A detailed analysis of the effect of the barrier height and other factors on the transmission coefficient of the S_N2 reaction is found in ref 12. Though it is difficult to conclude on the accuracy of transmission coefficient calculations, our value compares quite well with those obtained for other S_N2 processes with similar free energy barriers in aqueous solution.[12]

The existence of barrier recrossings in S_N2 reactions in aqueous solutions has been explained on the basis of the coupling between the solute and the solvent. In particular, for the symmetric $[Cl-CH_3-Cl]^-$ reaction, it has been found that the reaction outcome is largely determined by which of the two chlorine atoms is better solvated in the particular transition state configuration.[29] The questions now are what the difference is between the enzyme and the solvent and why there is a significantly smaller number of recrossings in the former. The magnitude of the solute-environment coupling can be determined from the time dependent friction coefficient, related to the random force exerted on the reaction coordinate:[49]

$$\zeta(t) = \frac{\langle FF(t)\rangle}{kT\mu} \tag{4}$$

where μ is the effective mass associated to the reaction coordinate and $\langle FF(t)\rangle$ is the autocorrelation function of the force acting on the reaction coordinate. We have obtained the initial value of the friction from simulations of the system restrained at the value of the reaction coordinate that defines the transition state, both in aqueous solution and in the enzyme. This value can be conveniently expressed as a frequency or a wavenumber just considering $\zeta(t = 0) = \omega_\zeta^2$. The values obtained for ω_ζ were 605 and 950 cm^{-1} for the enzymatic and the uncatalyzed processes, respectively. These numbers indicates a strong coupling of the reactant system with both media, but this coupling is clearly stronger in solution. One may compare these values with that obtained for the symmetric $[Cl-CH_3-Cl]^-$ reaction in solution, for which a friction of 890 cm^{-1} was predicted.[29] The recent results of Gao et al.[14] also show a stronger coupling in water than in the enzyme. Indeed, the ratio between the friction coefficients in water and in the enzyme reported by these authors show a similar tendency than in our case, although the absolute values are different.

To understand the different coupling between the reactant system and the environment in aqueous solution and in the enzyme and thus the origin of the differences in the transmission coefficient, we have turned our attention to the electrostatic effects. It has been shown that for S_N2 processes in aqueous solution the coupling arises by electrical forces associated with the charge switching between the attacking and leaving groups (the oxygen and the chlorine atoms in our case) and increases with the charge switching rate.[28,29] In Figure 3, we show the variation along the reaction coordinate of the averaged Mulliken charges on the attacking oxygen atom, the leaving chlorine atom, and the CH_2 moiety.[50] The vertical line displays the approximate position of the transition state. It can be seen that in both media the charges vary in the same way, the rate of charge-transfer

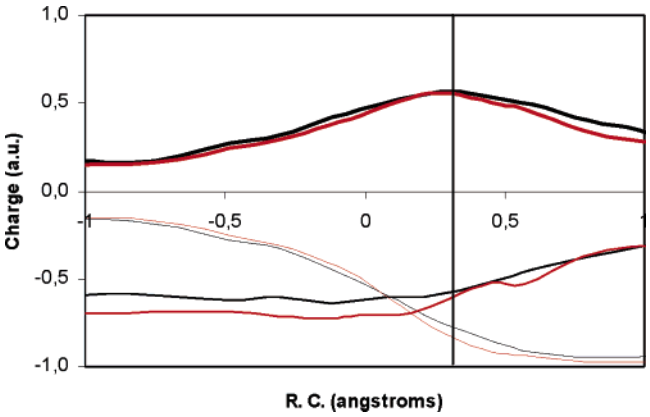


Figure 3. Evolution of the averaged Mulliken charges of the chlorine atom (dashed line), the nucleophilic oxygen (normal line), and the CH_2 group (bold line) with the reaction coordinate in solution (red) and in haloalkane dehalogenase (black).

being quite similar in both cases. In the transition state the averaged charge on the attacking oxygen (O1) and the leaving chlorine atoms are about −0.6 and −0.8 au, respectively, while for the CH_2 group it is about +0.6 au.

Then, the different coupling arising in both media is not due to differences in the chemical system but to the different response of the environment. We have evaluated this by means of the electric field created by water molecules or enzyme residues on the attacking oxygen, carbon, and chlorine atoms. This electric field is directly related to the force exerted by the surroundings on the chemical system. In addition, it depends on the local environment configuration around the reactive atoms, which has been shown to determine the reaction outcome in the symmetric S_N2 reaction in water.[51] In our work, we have chosen the component of the electric field projected along the vector defined by the leaving and attacking atoms (E_{OCl}), which is the component acting on the reaction process. Figure 4 displays a Gaussian fit to the probability distribution of E_{OCl} values obtained during a molecular dynamics simulation of the transition state in water and in the enzymatic active site. In particular, we have computed the values of this component of the electric field acting on the oxygen, chlorine, and carbon atoms. The pattern obtained in aqueous solution corresponds to a reaction field. The solvent electric field is essentially a response to the negative charge appearing on the chlorine atom, and its mean values diminish in the order Cl > C > O. The results are clearly different in the case of the enzyme, where the electric field is essentially the same on the chlorine and carbon atoms. This is due to the disposition of Trp125 and Trp175 residues: the hydrogen bonds established between the H^ϵ of these residues and the leaving chloride anion are nearly perpendicular to the $\vec{OCl}$ vector (see Figure 1). One may notice two other important differences between the electric field appearing in solution and in the enzyme which could be related to the different number of recrossings found in both media. First, in aqueous solution the difference between the mean values of the electric field on the attacking and the leaving atoms is larger than that in the enzyme. Second, the distribution of electric field

(49) Adelman, S. A. *Adv. Chem. Phys.* **1983**, *53*, 61−223.
(50) The averaged charge on the link atom was independent of the reaction coordinate and has a mean value of 0.28 ± 0.02 au. In addition we verified that the charges on the carboxylate group remained unchanged when the link atom was displaced to a more distant position increasing the size of the QM region, both in the reactant and in the transition state.
(51) In ref 29 the electrostatic potential was employed to analyze the influence of the solvent on the reaction outcome for the symmetric S_N2 process. Differences were evident when only the closest solvent molecules were considered. In our opinion this is more easily reflected by the electric field which depends on r^{-2} instead of r^{-1}.

