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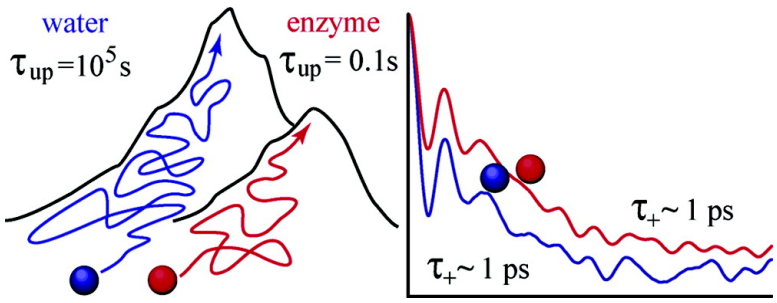

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Solute Solvent Dynamics and Energetics in Enzyme Catalysis: The S_N2 Reaction of Dehalogenase as a General Benchmark

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Abstract: One of the most useful ways of describing and analyzing enzyme catalysis is the description of the enzyme as an effective solvent for the reacting substrate. Here, we illustrate this concept by considering the S_N2 reaction of haloalkan dehalogenase (DhlA), analyze the energetics and dynamics of the solvent coordinate, and evaluate their relative catalytic effect. It is demonstrated that almost the entire catalytic effect is associated with the preorganization of the protein−solvent coordinate. It is clarified that this effect is associated with the fact that the transition state is "solvated" by the protein more than in the reference solution reaction. This effect is fundamentally different than the frequently proposed desolvation mechanism. The possible catalytic role of dynamical effects is analyzed by considering several reasonable ways of defining "dynamical contributions to catalysis". It is found that these contributions are small regardless of the definition used. It is also shown that the effect of the difference in the relaxation time of the solvent coordinate in the enzyme and solution reaction is rather trivial relative to the effect of the corresponding changes in reorganization free energy.

I. Introduction

Many proposals have been put forward to rationalize the origin of the large catalytic power of enzymes. Unarguably, this is a complex issue where many factors may be assumed to be important. Thus, it is important to find out which proposals account for the major effect in enzyme catalysis.[1−3] Our previous simulation studies have indicated that the major effect to enzyme catalysis comes from the preorganization of the protein environment, where the enzyme plays a role of a super solvent with smaller reorganization energy than the corresponding reaction in aqueous solutions.[4] This view has gained current support, e.g., refs 5 and 6, but it appears that the view of the enzyme as a solvent is still a concept that requires significant clarification. This work will reexamine the issue of enzyme catalysis in terms of general solvation concepts and uses one of the best-studied chemical reactions, namely the S_N2 reaction, to clarify some significant misunderstandings.

The S_N2 reaction is one of the simplest reactions and has also been one of the best studied reactions, see, e.g., references in ref 7. This reaction reveals an interesting solvent effect where the change of solvation energy along the reaction coordinate constitutes one of the main contributions to the large activation barrier. The reaction has been a benchmark for studies of solvation dynamics,[8−10] including nonequilibrium solvation effects,[3,9,10] and has also been used to define and discuss chemical reactivity in terms of solute/solvent reaction coordinate.[10] Thus, consideration of an enzymatic S_N2 reaction should provide an excellent opportunity to examine the energetics and dynamics of solvation effects. The present investigation uses the S_N2 step in the reaction of haloalkan dehalogenase (DhlA) as a benchmark to study solvation effects in enzyme catalysis. In particular, the desolvation proposal and the more recent dynamical proposal (see below) are addressed, whereas the closely related near attack conformation (NAC) proposal (which has been the subject of previous DhlA studies[11−13]) is left out since it recently has been examined extensively elsewhere.[14]

The role of solvation and desolvation in catalyzing enzyme reactions has been the subject of many studies; see, e.g., refs 15−17. The popular idea that enzymes work by desolvating their reactants has been shown to be problematic by Warshel and co-workers,[18,19] who pointed out that calculations that

(1) Borman, S. Much ado about enzyme mechanisms. *Chem. Eng. News* **2004**, *82*, 35−39.
(2) Warshel, A. *Computer Modeling of Chemical Reactions in Enzymes and Solutions*; John Wiley & Sons: New York, 1991.
(3) Villà, J.; Warshel, A. Energetics and dynamics of enzymatic reactions. *J. Phys. Chem. B* **2001**, *105*, 7887−7907.
(4) Warshel, A. Energetics of Enzyme Catalysis. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 5250−5254.
(5) Cannon, W. R.; Benkovic, S. J. Solvation, reorganization energy, and biological catalysis. *J. Biol. Chem.* **1998**, *273*, 26257−26260.
(6) Roca, M.; Marti, S.; Andres, J., et al. Theoretical modeling of enzyme catalytic power: Analysis of "cratic" and electrostatic factors in catechol O-methyltransferase. *J. Am. Chem. Soc.* **2003**, *125*, 7726−7737.
(7) Shaik, S. S.; Schlegel, H. B.; Wolfe, S. *Theoretical Aspects of Physical Organic Chemistry. Application to the SN2 Transition State*; Wiley-Interscience: New York, 1992.
(8) Gertner, B. J.; Bergsma, J. P.; Wilson, K. R.; Lee, S. Y.; Hynes, J. T. Nonadiabatic Solvation Model for S_N2 Reactions in Polar-Solvents. *J. Chem. Phys.* **1987**, *86*, 1377−1386.
(9) Gertner, B. J.; Wilson, K. R.; Hynes, J. T. Nonequilibrium Solvation Effects on Reaction-Rates for Model S_N2 Reactions in Water. *J. Chem. Phys.* **1989**, *90*, 3537−3558.
(10) Hwang, J. K.; King, G.; Creighton, S.; Warshel, A. Simulation of Free-Energy Relationships and Dynamics of S_N2 Reactions in Aqueous-Solution. *J. Am. Chem. Soc.* **1988**, *110*, 5297−5311.

seemed to support this idea have been based on an incorrect thermodynamic cycle; see the discussion in ref 2. It has been demonstrated that, rather, enzymes work by solvating their transition state (TS) more than water does. Nevertheless, the idea that enzymes work by desolvation effects reappears in the scientific literature in different forms every now and then. The most recent version has been provided in a study of haloalkene dehalogenase (DhlA) by Devi-Kesavan and Gao.[20] This study is of particular interest in view of the fact that it addresses an S_N2 reaction, which is one of the simplest and most well-defined chemical reactions. Furthermore, the activation barrier of this reaction in water is modulated by a major solvation effect, thus making it an ideal system to study the desolvation proposal.

The idea that dynamical effects play a major role in enzyme catalysis has also been put forward repeatedly.[1,21−31] Early simulation studies[32,33] indicated, however, that they do not contribute to catalysis. In particular, it was found that the dynamical effects in the enzyme and in the uncatalyzed reference solution reaction are similar and that the difference in the rate constants is determined by the difference between the activation free energies. In subsequent studies, it was confirmed that the transmission factor, which is one of the primary measures of dynamical effects, is similar in enzymes and solution.[2,3,34,35] However, some controversy remains with regard to the nature of other measures of dynamical effects and their role in catalysis. For example, a recent work of Nam et al.[36] agreed that the catalytic rate of DhlA is not due to dynamical effects but concluded that the dynamics in the enzyme and the reference solution reaction are very different and that "In aqueous solution there is a significant electrostatic effect, which is reflected by the slow relaxation of the solvent. On the other hand, there is no strong electrostatic coupling in the enzyme and the major effect on the reaction coordinate motion is intramolecular energy relaxation". This proposal might have created the impression that enzyme catalysis originates from the slow dynamics of the solvent reaction. However, the dynamical effects (that reflect the time of arrival of a reactive trajectory to the TS) are, as will be shown in this study, similar in the enzyme and solution reaction, and the catalysis originates from the difference in producing a reactive trajectory, which is directly correlated to the solvent reorganization free energy.

The present work will clarify that solvation effects represent the total electrostatic interaction between the enzyme and the reacting substrate. Thus, assessing the electrostatic contribution to catalysis is equivalent to evaluating the difference between the solvation free energy of the substrate in the protein and in water. In contrast to recent implications,[36] this effect cannot be assessed without calculating the difference in solvation of the TS and RS in the enzyme and water, and ignoring this point can lead to problematic conclusions.

The present work will place special emphasis on enzyme dynamical effects; it will be shown that the dynamical nature of enzymatic reactions should be analyzed in terms of coupled dynamics of the solute−solvent system and that the dynamical behavior of the enzyme as an effective solvent is similar to the dynamics of the solvent coordinate in water. The significant difference lies instead in the amplitude of the solvent modes, which are entirely determined by the reorganization free energy (see analysis in section II).

The paper is constructed in the following way: section II reviews our simulation approaches and conceptual tools. Section III examines the energetics of the DhlA reaction and demonstrates the effect of enzyme solvation (i.e., the protein solvates the reacting fragments better than water, which is opposite to the desolvation hypothesis). Section IV examines the dynamics of the DhlA reaction and the corresponding solution reaction and demonstrates that the solvation dynamics are similar and that dynamical effects do not contribute to catalysis in a substantial way.

II. Methods for Simulating S_N2 Reactions in Solution and in Enzymes

A prerequisite to any attempt of analyzing enzyme catalysis is to have a method that is able to reproduce the observed

(11) Lau, E. Y.; Kahn, K.; Bash, P. A.; Bruice, T. C. The importance of reactant positioning in enzyme catalysis: A hybrid quantum mechanics/molecular mechanics study of a haloalkane dehalogenase. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, **97**, 9937−9942.
(12) Bruice, T. C. A view at the millennium: The efficiency of enzymatic catalysis. *Acc. Chem. Res.* **2002**, *35*, 139−148.
(13) Lightstone, F. C.; Zheng, Y. J.; Maulitz, A. H.; Bruice, T. C. Nonenzymatic and enzymatic hydrolysis of alkyl halides: A haloalkane dehalogenation enzyme evolved to stabilize the gas-phase transition state of an S_N2 displacement reaction. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 8417−8420.
(14) Shurki, A.; Strajbl, M.; Villa, J.; Warshel, A. How much do enzymes really gain by restraining their reacting fragments? *J. Am. Chem. Soc.* **2002**, *124*, 4097−4107.
(15) Dewar, M. J. S.; Storch, D. M. Alternative View of Enzyme-Reactions. *Proc. Natl Acad. Sci. U.S.A.* **1985**, *82*, 2225−2229.
(16) Warshel, A.; Strajbl, M.; Villa, J.; Florian, J. Remarkable rate enhancement of orotidine 5′-monophosphate decarboxylase is due to transition-state stabilization rather than to ground-state destabilization. *Biochemistry* **2000**, *39*, 14728−14738.
(17) Jencks, W. P. *Catalysis in Chemistry and Enzymology*; Dover: New York, 1987.
(18) Aqvist, J.; Luecke, H.; Quiocho, F. A.; Warshel, A. Dipoles Localized at Helix Termini of Proteins Stabilize Charges. *Proc. Natl Acad. Sci. U.S.A.* **1991**, *88*, 2026−2030.
(19) Warshel, A.; Åqvist, J.; Creighton, S. Enzymes Work by Solvation Substitution Rather Than by Desolvation. *Proc. Nat. Acad. Sci. U.S.A.* **1989**, *86*, 5820−5824.
(20) Devi-Kesavan, L. S.; Gao, J. L. Combined QM/MM study of the mechanism and kinetic isotope effect of the nucleophilic substitution reaction in haloalkane dehalogenase. *J. Am. Chem. Soc.* **2003**, *125*, 1532−1540.
(21) Kohen, A.; Cannio, R.; Bartolucci, S.; Klinman, J. P. Enzyme dynamics and hydrogen tunnelling in a thermophilic alcohol dehydrogenase. *Nature* **1999**, *399*, 496−499.
(22) Basran, J.; Sutcliffe, M. J.; Scrutton, N. S. Enzymatic H-transfer requires vibration-driven extreme tunneling. *Biochemistry* **1999**, *38*, 3218−3222.
(23) Wilson, E. K. Enzyme dynamics. *Chem. Eng. News* **2000**, *78*, 42−45.
(24) Berendsen, H. J. C.; Hayward, S. Collective protein dynamics in relation to function. *Curr. Opin. Struct. Biol.* **2000**, *10*, 165−169.
(25) Cameron, C. E.; Benkovic, S. J. Evidence for a functional role of the dynamics of glycine-121 of Escherichia coli dihydrofolate reductase obtained from kinetic analysis of a site-directed mutant. *Biochemistry* **1997**, *36*, 15792−15800.
(26) Cannon, W. R.; Singleton, S. F.; Benkovic, S. J. A perspective on biological catalysis. *Nat. Struct. Biol.* **1996**, *3*, 821−833.
(27) Careri, G.; Fasella, P.; Gratton, E. Enzyme Dynamics − Statistical Physics Approach. *Ann. Rev.f Biophys. Bioeng.* **1979**, *8*, 69−97.
(28) Karplus, M.; McCammon, J. A. Dynamics of Proteins − Elements and Function. *Ann. Rev. Biochem.* **1983**, *52*, 263−300.
(29) Kurzynski, M. Importance of intramolecular protein dynamics to kinetics of biochemical processes. *Cell. Mol. Biol. Lett.* **1999**, *4*, 117−130.
(30) Radkiewicz, J. L.; Brooks, C. L. Protein dynamics in enzymatic catalysis: Exploration of dihydrofolate reductase. *J. Am. Chem. Soc.* **2000**, *122*, 225−231.
(31) Daniel, R. M.; Dunn, R. V.; Finney, J. L.; Smith, J. C. The role of dynamics in enzyme activity. *Ann. Rev. Biophys. Biomol. Struct.* **2003**, *32*, 69−92.
(32) Warshel, A.; Sussman, F.; Hwang, J. K. Evaluation of Catalytic Free-Energies in Genetically Modified Proteins. *J. Mol. Biol.* **1988**, *201*, 139−159.
(33) Warshel, A. Dynamics of Enzymatic-Reactions. *Proc. Nat. Acad. Sci. U.S.A.−Biol. Sci.* **1984**, *81*, 444−448.
(34) Neria, E.; Karplus, M. Molecular dynamics of an enzyme reaction: Proton transfer in TIM. *Chem. Phys. Lett.* **1997**, *267*, 23−30.
(35) Billeter, S. R.; Webb, S. P.; Agarwal, P. K.; Iordanov, T.; Hammes-Schiffer, S. Hydride transfer in liver alcohol dehydrogenase: Quantum dynamics, kinetic isotope effects, and role of enzyme motion. *J. Am. Chem. Soc.* **2001**, *123*, 11262−11272.
(36) Nam, K.; Prat-Resina, X.; Garcia-Viloca, M.; Devi-Kesavan, L. S.; Gao, J. L. Dynamics of an enzymatic substitution reaction in haloalkane dehalogenase. *J. Am. Chem. Soc.* **2004**, *126*, 1369−1376.