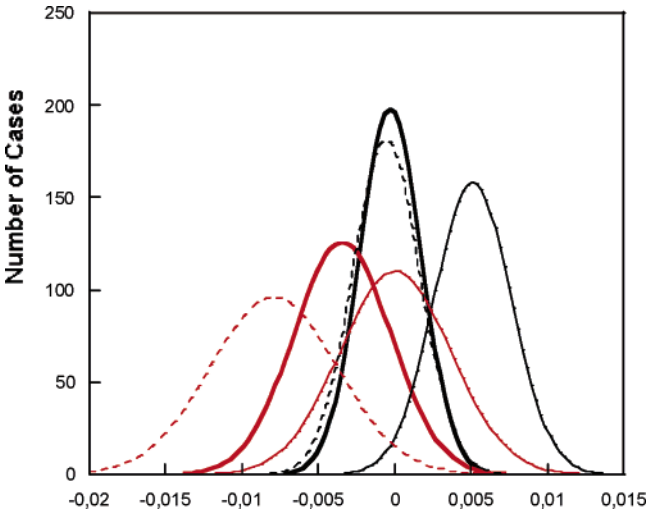


Figure 4. Probability distribution of the component of the electric field created by the environment along the OCl vector on the chlorine atom (dashed line), the nucleophilic oxygen (normal line), and the carbon atom (bold line) in solution (red) and in haloalkane dehalogenase (black).

values is also wider in solution. This means that it is more probable to find configurations with large differences in the interaction of the leaving and attacking atoms with their surroundings in water than in the enzyme. For example, in water it will be more probable to find configurations where the interaction of the chlorine atom with the environment is stronger than that for the oxygen atom, and then the trajectory starting at this configuration will lead to products irrespectively of the initial velocity along the reaction coordinate. Of course it will be also more probable to find configurations with the opposite interaction pattern leading always to reactants and consequently the transmission coefficient is smaller than in the enzyme. Water solution is more flexible than the enzyme, and it adapts to the charge distribution of the solute creating a solvent reaction field. Equilibrium fluctuations of this electric field are larger than those in the enzyme (as reflected in the wider distributions) and can lead to important departures of transition state theory predictions by means of trajectory recrossings provoked by differential stabilization of the attacking and leaving groups. Fluctuations in the enzyme are smaller, and thus it is also smaller the probability of environment-induced recrossings.

Analysis of environment-induced recrossings shows an interesting difference between solvent and enzyme reactions. We have compared the time evolution of E_{OCl} on both the leaving and the attacking atoms averaging over reactive (RP) and nonreactive (RR and PP) trajectories. Results are presented in Figure 5. The transition state defines the initial configuration ($t = 0$), while negative and positive times corresponds to the evolution toward reactants ($R{-}O^- + CH_2R'{-}Cl$) and products ($R{-}O{-}CH_2R' + Cl^-$) in a reactive trajectory (a RR trajectory evolves toward reactants irrespectively of the sign of time, while a PP trajectory always evolves toward products). It is interesting to note that, in the enzyme, reactive and nonreactive trajectories differ in the initial value and the evolution of the electric field (the component along the $\vec{O}Cl$ vector) acting on the oxygen atom but not in the electric field on the chlorine atom. A large positive electric field on the oxygen atom indicates an environmental force acting against the approach of the negative charge to dichloroethane, defining then an RR trajectory. In an RP trajectory this component of the electric field changes notably before $t = 0$. In solution we have just the opposite situation. Reactive and nonreactive trajectories clearly differ in the initial value and the evolution of the electric field acting on chlorine atom. In this case the negative electric field corresponds to a good solvation of the leaving chloride anion and thus to an environmental force against the approach of the anion to the ester formed. Thus, recrossings in the enzyme seem to be essentially due to fluctuations in the interactions of the environment with the attacking oxygen atom, while in solution these seem to be also due to changes in the solvation of the leaving group. This result indicates a different mixture of reacting system and environmental coordinates in the enzyme and in solution that can contribute to the differences found in the transmission coefficient. As we shall discuss below this can be related to the