catalytic effect. Such methods include molecular orbital quantum mechanical/molecular mechanics (QM/MM) approaches[37−43] and the empirical valence bond (EVB) methods.[2,44,45] The present work will use the EVB method and related strategies that have been reviewed extensively in the past, e.g., refs 2 and 45. Thus, we will only discuss these methods briefly and will emphasize the modeling of S_N2 reactions.

II.1. Evaluating Activation Barriers and Binding Energies. The EVB method is a QM/MM approach that describes reactions by mixing resonance states (or more precisely diabatic states that correspond to classical valence-bond (VB) structures) that represent the reactant intermediate (or intermediates) and product states. In the case of an S_N2 reaction in the form of

$$X^- + CH_3Y \rightarrow XCH_3 + Y^- \tag{1}$$

it is frequently sufficient to use two diabatic states of the form

$$\phi_1 = X^- \quad C - Y$$

$$\phi_2 = X - C \quad Y^- \tag{2}$$

The potential energies of these diabatic states and their mixing term are represented by

$$H_{ii} = \epsilon_i = \alpha^i_{gas} + U^i_{intra}(\boldsymbol{R}, \boldsymbol{Q}) + U^i_{Ss}(\boldsymbol{R}, \boldsymbol{Q}, \boldsymbol{r}, \boldsymbol{q}) + U_{ss}(\boldsymbol{r}, \boldsymbol{q})$$

$$H_{12} = A \exp(-\mu|\Delta R'|) \tag{3}$$

Here, $\boldsymbol{R}$ and $\boldsymbol{Q}$ represent the atomic coordinates and charges of the reactive fragment's diabatic states, and $\boldsymbol{r}$ and $\boldsymbol{q}$ are those of the surrounding protein and solvent. α^i_{gas} is the gas-phase energy of the ith diabatic state (where all the fragments are taken to be at infinity), $U^i_{intra}(\boldsymbol{R}, \boldsymbol{Q})$ is the intramolecular potential of the solute system (relative to its minimum), $U^i_{Ss}(\boldsymbol{R}, \boldsymbol{Q}, \boldsymbol{r}, \boldsymbol{q})$ represents the interaction between the solute (S) atoms and the surrounding (s) solvent and protein atoms. $U^i_{Ss}(\boldsymbol{r}, \boldsymbol{q})$ represents the potential energy of the protein/solvent system ("ss" designates surrounding-surrounding), and ϵ_i given by eq 3 from the diagonal elements of the EVB Hamiltonian (H_{EVB}). The off-diagonal elements of this Hamiltonian, H_{ij}, are usually assumed to be constant but can also be represented by an exponential function of the distances between the reacting atoms. In the present case, we express H_{ij} as a function of the distance $\Delta R'$ that express the difference between the O−C and the C−Cl bond lengths. In addition, the H_{ij} elements are assumed to be the same in the gas phase, in solutions, and in the proteins. The adiabatic ground-state energy E_g and the corresponding eigenvector $\boldsymbol{C_g}$ are obtained by solving the secular equation

$$\boldsymbol{H_{EVB}C_g} = E_g\boldsymbol{C_g} \tag{4}$$

The EVB treatment provides a natural picture of intersecting electronic states, which is useful for exploring environmental effects on chemical reactions in condensed phases.[46] The ground-state charge distribution of the reacting species, the "solute", polarizes the surroundings, the "solvent", and the charges of each resonance structure of the solute then interacts with the polarized solvent.[2] This coupling enables the EVB model to capture the effect of the solvent on the quantum mechanical mixing of the different states of the solute. For example, in cases where ionic and covalent states are describing the solute, the interaction of the solvent with the ionic state will lead to a more consistent ground state charge distribution than that obtained by alternative molecular orbital treatments (see ref 10).

The simplicity of the EVB formulation makes it easy to obtain its analytical derivatives (using the Hellmann−Feynman theorem for eq 4) and thus to sample the EVB energy surface by MD simulations. Running such MD trajectories on the EVB surface of the reactant state can provide the free energy function Δg that is needed to calculate the activation energy $\Delta g^{\ddagger}$. However, since trajectories on the reactant surface will reach the transition state only rarely, it is usually necessary to run a series of trajectories on potential surfaces that gradually drive the system from the reactant to the product state.[10,46] The EVB approach accomplishes this by changing the system adiabatically from one diabatic state to another. In the simple case of two diabatic states, this "mapping" potential, ϵ_m, can be written as a linear combination of the reactant and product potentials, ϵ_1 and ϵ_2:

$$\epsilon_m = (1 - \lambda_m)\epsilon_1 + \lambda_m\epsilon_2 \qquad (0 \le \lambda_m \le 1) \tag{5}$$

When λ_m is changed from 0 to 1 in $n + 1$ fixed increments ($\lambda_m = 0/n, 1/n, 2/n, ..., n/n$), potentials with one or more of the intermediate values of λ_m will force the system to fluctuate near the TS.

The free energy ΔG_m associated with changing λ from 0 to m/n is evaluated by the FEP procedure and is described elsewhere (see, e.g., Chapter 3 in ref 2). The free energy functional that corresponds to the adiabatic ground-state surface E_g (eq 4) is then obtained by the FEP-umbrella sampling (FEP/US) method[2,46] that can be written as

$$\Delta g(x') = \Delta G_m - \beta^{-1} \ln\langle\delta(x - x') \exp[-\beta(E_g(x) - \epsilon_m(x))]\rangle_m \tag{6}$$

where ϵ_m is the mapping potential that keeps x in the region of x'. If the changes in ϵ_m are sufficiently gradual, the free energy functional $\Delta g(x')$ obtained with several values of m overlap over a range of x', and patching together the full set of $\Delta g(x')$ gives the complete free energy curve for the reaction. The FEP/US

(37) Warshel, A.; Levitt, M. Theoretical Studies of Enzymic Reactions − Dielectric, Electrostatic and Steric Stabilization of Carbonium-Ion in Reaction of Lysozyme. *J. Mol. Biol.* **1976**, *103*, 227−249.
(38) Gao, J. L. Hybrid quantum and molecular mechanical simulations: An alternative avenue to solvent effects in organic chemistry. *Acc. Chem. Res.* **1996**, *29*, 298−305.
(39) Field, M. J.; Bash, P. A.; Karplus, M. A Combined Quantum-Mechanical and Molecular Mechanical Potential for Molecular-Dynamics Simulations. *J. Comput. Chem.* **1990**, *11*, 700−733.
(40) Friesner, R. A.; Beachy, M. D. Quantum mechanical calculations on biological systems. *Curr. Opin. Struct. Biol.* **1998**, *8*, 257−262.
(41) Monard, G.; Merz, K. M. Combined quantum mechanical/molecular mechanical methodologies applied to biomolecular systems. *Acc. Chem. Res.* **1999**, *32*, 904−911.
(42) Field, M. J. Simulating enzyme reactions: Challenges and perspectives. *J. Comput. Chem.* **2002**, *23*, 48−58.
(43) Cui, Q.; Elstner, M.; Kaxiras, E.; Frauenheim, T.; Karplus, M. A QM/MM implementation of the self-consistent charge density functional tight binding (SCC-DFTB) method. *J. Phys. Chem. B* **2001**, *105*, 569−585.
(44) Shurki, A.; Warshel, A. Structure/function correlations of proteins using MM, QM/MM, and related approaches: current progress. *Adv. Protein Chem.* **2003**, *66*, 249−313.
(45) Aqvist, J.; Warshel, A. Simulation of Enzyme-Reactions Using Valence-Bond Force-Fields and Other Hybrid Quantum-Classical Approaches. *Chem. Rev.* **1993**, *93*, 2523−2544.
(46) Hwang, J. K.; Creighton, S.; King, G.; Whitney, D.; Warshel, A. Effects of Solute Solvent Coupling and Solvent Saturation on Solvation Dynamics of Charge-Transfer Reactions. *J. Chem. Phys.* **1988**, *89*, 859−865.
(47) King, G.; Warshel, A. Investigation of the Free Energy Functions for Electron Transfer Reactions. *J. Chem. Phys.* **1990**, *93*, 8682−8692.

approach may also be used to obtain the free energy functional of the isolated diabatic states. For example, the diabatic free energy Δg_1 of the reactant state can be calculated as

$$\Delta g_1(x') = \Delta G_m - \beta^{-1} \ln\langle \delta(x - x') \exp[-\beta(\epsilon_1(x) - \epsilon_m(x))]\rangle_m \quad (7)$$

The diabatic free energy profiles of the reactant and product states represent microscopic equivalents of the Marcus parabolas.[47−49]

The EVB method satisfies some of the main requirements for reliable studies of enzymatic reactions. Among the obvious advantages of the EVB approach is the facilitation of proper configurational sampling and converging free energy calculations. This includes the inherent ability to evaluate nonequilibrium solvation effects.[3] It should also be noted that the reliability of any method addressing enzyme catalysis lies in its ability to obtain accurate free energy differences between the enzyme and solution reaction. This is hard to accomplish by, for example, current ab initio QM/MM methods, which are often perceived as more accurate even though they suffer from significant sampling difficulties. The use of semiempirical QM/MM approaches can greatly improve this, but also these methods require calibration, which is easier to accomplish with the EVB formulation. Finally, the EVB benefits from the above-mentioned ability to treat the solute−solvent coupling consistently. This feature is essential for study of dynamical contributions to catalysis, which is one of the primary objectives of the present work.

In studying enzyme catalysis, it is frequently essential to determine if the catalysis is due to reactant-state destabilization (RSD) or to transition-state stabilization (TSS). This can only be done by calculating the binding energy of the RS and the TS[14,50] in some form. This type of calculation is extremely challenging, and one of the best ways to perform it is to use the linear response approximation (LRA) treatment.[51] This approach provides a good estimate for the free energy associated with the change between two potential surfaces (U_1 and U_2) by (see ref 51)

$$\Delta G(U_1 \rightarrow U_2) = \frac{1}{2}(\langle U_2 - U_1\rangle_1 + \langle U_2 - U_1\rangle_2) \quad (8)$$

The notation $\langle\ \rangle_i$ designates an average over trajectories propagated on the potential energy surface U_i. The use of the LRA offers the unique ability to decompose free energies to their individual additive contributions.[52] Such a treatment cannot be accomplished by FEP approaches due to their nonadditive nature. The individual LRA contribution of the ith energy term is given by

$$\Delta G_i(U_1 \rightarrow U_2) = \frac{1}{2}(\langle U_2^i - U_1^i\rangle_1 + \langle U_2^i - U_1^i\rangle_2) \quad (9)$$

II.2. Evaluating Dynamical Effects in Terms of the EVB Energy Gap. The EVB approach provides a very convenient way to evaluate and analyze the rate constants of the reaction in the enzyme and the corresponding reference reaction in water. Our approach for evaluating the rate constant involves the well-known expression

$$k_{12} = \kappa k_{TST} \quad (10)$$

where κ is the "transmission coefficient" and k_{TST} is the transition-state theory rate constant, which is given by

$$k_{TST} = \frac{1}{2}\langle|\dot{x}|\rangle_{TS} \exp(-\Delta g^{\ddagger}\beta)/\int_{-\infty}^{x^{\ddagger}} \exp(-\Delta g(x)\beta)\mathrm{d}x \quad (11)$$

where $\Delta g^{\ddagger} = \Delta g(x^{\ddagger})$.

The transmission coefficient depends on two interrelated factors: the probability that a system arriving at the transition state (TS) from the reactant state (RS) will end up in the product state (PS) rather than returning to the RS and the average number of times that a productive trajectory passes back and forth across $x^{\ddagger}$ before it moves permanently to the PS. These factors can be evaluated by examining a family of MD trajectories that start at the TS with a distribution of velocities.[34,53−60] A practical way to obtain the transmission factor is to save multiple sets of the atomic coordinates and velocities during a parent MD trajectory on an artificial potential surface (composed of the EVB surface plus a restraint potential) that holds the system in the TS region. A new trajectory is then started from each of these structures and is propagated both forward and backward in time until both segments have settled in either the reactant or the product state. Combining the forward and backward segments gives a complete trajectory with the desired properties.