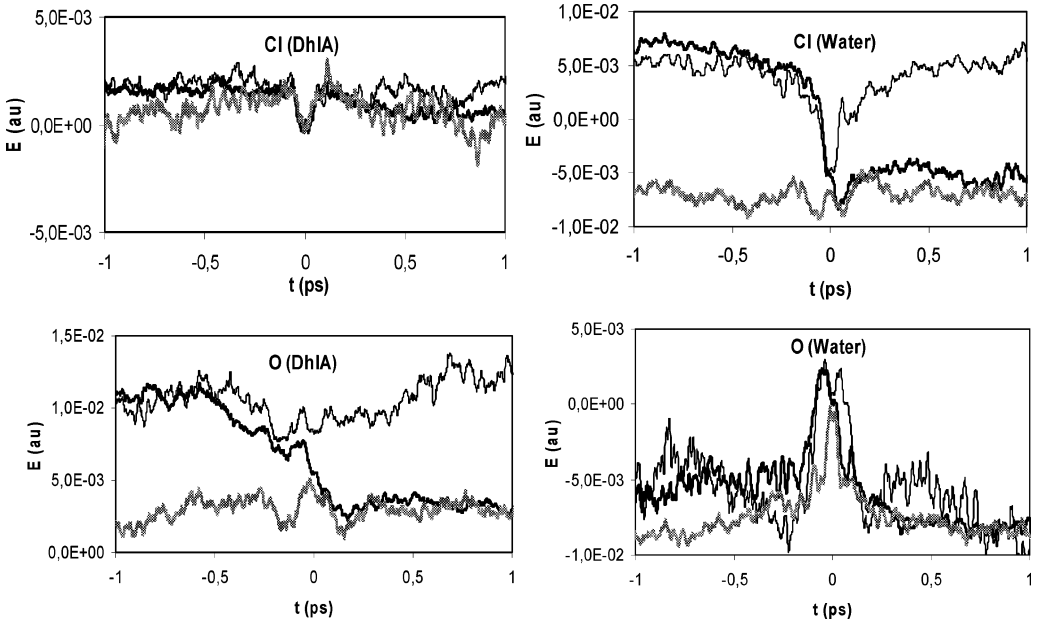


Figure 5. Averaged time evolution of the of the component of the electric field created by the environment along the OCl vector on the chlorine and oxygen atoms in aqueous solution and in the enzyme. Bold, normal, and gray lines correspond to averaged values over RP, RR, and PP trajectories.

Table 1. Wave Numbers Associated with the Reaction Dynamics (Equilibrium, Friction at $t = 0$ and Nonadiabatic Limit) in cm^{-1} and Transmission Coefficients Calculated from Molecular Dynamics and in the Nonadiabatic Limit

	ω_{eq}	ω_{ζ}	ω_{na}	κ_{MD}	κ_{na}
solution	1150	950	650	0.56	0.57
enzyme	1050	605	860	0.77	0.82

different evolution of the hydrogen bonds interaction pattern between the solute/substrate and the environment in aqueous solution and in the enzyme.

Interesting differences between the dynamics in solution and in the enzyme are also evidenced when comparing the magnitude of friction and the equilibrium forces in both media. At $t = 0$, the friction in solution is about 950 cm^{-1} and 605 cm^{-1} in the enzyme. The equilibrium frequency, derived from the curvature of the potential of mean force at the top of the barrier, is 1150 and 1050 cm^{-1} in solution and in the enzyme, respectively. From these values we can obtain the force acting on the reaction coordinate assuming that the environment is completely frozen, that is, in the nonadiabatic regime.[12] In this case the friction is always equal to the $t = 0$ value, and then the net force, expressed as a frequency, is

$$\omega_{na}^2 = \omega_{eq}^2 - \omega_{\zeta}^2 \quad (5)$$

From this nonadiabatic frequency of passage over the barrier top, we can estimate the nonadiabatic limit to the transmission coefficient.[52] Results are summarized in Table 1. As it can be seen, the transmission coefficient obtained from molecular dynamics rare event simulations is slightly lower than the value obtained in the nonadiabatic limit. This could be due to dynamical effects in the rearrangement of the environment around the other oxygen atom of the carboxylate group as the reaction advances. As we shall discuss in the following section, environment interactions with this atom play also a fundamental role in the reaction progress, but these effects are not included in our estimation of the friction as far as the coordinates of this atom are not included in the definition of the reaction coordinate.

Analysis of Reactive Trajectories. In this section we analyze the differences between reactive trajectories in aqueous solution and in the enzyme in order to understand the molecular mechanism of catalysis. For this purpose, we have averaged over the reactive trajectories in both media.

Figure 6 shows the evolution of the most important atomic distances during the reaction: the bond breaking distance (CCl), the bond forming distance (OC), and the distance between the attacking and leaving groups (OCl). In both media the description of the reaction dynamics is quite similar. There are four stages common to the chemical reaction both in the enzyme and in water: (i) an activation of the chlorine−carbon bond reflected in the oscillations of its distance starting at about −4 ps (not presented in the figure);[31] (ii) the approach of the reactant fragments reflected in the diminution of the OCl distance, which takes place between −130 and −40 fs, when the OCl distance diminishes from 5 to 4.2 Å; (iii) the transfer of the methyl group from the leaving chlorine atom to the attacking oxygen one, which requires about 80 fs (from the last CCl vibration at about −40 fs to the first CO vibration at +40 fs) and can be seen as

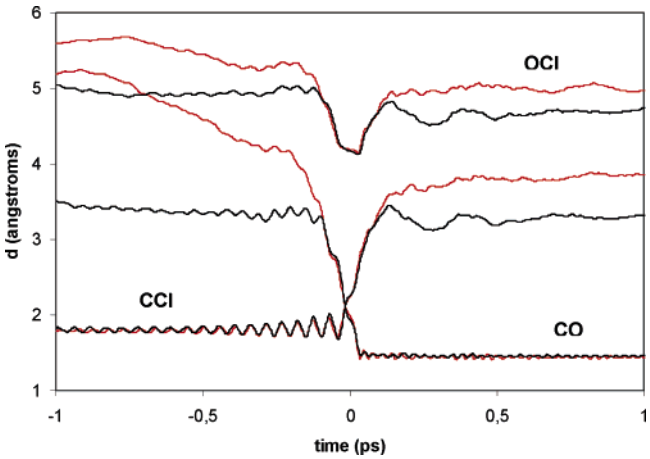


Figure 6. Time evolution of the most significant atomic distances in aqueous solution (red line) and in haloalkane dehalogenase (black line) averaged for the reactive trajectories. The distances are named on the plot.

a positive charge moving from one negatively charged center to the other [O^-- -C^+- -Cl^-]; (iv) the departure of the leaving group once the new carbon−oxygen bond has been formed, taking place roughly from +40 to +140 fs, when the OCl distance has been lengthened up to 5 Å.