A general analysis of the role of the transmission factor in enzymatic catalysis has been developed by considering the interactions of a reacting substrate with its surroundings at the TS.[2,10,32,46,61] This treatment starts by considering the relationship between κ and τ_+, the average time that productive trajectories take to move away from the TS: $\kappa \approx \Delta x^{\ddagger}\tau_+^{-1}(2k_BT/\pi m)^{-1/2}$. According to this expression, any significant difference between the transmission factors for an enzymatic reaction and the corresponding reaction in solution must reflect a difference in τ_+. Further, since the solute is the same in the enzyme and solution, a difference in τ_+ must originate from the interactions

(48) Marcus, R. A. Chemical and electrochemical electron transfer theory. *Ann. Rev. Phys. Chem.* **1964**, *15*, 155−196.
(49) Marcus, R. A. Electron-Transfer Reactions in Chemistry − Theory and Experiment (Nobel Lecture). *Angew. Chem., Inte. Ed. Engl.* **1993**, *32*, 1111−1121.
(50) Shurki, A.; Warshel, A. Structure/Function Correlations of Protreins using MM, QM/MM and Related Approaches; Methods, Concepts, Pitfalls and Current Progress. *Adv. Protein Chem.* **2003**, *66*, 249−312.
(51) Lee, F. S.; Chu, Z. T.; Bolger, M. B.; Warshel, A. Calculations of Antibody−Antigen Interactions: Microscopic and Semi-Microscopic Evaluation of the Free Energies of Binding of Phosphorylcholine Analogues to McPC603. *Prot. Eng.* **1992**, *5*, 215−228.
(52) Florian, J.; Goodman, M. F.; Warshel, A. Theoretical Investigation of the Binding Free Energies and Key Substrate-Recognition Components of the Replication Fidelity of Human DNA Polymerase β. *J. Phys. Chem. B* **2002**, *106*, 5739−5753.
(53) Keck, J. C. Variational theory of reaction rates. *Adv. Chem. Phys.* **1966**, *13*, 85−121.
(54) Anderson, J. B. Statistical theoreticals of chemical reactions. Distributions in the transition region. *J. Chem. Phys.* **1973**, *58*, 4684−4692.
(55) Bennett, C. H. Molecular dynamics and transition state theory: the simulation of infrequent events. In *Algorithms for chemical computations*; Christofferson, R. E., Ed.; American Chemical Society: Washington, DC, 1977; pp 63−97.
(56) Chandler, D. Statistical-Mechanics of Isomerization Dynamics in Liquids and Transition-State Approximation. *J. Chem. Phys.* **1978**, *68*, 2959−2970.
(57) Cline, R. E.; Wolynes, P. G. Stochastic Dynamic-Models of Curve Crossing Phenomena in Condensed Phases. *J. Chem. Phys.* **1987**, *86*, 3836−3844.
(58) Straub, J. E.; Berne, B. J. A Rapid Method for Determining Rate Constants by Molecular-Dynamics. *J. Chem. Phys.* **1985**, *83*, 1138−1139.
(59) Grimmelmann, E. K.; Tully, J. C.; Helfand, E. Molecular-Dynamics of Infrequent Events − Thermal-Desorption of Xenon from a Platinum Surface. *J. Chem. Phys.* **1981**, *74*, 5300−5310.
(60) Warshel, A.; Parson, W. W. Dynamics of biochemical and biophysical reactions: insight from computer simulations. *Quart. Rev. Biophys.* **2001**, *34*, 563−679.

of the reacting groups with their respective surroundings in the enzyme and solution transition states.

To treat the reaction in an efficient way, we define a generalized, time-dependent reaction coordinate $x(t)$ as the energy gap between the reactant and product VB states, $\Delta\epsilon(t) = \epsilon_2(t) - \epsilon_1(t)$. This coordinate can quite easily be divided into a solute coordinate, $R(t)$, reflecting internal bonds of the reacting EVB groups, and a solvent coordinate, $Q(t)$, representing interactions of the solute with the solvent (the "solvent" again refers to the surroundings of the reacting atoms in either the enzyme or the solution). In this notation, the solvent coordinate depends mainly on the difference in electrostatic energies, $\Delta\epsilon_{el}$:[10]

$$-(\hbar\omega_Q\delta_Q)\, Q(t) \approx \Delta\epsilon_{el}(t) = \epsilon_{el,2}(t) - \epsilon_{el,1}(t) \qquad (12)$$

where ω and δ will be defined in section III.4.

Thus, in the common case that the relaxation time for solvent motions is equal to or longer than that for the solute dipole, τ_+ is given to a good approximation by (see ref 10)

$$\tau_+^{-1} \simeq \frac{\partial\langle\Delta\epsilon_{el}(t)\rangle_{TS}}{\partial t}/\Delta x_S^{\ddagger} \qquad (13)$$

where $\langle ... \rangle_{TS}$ denotes an average over a trajectory on a mapping potential that keeps the system in the region of the transition state, and $\Delta x_S^{\ddagger}$ is the width of the TS region along the solvent coordinate.

By starting with coupled equations for the time dependence of the solvent and solute coordinates and using the linear-response approximation,[10,62] we obtain a relationship between the electric dipole of the solute ($\vec{\mu}$) and the average time dependence of the solvent reaction coordinate after a system enters the TS:

$$\langle\Delta\epsilon_{el}(t)\rangle_{TS} = \frac{\langle\Delta\epsilon_{el}^{max}\rangle}{\langle\Delta\vec{\mu}_{max}\rangle}\frac{\int_0^t\{\langle\Delta\dot{\epsilon}_{el}(t)\Delta\epsilon_{el}(t+\tau)\rangle_{TS}\langle\Delta\vec{\mu}(\tau)\rangle_{TS}\}d\tau}{\langle\Delta\dot{\epsilon}_{el}(t)\Delta\epsilon_{el}(t)\rangle_{TS}} \qquad (14)$$

In this expression, $\langle\Delta\vec{\mu}_{max}\rangle$ is the difference between the solute dipoles in the product and transition states ($\langle\vec{\mu}\rangle_2 - \langle\vec{\mu}\rangle_{TS}$) and $\langle\Delta\epsilon_{el}^{max}\rangle$ is the average change in $\Delta\epsilon_{el}$ between these two states ($\langle\epsilon_{el}\rangle_2 - \langle\epsilon_{el}\rangle_{TS}$). The integral in the numerator depends on the response function $\langle\Delta\dot{\epsilon}_{el}(0)\Delta\epsilon_{el}(t)\rangle$, which is related to the time derivative of the classical autocorrelation function of $\Delta\epsilon_{el}$ by

$$\frac{\partial C(\tau)_{el}}{\partial t} = \frac{\partial}{\partial t}\langle\Delta\epsilon_{el}(t)\Delta\epsilon_{el}(t+\tau)\rangle = -\Delta\dot{\epsilon}_{el}(t)\Delta\epsilon_{el}(t+\tau) \qquad (15)$$

The above treatment indicates that we can deduce the importance of dynamical effects in an enzymatic reaction simply from the autocorrelation function of $\Delta\epsilon_{el}$. If the autocorrelation functions are similar in the enzyme and in solution, the transmission factors must be similar.

The EVB also allows one to evaluate the projection of the protein (or solvent) motion along the reaction coordinate. This can be done by considering the fluctuations of $\Delta\epsilon_{12}$ during a MD trajectory in state i and relating it to the fluctuations of an equivalent harmonic system by evaluating the autocorrelation function

$$C_i(t) = \langle u(\tau + t)u(\tau)\rangle_i \qquad (16)$$

where $u(t) = \Delta\epsilon_{12}(t) - \langle\Delta\epsilon_{12}\rangle_i$. According to the Wiener–Khintchine theorem, the magnitude of the Fourier transform of the autocorrelation function is the power spectrum, $J(\omega)$, of the fluctuations:

$$J(\omega) = \left|\int_{-\infty}^{\infty} C(t)\exp(i\omega t)dt\right| \qquad (17)$$

The power spectrum as defined here is the magnitude (squared amplitude) of the fluctuations of $\Delta\epsilon_{12}$ at a given angular frequency (ω; related expressions in the literature often use the dimensionless spectral density function, $\omega J(\omega)$). Some manipulations of $J(\omega)$[63] give at the high-temperature limit the following expression

$$J(\omega) = \pi\beta^{-1}\sum_j \hbar\omega_j d_j^2\delta(\omega - \omega_j) \qquad (18)$$

A Fourier transform of $C_i(t)$ thus picks out the vibrational modes that are coupled to the electron-transfer reaction because they have significant nuclear displacements (d_j) between the reactant and product states. Mode j contributes a Fourier component at frequency ω_j with a magnitude proportional to $\omega_j d_j^2$.

The Fourier magnitudes obtained by eq 18 can be scaled by relating the area under the spectral density function to the overall reorganization energy (λ):

$$\lambda = \frac{1}{2}\sum_j \hbar\omega_j d_j^2 = \frac{\beta}{2\pi}\left|\int_{-\infty}^{\infty} J(\omega)d\omega\right| \qquad (19)$$

The reorganization energy can also be obtained independently from the difference between the average values of $\Delta\epsilon_{12}$ in the reactant and product states ($\langle\Delta\epsilon_{12}\rangle_1$ and $\langle\Delta\epsilon_{12}\rangle_2$), as described in eq 8.

The specific EVB parameters used in this work constitute a small modification of the parameters that are described in the Supporting Information of ref 14. The EVB MD simulations were performed using the MOLARIS program with a simulation sphere of 18 Å described by the ENZYMIX[64] force field and subject to the SCAAS[65] and LRF long-range treatment.[51] For the free energy profile, the reaction was divided into 21 frames (i.e., 21 values of λ_m in eq 5), and each frame was simulated for 50 ps with a time step of 1 fs. Before acquiring the statistics, these simulations were initially relaxed and equilibrated equally long to avoid getting trapped in unphysical configurations. This gives a reaction barrier that is based on more than 1 ns simulations (21×50000 steps with 1 fs step size), which is significantly more than what is normally run, e.g., ref 14. For the dynamical analyses, however, it was found that this approach was inadequate, and therefore, the autocorrelation functions and

(61) Bentzien, J.; Muller, R. P.; Florian, J.; Warshel, A. Hybrid ab initio quantum mechanics molecular mechanics calculations of free energy surfaces for enzymatic reactions: The nucleophilic attack in subtilisin. *J. Phys. Chem. B* **1998**, *102*, 2293–2301.
(62) Kubo, R. The fluctuation–dissipation theorem. *Rep. Prog. Phys.* **1966**, *29*, 255–284.
(63) Warshel, A.; Hwang, J. K. Simulation of the Dynamics of Electron-Transfer Reactions in Polar-Solvents – Semiclassical Trajectories and Dispersed Polaron Approaches. *J. Chem. Phys.* **1986**, *84*, 4938–4957.
(64) Lee, F. S.; Chu, Z. T.; Warshel, A. Microscopic and Semimicroscopic Calculations of Electrostatic Energies in Proteins by the Polaris and Enzymix Programs. *J. Comput. Chem.* **1993**, *14*, 161–185.
(65) King, G.; Warshel, A. A Surface Constrained All-Atom Solvent Model for Effective Simulations of Polar Solutions. *Jo. Chem. Phys.* **1989**, *91*, 3647–3661.

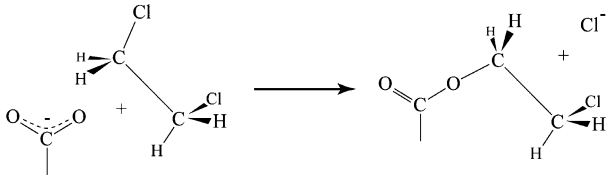

Figure 1. Schematic description of the S_N2 step in the DhlA reaction where the nucleophile carboxylate (Asp 124) replaces the halide group.

power spectrum was obtained by simulating the system for 50 ps with a time step of 0.1 fs, i.e., 500000 steps on the RS or TS geometry. Again, all simulation runs have been relaxed and equilibrated thoroughly before collecting the statistics.

Finally, one may wonder what relationship there is between the starting structure in the EVB simulations and the actual configurations of the reactant and product. The underlying approach we use is a standard approach that is robust and previously well examined (FEP/US). The issue is not the coordinates found by some arbitrary MD simulation but a process where the system is driven (by the EVB potential) from the product to the reactant state. This forces the transition to accrue rather fast, but the main point is to obtain the correct converging free energy for this transition. In fact, since 1986 we have examined and established repeatedly that the EVB end points are fully relaxed configurations. At any rate, the final simulation that was used to get the reaction barrier is based on more than 1 ns simulations that previously have been equilibrated equally long, and surely any significant changes in atom position that are in the range of some Å movement have been taken into account by this approach. Note also that all atoms have been free to move in accordance with the forces calculated in the MD simulations in all these calculations.

III. The Energetics of the S_N2 Reaction Step in DhlA and in Solution

III.1. General Considerations. The enzyme haloalkane dehalogenase (DhlA) from *Xanthobacter autotrophicus* GJ10, whose structure was solved by Verschueren et al. (PDB entry code 2DHD[66]), catalyses the reaction depicted in Figure 1.