Obviously, we have observed differences between both media for longer reaction due to the diffusion of the reactants or products fragments in water. As a consequence of this diffusion process, the carbon−oxygen distance in water does not present an oscillatory behavior in the reactant state ($t < 0$), which is clearly apparent in the enzyme.

Let us now discuss the dynamical behavior of the environment. As far as the electrostatic interaction is determined in the evolution of the reaction, we have focused our attention on the hydrogen bonds established between the reactant system and the environment. In aqueous solution we have computed the number of hydrogen bonds established between the different atoms of the reactants and water molecules and also the averaged shortest distance between the solute's atoms and water hydrogen atoms. In particular we have focused on the hydrogen bonds established with the attacking oxygen atom (O1), the leaving chlorine atom (Cl), and the other oxygen atom of carboxylate (O2). The hydrogen bonds have been defined using a simple criterion based on the distance between participating atoms.[53] Figure 7a displays the number of hydrogen bonds as a function of time, averaged for the reactive trajectories, while Figure 7b shows the time evolutions of the averaged shortest distance. In the reactant state, we find approximately 3.6 hydrogen bonds between water molecules and the carboxylate oxygen atoms (O1 and O2) and 1.9 for the chlorine atom. In the product state, the averaged number of hydrogen bonds established by O1, O2, and Cl are 0.3, 1.6, and 6.5 approximately. When the transition state is reached, the averaged number of hydrogen bonds of the chlorine atom has to be increased up to 4.5. In contrast, to climb up the barrier, both oxygen atoms must be desolvated, reducing the number of hydrogen bonds up to 1.2 for the attacking oxygen atom (O1) and 3.0 for the other one (O2). In average, the system has lost about half hydrogen bond when

(52) Zwan, G.; Hynes, J. T. *Chem. Phys.* **1984**, *90*, 21−35.

(53) The criterion was the distance of the first minimum of the radial distribution function for acetate and chloride anions in water as given in refs 54 and 55, respectively. These distances can lead to a slight underestimation of the coordination number for the neutral atoms.

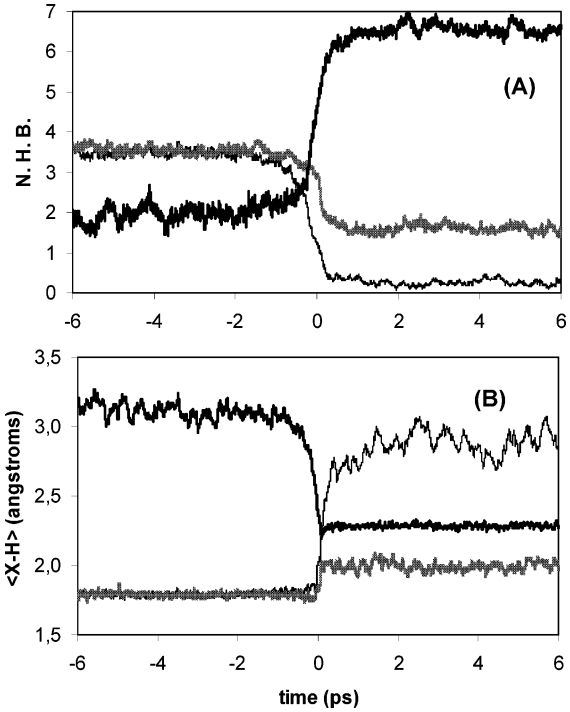


Figure 7. Time evolution of the number of hydrogen bonds established among chlorine atom (bold line), O1 (normal line) and O2 (grey line), and water molecules averaged over reactive trajectories in solution. (A) Time evolution of the averaged number of hydrogen bonds (see ref 53). (B) Time evolution of the averaged shortest distance between the QM atom and a hydrogen atom of solvent molecules.

reaching the transition state and then the energy barrier is consequently larger than in the gas phase. If we look at the distances we can see that the evolution of hydrogen bond interactions follow the charge flow from acetate to chlorine. As the acetate anion loses its initial negative charge, hydrogen bond interactions are weakened and distances are consequently lengthened. For the chlorine atom we have the opposite evolution because it becomes more charged as the reaction advances, and then the interaction is strengthened and the distances shortened. Accordingly, solvation of the chlorine atom appears to be a major driving force for the reaction in solution while desolvation of acetate anion hinders the process. Figure 7 also illustrates the dynamical evolution of these hydrogen bonds. It can be seen that changes in the solvation of these atoms start as soon as 1−2 ps prior to the passage over the barrier top. This suggests an important solvent fluctuation preceding the bond breaking and forming process, and it is consistent with the existence of water reorientation motions in a similar time scale.[56,57]

In the enzyme the two carboxylate oxygen atoms can, in principle, establish hydrogen bond interactions with the H^N atoms of Glu56 and Trp125, while the leaving chlorine atom can form hydrogen bonds with the H^ϵ atoms of Trp125 and Trp175 (see Figure 1). Figure 8 shows the averaged evolution of the corresponding distances for O1 (8a), O2 (8b), and Cl (8c). A comparative analysis of the evolution of hydrogen bonds