To study the origin of the catalytic effect in this enzyme (or in any other enzyme), it is essential to have a clear idea about the activation barrier of the reaction in the enzyme active site and the reference reaction in solution.

Our estimate of the energetics of the S_N2 step in solution is based on the classical work of Swain and Scott.[67] This work examined the rate constants for series of closely related S_N2 reactions with different nucleophiles. The results give a difference of 0.44 kcal/mol between the S_N2 reactions with CH_3COO^- and Cl^- as nucleophiles. Since the observed $\Delta g^{\ddagger}$ for the S_N2 reaction $Cl^- + CH_3Cl \rightarrow ClCH_3 + Cl^-$ is 26.6 kcal/mol (from ref 10), we use the above difference and estimate $\Delta g^{\ddagger}_w$ to be about 27 kcal/mol for $CH_3COO^- + CH_3Cl \rightarrow CH_3COOCH_3 + Cl^-$. Alternatively, one can use the enthalpy and entropy of the S_N2 reaction of ethyl chloride with sodium acetate at temperatures above 100 °C. This gives $\Delta g^{\ddagger} \sim 28.6$ kcal/mol. It is not clear, however, that one can interpret $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ from 100 to 30 °C. Thus, we prefer the estimate of 27 kcal/mol. For the enzyme we obtain $\Delta g^{\ddagger}_{cat} = 15.3$ kcal/mol from the observed k_2[14] by using transition-state theory. Thus, the catalytic effect ($\Delta g^{\ddagger}_{cat} - \Delta g^{\ddagger}_{wat}$) is ~11.7 kcal/mol. If one considers the fact that it costs about 2.5 kcal/mol to bring the reactants to the same solvent cage (as was found by the rigorous calculations of ref 14), we obtain $\Delta g^{\ddagger}_{cage}$ ~24.5 kcal/mol which gives $\Delta g^{\ddagger}_{cat} - \Delta g^{\ddagger}_{cage}$ ~9.2 kcal/mol. Our task is to determine the origin of this effect. Note that the 2.5 kcal/mol cage effect must be added to either the calculated $\Delta g^{\ddagger}_{cage}$ in order to compare the calculated and observed $\Delta g^{\ddagger}_w$, or to be subtracted from the observed $\Delta g^{\ddagger}_w$ in order to compare the calculated $\Delta g^{\ddagger}_{cage}$ to the corresponding $(\Delta g^{\ddagger}_w)_{obs} - \Delta g_{cage}$. These considerations were not taken into account in ref 20.

Table 1. Activation Free Energies[a] for the S_N2 Step of the Reaction of DhlA and the Corresponding Reference Reaction

	$\Delta g^{\ddagger}_{calc}$	$\Delta g^{\ddagger}_{expt}$[c]
water[b]	26.8	27
water cage	24.2	24.6
protein	15.2	15.3

[a] Energies in kcal/mol. The calculated values were obtained by the EVB approach with the parameters given as Supporting Information in ref 14. [b] Corresponds to 1 M concentration of the substrate, namely $\Delta g^{\ddagger}$ refers to $\Delta g^{\ddagger}_w$. [c] The sources for the experimental results are discussed in the text (section III.1).

III.2. Analyzing the Catalytic Effect and the Solvation Contribution to This Effect. The results of the present EVB study of the activation free energies in the enzyme and solution are given in Table 1. In this study, we have run longer simulations and obtain a catalytic effect of 11.6 kcal/mol in good agreement with the estimated experimental results (11.7 kcal/mol). A recent QM/MM study of the system[20] reports a catalytic effect of about 16 kcal/mol, which is apparently somewhat of an overestimation of the observed value. At any rate, our potential surfaces and those of the more recent calculation of ref 36 accounts for the observed catalytic effect and thus can be used to examine the actual origin of the catalytic effect (here, it is also useful to clarify a misunderstanding of ref 36 where it was asserted that the gas-phase EVB calculation must be incorrect[68]).

The calculated activation barriers for the protein and solution reaction cannot tell us if the catalytic effect is due to TSS or RSD. They also cannot tell us if the catalysis is due to electrostatic effects or to other factors. To explore this issue

(66) Verschueren, K. H. G.; Seljee, F.; Rozeboom, H. J.; Kalk, K. H.; Dijkstra, B. W. Crystallographic Analysis of the Catalytic Mechanism of Haloalkane Dehalogenase. *Nature* **1993**, *363*, 693−698.
(67) Swain, C. G.; Scott, C. B. Qualitative correlation of relative rates. Comparison of hydroxide ion with other nucleophilic reagents toward alkyl halides, esters, epoxides and acyl halides. *J. Am. Chem. Soc.* **1953**, *75*, 141−147.

(68) Some misunderstanding has been associated with our previous attempt to move from the energies evaluated in our early studies of the energy of the DhlA in a hypothetical nonplanar enzyme active site (Shurki et al. *J. Am. Chem. Soc.* **2002**, *124*, 4097−4107). That is, while the TS structure is similar in the gas phase and in solution the RS structure is quite different. In fact, as indicated schematically in Figure 3, the free energy of the gas-phase potential at the minimum of the gas-phase potential, $\langle R\rangle_{RS}{}^{p}$ are different and the corresponding energy at $\langle R\rangle_{RS}{}^{g}$ has been found to be higher by about 6 kcal/mol. Similarly, the gas-phase free energy at $\langle R\rangle_{RS}{}^{w}$ is about 13 kcal/mol higher than the corresponding energy at the gas-phase minimum ($\langle R\rangle_{RS}{}^{g}$). The energy difference between $\langle R\rangle_{RS}{}^{w}$ and $\langle R\rangle_{RS}{}^{p}$ in solution has already been considered in ref 14 and referred to as the solvation induced NAC effect. This value of about 2.5 kcal/mol reflects the compensation of a much larger gas-phase effect by the solvent dielectric. The value of the gas-phase activation barrier deduced from our thermodynamic cycle is in an excellent agreement with ab initio estimates of this barrier (about 17 kcal/mol) for a formate nucleophile (Lewandowicz et al. *J. Am. Chem. Soc.* **2001**, *123*, 4550−4555). Note that the EVB includes in the quantum region a formate ion rather than an aspartate. This is fully justified since the activation barrier in solution is almost identical for formate and aspartate, and more importantly the catalytic effects are almost identical when the QM part includes only the COO^- group of Asp 124 or a CH_2COO^- group.

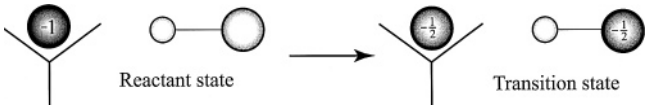

Figure 2. Schematic description of the change in charge distribution during the S_N2 reaction of DhlA. In the reactant state, the charge is localized on Asp 124, whereas it is delocalized over the reaction fragments in the transition state.