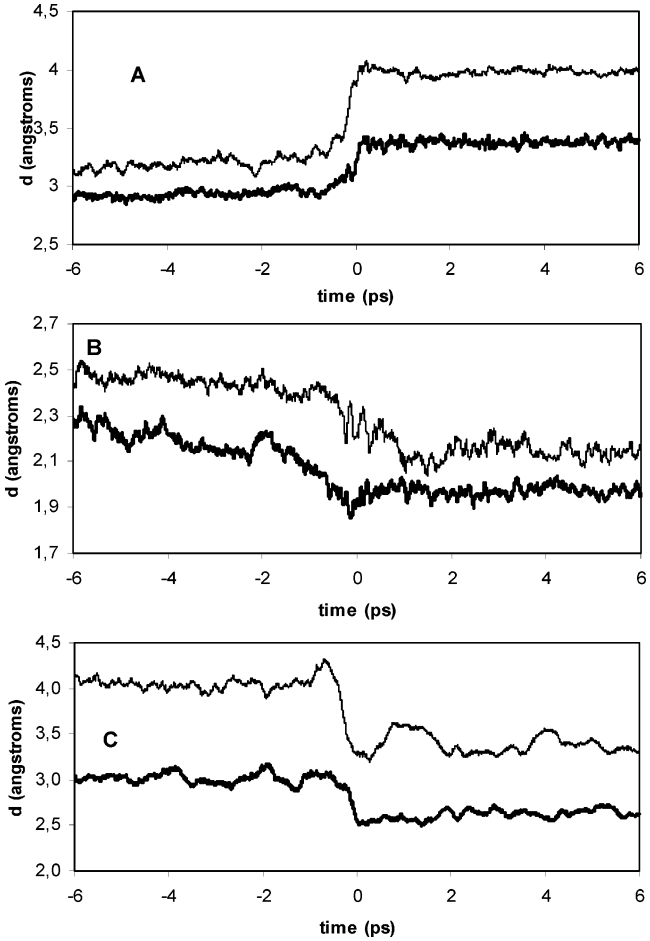


Figure 8. Time evolution of hydrogen bond distances averaged over reactive trajectories in haloalkane dehalogenase. (A) Distances between the nucleophilic oxygen (O1) and H^N atoms of Glu56 (normal line) and of Trp125 (bold line). (B) Distances between the O2 oxygen atom and H^N atoms of Glu56 (bold line) and of Trp125 (normal line). (C) Distances between the leaving chloride anion and H^ϵ of Trp125 (normal line) and Trp175 (bold line).

in the enzyme and in water solution can be very useful to understand the origin of one important contribution to catalysis. For O1 and Cl the evolution of the interactions with polar hydrogen atoms during the enzymatic reaction is similar to what we found in solution. The leaving chlorine atom establishes shorter interactions in the product state than in the reactant state, although the effect becomes evident only after $t = -600$ fs. As the absolute charge of this atom is also increased, we expect a favorable contribution of the interaction of this atom with the environment to the reaction progress. For the attacking oxygen atom (O1) the distances to the amide hydrogens are lengthened as this atom approaches dichloroethane, just as in aqueous solution. The main difference with respect to the reaction in solution concerns the other oxygen atom (O2), which establishes shorter interactions with the H^N atoms as the reaction advances. We also observed this behavior comparing the averaged H^N−O2 distances obtained from 100 ps MD simulations of the transition and reactant states. Hydrogen bond distances of the O2 atom follow opposite trends in solution and in the enzyme, and thus, in this last environment, these interactions do not hinder the reaction progress as much as in solution, making a contribution to catalysis. The shortening of the hydrogen bond distances can partly compensate the weakening of the interaction

(54) Jorgensen, W. L.; Gao, J. *J. Phys. Chem.* **1986**, *90*, 2174−2182.
(55) Tuñón, I.; Martins-Costa, M. T. C.; Millot, C.; Ruiz-López, M. F. *Chem. Phys. Lett.* **1995**, *241*, 450−456.
(56) Mosyak, A. A.; Prezhdo, O. V.; Rossky, P. J. *J. Chem. Phys.* **1998**, *109*, 6390−6395.
(57) Balbuena, P. B.; Johnston, K. P.; Rossky, P. J.; Hyun, J. K. *J. Phys. Chem. B* **1998**, *102*, 3806−3814.

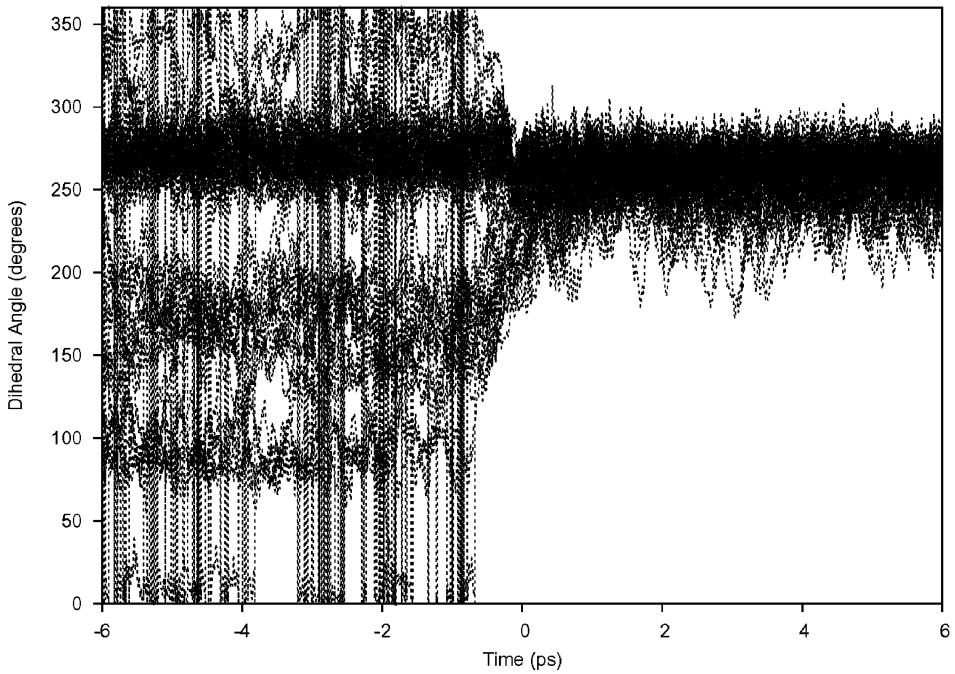


Figure 9. Evolution of the O2−CG−CB−CA dihedral angle (see Figure 1) versus time for all the reactive trajectories in haloalkane dehalogenase.

due to the loss of negative charge on the acetate anion as the reaction advances.

To understand this surprising evolution of the H-bond interactions of the O2 atom in the enzyme, we have analyzed the individual reactive trajectories. The change of the averaged hydrogen bond distances established by O1 and O2 atoms in the enzyme is the result of different types of trajectories. At $t = -6$ ps, 32% of the reactive trajectories presented a hydrogen bond between O1 and the amide group of Trp125, and 22%, with the amide group of Glu56. At $t = 0$, this is when the transition state is reached, only 10% of the structures showed a hydrogen bond between O1 and the amide group of Trp125, and none, with Glu56. This desolvation of the attacking oxygen is assisted by the solvation of the O2 atom. At $t = -6$ ps, 75% of the reactive trajectories presented a hydrogen bond between O2 and the amide group of Glu56, and 63%, with the amide group of Trp125 (obviously, in some cases the O2 atoms displays short distances with both amide groups). At $t = 0$, this is when the transition state is reached, and the percentages are increased up to 97% and 69%, respectively. The reason for this compensation between the hydrogen bonds of O1 and O2 is that the positioning of the attacking oxygen with respect to the dichloroethane is essentially accomplished by means of a rotation of the carboxylate group around the CG−CB bond (see Figure 1). When this bond is rotated to place the O1 atom close to dichloroethane, the O2 atom is better exposed to the polar hydrogen atoms of Glu56 and Trp125 amide groups. The evolution of the dihedral angle that defines the rotation around this bond (O2−CG−CB−CA) is shown in Figure 9 for all the reactive trajectories. When the transition state is reached, all the trajectories present a dihedral angle close to 250°, which is also kept for positive reaction times. However, in the reactant state ($t < 0$), we can observe different behaviors. Most of the cases have a dihedral angle close to 270°, but the carboxylate group can rotate around the C_G-C_B bond and other conformations (0°, 90°, and 180°) present a significant population. Then, although the evolution of these hydrogen bonds could seem to be strange as far as they are shorter when the O2 atoms loses negative charge, the reason of this behavior is found in the structure of the enzyme, which is prepared to accommodate the reaction transition structure. To avoid the shortening of the hydrogen bonds established with the O2 atom in the transition state the enzyme structure should be deformed with an associated energy cost. Obviously, the behavior is completely different in aqueous solution as water molecules initially hydrogen-bonded to O2 can establish new interactions with other solvent molecules. Thus, the O2 atom plays a completely different role in solution and in the enzyme. In solution, this atom presents also an important desolvation at the top of the barrier, and thus this means an energy penalty to reach the transition state. In the enzyme, when the attacking O1 atom approaches the substrate, the carboxyl group rotates and then the O2 atom can form shorter hydrogen bonds than those in the reactant state and then the energy penalty is reduced. To quantify this effect we have evaluated the contribution of the hydrogen bonds of the O2 atom to the energy barrier in aqueous solution and in the enzyme by means of PM3/MM calculations including only those water molecules or amino acids directly hydrogen-bonded to this atom.[58] In aqueous solution the desolvation of this atom means a loss of 12 kcal/mol in interaction energy, while in the enzyme this amounts to 4 kcal/mol. This means that the different evolution of the hydrogen bonds of the O2 atom in the enzyme with respect to the aqueous solution makes a catalytic contribution of 8 kcal/mol in interaction energy. The importance of these hydrogen bonds on the catalytic process has also been previously suggested from X-ray crystallographic data.[43,44]

We have also found a contribution to catalysis during the positive methyl group transfer. As we discussed above, from

(58) The averaged interaction energies were obtained from 10 000 structures taken from MD simulations of the transition and reactant states in aqueous solution and in the enzyme. In these calculations we included all the QM region and those water molecules or residues presenting a hydrogen bond with the O2 atom. In the case of the enzyme the side chains of residues Glu56 and Trp125 were not considered in order to avoid the interaction with the chlorine atom.

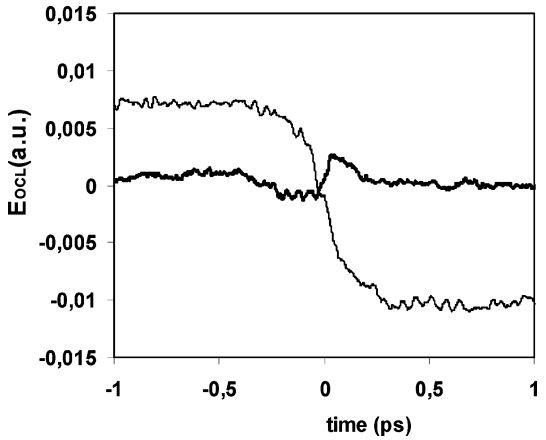
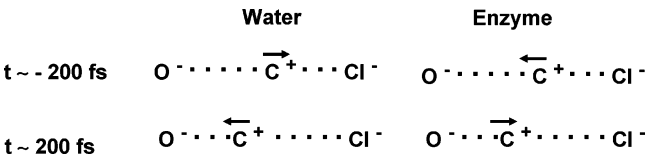


Figure 10. Time evolution of the component of the electric field created by the environment on the attacked carbon atom along the OCl vector, averaged for reactive trajectories in aqueous solution (normal line) and in haloalkane dehalogenase (bold line).

−40 to +40 fs the distance between the attacking and the leaving groups remains essentially constant (at a mean value of 4.2 Å) and, in this period, the reaction can be seen as the movement of a positively charged particle from one negative center (the chlorine atom) to another (the O1 atom). According to the charges showed in Figure 3 the chemical system can be described at this step approximately as [O$^-$−C$^+$−Cl$^-$]. In solution, the electrostatic reaction field of the solvent hinders this transfer. When the carbon atom is still bonded to the chlorine atom it experiences a solvent-induced force against the transfer to the oxygen atom. Once the barrier top has been passed over, the force acts in the opposite sense; this is against the transfer toward the chlorine atom. Then, we have an electrostatic contribution that destabilizes the transition structure. This solvent electrostatic effect can be seen in Figure 10 where the component of the solvent electric field along the $\vec{\text{O}}$Cl vector acting on the carbon atom is presented. This component of the field is positive on the reactant side ($t < 0$) and negative on the product side ($t > 0$). The solvent field in aqueous solution favors the reactant and product states where the charge is more localized ([OC- -Cl$^-$] or [O$^-$- -CCl]) than in the transition state. In the same Figure 10 we also present the component of the electric field acting on the carbon atom in the enzyme. Here we can observe a very different behavior of the electric field. At long times this component of the electric field is close to zero on both the reactant side and the product side. However, about 200 fs before the passage over the barrier top, there is a subtle change in the electric field created by the environment. At $t \approx -200$ fs, the electric field changes and it becomes slightly negative. This component of the electric field acts as a force on the positively charged carbon atom favoring the displacement toward the nucleophilic oxygen. If we look at the behavior on the opposite side, we find similar features. Up to $t \approx 200$ fs, the electric field is slightly positive and then it favors the movement of the positive charge toward the chlorine atoms. Thus, in the proximity of the passage over the barrier top, the structure of the enzyme creates an electrostatic force that effectively stabilizes this transition state. The different behaviors of the forces exerted by the surroundings on the transferred methyl group near the passage over the top in aqueous solution and in the enzyme are shown in Scheme 2.

Scheme 2



In terms of dynamical contributions to the transmission coefficient, this enzymatic electric field reduces the friction of the environment as compared to water solution, where the electric field always acts against the passage of the system over the barrier top, and then it could be one of the important terms contributing to the differences found in the friction between the solvent and the enzyme.

To confirm the discussion about the different evolution of the interaction patterns for the reaction in water and in the enzyme, we have evaluated the contribution of the QM/MM interaction to the energy barrier ($\Delta E^{\ddagger}_{\text{QM/MM}} = E^{\text{TS}}_{\text{QM/MM}} - E^{\text{RS}}_{\text{QM/MM}}$) in the enzyme and in solution using 1000 structures belonging to the transition and reactant states. In water solution this contribution amount to up 36 ± 8 kcal/mol, while in the enzyme it is only 4 ± 4 kcal/mol. This means that in the enzyme the substrate loses much less interaction energy than in solution when it evolves from the reactant to the transition state, and thus the energy barrier is expected to be smaller. Of course it should be taken into account that in water a large fraction of the interaction energy cost is recovered as solvent−solvent interaction energy. This is so because as solvent molecules break interactions with the solute they can establish new interactions with other solvent molecules (in continuum models the free energy of polarizing the environment is one-half of the interaction term). In the enzyme, the fraction of energy spent or recovered changing the environment according to the changes in the charge distribution during the reaction progress is expected to be significantly smaller, as far as the active site is prepared to accommodate the transition structure.[59,60] Thus, in a very crude way, neglecting changes in the QM energy, we could estimate the catalytic effect coming from these calculated interaction energies as

$$\Delta\Delta E^{\ddagger} = \Delta E^{\ddagger}_{\text{w}} - \Delta E^{\ddagger}_{\text{e}} \approx \frac{1}{2}\Delta E^{\ddagger}_{\text{QM/MM,w}} - \Delta E^{\ddagger}_{\text{QM/MM,e}} = 18 - 4 = 14 \text{ kcal/mol} \quad (6)$$

The good agreement with the catalytic effect obtained from PMFs calculations[26] and experimental estimations[27,28] gives us some confidence in our interaction energies and our analysis of the origin of catalysis in DhlA. The values provided for the interaction energies obviously mean that the catalytic effect is not due to a stronger interaction of the enzymatic transition state with respect to the enzymatic reactant state. However, using only interaction energies, we cannot conclude if the origin of catalysis is due to a destabilization of the reactant state[22] or to a transition state stabilization[24] with respect to the in solution reaction. Other energetic terms must be considered to complete an adequate thermodynamic cycle. This analysis has been very

(59) (a) Warshel, A. *P. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 5250−5254. (b) Warshel, A. *J. Biol. Chem.* **1998**, *273*, 27035−27038.
(60) In chorismate mutase, we evaluated this fraction as being less than 10%. Martí, S.; Andrés, J.; Moliner, V.; Silla, E.; Tuñón, I.; Bertrán, J. *Chem.−Eur. J.* **2003**, *9*, 984−991.

recently carried out for this reaction by Warshel and coworkers[25] using a linear response approximation treatment concluding that the transition state is better "solvated" by the protein than in solution. In the analysis presented in that work, catalysis is mainly attributed to the large differences in the reorganization energy between the aqueous and the enzymatic environments.[61] According to these authors the relative contributions to the activation free energy due to the "reorganization" of the environment[62] (which accounts also for the change of the interactions with the substrate) are ~17 and 5 kcal/mol in aqueous solution and in the enzyme, respectively. In this paper, we have stressed the microscopic evolution of the interactions established by the substrate with its surroundings to analyze the origin of catalysis. However, the energetic differences proposed in ref 25 to explain catalysis compare very well with our estimations, given in eq 6, based on the balance of the interaction energies between the reactant and the transition states and the fraction of energy invested in or recovered from the environment in both media.

4. Conclusions

The amazing ability of enzymes to catalyze chemical reactions is attained by a substantial reduction of the activation free energy. This effect is clearly stressed when the potential of mean force of the catalyzed reaction is compared to the counterpart process in solution. In the case of the nucleophilic displacement of a chloride anion in 1,2-dichloroethane catalyzed by DhlA, our previous calculations[26] predicted a reduction of the activation free energy of about 11−13 kcal/mol which means an increase of the rate constant in a factor of 10^8-10^9 relative to the uncatalyzed reaction. Obviously, the effect of the enzyme on the rate constant through changes in the transmission coefficient is expected to be much more modest, and this can be one of the reasons to explain why PMF's calculations are much more popular than the study of reactive trajectories.

We have here presented an analysis of rare event trajectories for the nucleophilic displacement of a chloride anion of 1,2-dichloroethane by a carboxylate group in DhlA and in aqueous solution. These trajectories are started at a transition state configuration obtained from an equilibrium distribution with the system placed at the top of the respective PMFs. As it has been discussed, analysis of these trajectories provides valuable information not only about changes in the transmission coefficient but also on the microscopic details of the reduction of the activation free energy in the enzyme. With respect to the first subject, and in agreement with a recent similar study,[14] we have found that the transmission coefficient in the enzyme is larger than that in water, contributing, modestly, to the acceleration of the chemical process. The origin of the differences in the transmission coefficient has been analyzed in terms of the different electrostatic couplings between the chemical system and the environment in both media. The enzyme is less flexible than water solution, and then equilibrium fluctuations of the electric field acting on the leaving and the attacking groups are smaller. Thus it will be less probable to find large differences in the solvation of these groups with respect to the equilibrium situation in the enzyme, and then it will also be less probable to find recrossing trajectories. The nonadiabatic limit provides a reasonable approximation for the calculation of the transmission coefficient.

From the detailed analysis of the reactive trajectories, we have also found interesting differences that can explain the way in which the enzyme attains a large diminution in the activation free energy. In aqueous solution the reaction requires the desolvation of the two oxygen atoms of the carboxylate group as the system advances toward the transition state, while this climbing is assisted by the formation of an increasing number of hydrogen bonds with the leaving group (the chlorine atom). In the enzymatic process the changes taking place in the interactions established between the substrate and the environment during the reaction progress are different. While the chlorine atom also forms stronger hydrogen bonds as the reaction goes from the reactant state to the product state, a striking difference is found in the desolvation of the attacking group. Effectively, in the enzyme the correct alignment of the nucleophilic oxygen is attained by means of a rotation around the C_G-C_B bond. This rotation implies the desolvation of the attacking oxygen, just as in water solution. However, this desolvation is now assisted by the formation of shorter hydrogen bonds with the other oxygen atom of the carboxylate group, an effect which is expected to partly compensate the loss of charge in the acetate anion. Then we have an additional contribution for the reaction in the enzyme that can explain the lower activation free energy. In other words, the enzyme provides an effective electrostatic transition state stabilization when compared to the in solution process, due to the different hydrogen bonds pattern. An additional difference has been found for the movement of the positively charged methyl group between the chlorine and the oxygen atoms just during the pass over the top of the barrier. In this case, the enzymatic electric field seems to be designed to stabilize the transition state charge distribution $[O^-\text{- -}C^+\text{- -}Cl^-]$, while the solvent reaction field obviously acts favoring a charge localized configuration ($[OC\text{- -}Cl^-]$ or $[O^-\text{- -}CCl]$). As a result, when the reaction takes place in the enzyme the evolution of the substrate from the reactant to the transition states involves a significantly smaller cost in interaction energy, making this term a decisive contribution to catalysis.

As a general conclusion, the analysis of detailed trajectories for a particular enzymatic process does not provide only information about dynamical effects (transmission coefficients), but it is a complementary tool to the study of the PMF. The detailed description of the evolution of the chemical system and of the environment is essential to understand the origins of enzymatic catalysis as it allows us to follow the changes that take place in the chemical system and in its surroundings.

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(61) In ref 25 the solvent reorganization energy (defined from the diabatic curves corresponding to the product and reactant states) is calculated to be considerably smaller in the enzyme than in solution (being both positive quantities that contribute to increase the free energy barrier). In our approach, the change in the environment energy when passing from the reactant state to the transition state ($\Delta E^{\ddagger}_{MM}$) favours the reaction in solution for this process where one moves from a charge-localized reactant state to a charge-delocalized transition state in which the solvent structure is less distorted. Note that both concepts are different as the reorganization energy also accounts for changes in the interactions.

(62) Estimated as $^1/_4$ of the reorganization energy.

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