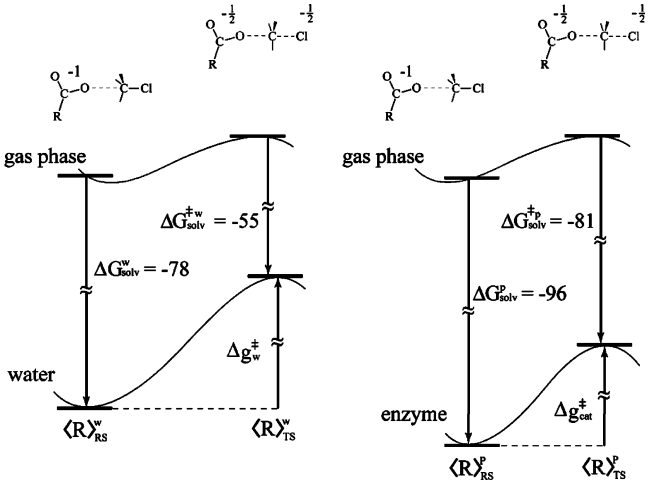

Figure 3. Schematic description of the energetics of the S_N2 reaction in DhlA and the reference water system. The figure focuses on the effects of the solvation free energies. As seen from the figure, the solvation of both the RS and TS is larger in the enzyme than in solution. Moreover, the solvation of the TS is considerably larger in the enzyme than in water. This is the origin of the catalytic effect; note, however, that the catalytic effect is not directly given by this solvation difference or by the difference between the RS and TS in the given system, but by the difference between the relevant $\Delta\Delta G_{solv}$ in the enzyme and solution reaction (see also the text).

one must be able to calculate the binding energies of the RS and TS in both the protein active site and the reference solvent cage. In doing so, it is important to evaluate the different energy contributions to these binding energies. Now, before we describe such an analysis, it is useful to recognize the challenge that the enzyme must face in catalyzing an S_N2 reaction. That is, in a typical S_N2 reaction the charge distribution of the reactant changes (see Figure 2) from a localized charge of $(-1, 0)$ in the RS to delocalized distribution $(-1/2, -1/2)$ at the TS. Thus, the RS in the reference solution reaction is solvated much more strongly than the corresponding TS. In this situation it is hard for the enzyme dipole to solvate the TS more than the RS, but they can at least increase the solvation of the TS relative to the corresponding solvation in water. This situation is fundamentally different from the well-known desolvation hypothesis,[15−17] which proposes that the enzyme work by RSD providing *less solvation* to the RS than water does. The difference between the desolvation proposal promoted by ref 20 and the solvation substitution proposal[19] will be further discussed below. However, our first priority is to find out what is actually happening in DhlA.

Our analysis of the energetics of the RS and TS were performed by the LRA method and is summarized in Figure 3. The figure considers the solvation free energies of the RS and TS and basically reproduces most of the catalytic effect (see also ref 14). In analyzing the results of our analysis, it is important to realize that both the enzymes and the solvent cage should be considered as "solvents" for the substrates. This view,[4] which is now shared by other workers, e.g., refs 5 and 6, simply considers the interaction between the environment and solute charges as a general solvation effect, which can be evaluated conveniently by the LRA approach or by more demanding FEP calculations.

As can be seen from Figure 3, the enzyme solvates the RS more (rather than less) than the solvent cage. Thus, the calculations reported in Figure 3 are inconsistent with the desolvation proposal. Instead, as is the case with other systems that were proposed to work by the desolvation proposal (see refs 2 and 16 for discussion), the enzyme solvates the delocalized TS much more than water does (-81 versus ~ -55). Thus, TSS is the source of the catalytic power of the enzyme (see also below for the relationship of this effect to the protein preorganization). Interestingly, the desolvation proposal requires that the charge on Asp124 (the nucleophile in the reaction) will be much less stable in the enzyme than in water. This will lead to an increase in its pK_a value and will force the Asp to accept a proton at pH 7 (see a related discussion in ref 16).

At this point, it is useful to clarify some confusion with regard to the origin of the catalytic effect of DhlA (see also ref 68 for a misunderstanding with regard to the EVB gas-phase energy). That is, Devi-Kasavan and Gao[20] examined the origin of the catalytic power of haloalkane dehalogenase (DhlA) by a QM/MM approach. The calculations reproduce the correct trend of the catalytic effect indicating that it is due to electrostatic effects. It was also found the activation barrier is higher in water than in the enzyme and that the reaction in water involves loss of solvation energy (the corresponding solvation analysis was not done in the enzyme). However, the suggestion of ref 20 that these findings are consistent with RSD desolvation proposal is problematic. That is, the desolvation proposal states very clearly and unambiguously that the enzyme solvates the reactant state (RS) less than water does regardless of the relative solvation of the RS and TS in water; see, e.g., refs 2,17, and 69. Thus, an examination of the origin of the catalytic effect should compare the solvation of the RS in the enzyme and in water as well as the TS in the enzyme and in water (rather than comparing the activation barriers in enzyme and in water). However, our study (Figure 3) shows clearly that DhlA stabilizes (solvates) its RS more than water does and solvates its TS much more than water does.

The problem with the proposal of ref 20 can best be illustrated by realizing that the catalytic effect is determined by $\Delta\Delta g^{\ddagger}_{cat} - \Delta\Delta g^{\ddagger}_{w}$. This quantity can be evaluated by considering the following cycle

$$\Delta g^{\ddagger}_{cat} = (\Delta g^{\ddagger}_{g}) + (\Delta G^{p}_{solv})_{TS} - (\Delta G^{p}_{solv})_{RS} - \Delta\Delta G^{p}_{RS^{g}\rightarrow RS^{p}}$$

$$\Delta g^{\ddagger}_{w} = (\Delta g^{\ddagger}_{g}) + (\Delta G^{w}_{solv})_{TS} - (\Delta G^{w}_{solv})_{RS} - \Delta\Delta G^{w}_{RS^{g}\rightarrow RS^{w}} \quad (20)$$

where $\Delta g^{\ddagger}_{g}$ is the activation energy in the gas phase and the $\Delta\Delta G$ designates the change in solvation energies moving from the gas-phase ground-state geometry to the RS geometry in the protein or solution system. For convenience, it is assumed here that the TS geometry is similar in the gas phase, the protein, and solution, which was also found in other studies, e.g., refs

(69) Crosby, J.; Stone, R.; Lienhard, G. E. Mechanisms of Thiamine-Catalyzed Reactions. Decarboxylation of 2-(1-Carboxy-1-hydroxyethyl)-3,4-dimethylthiazolium Chloride. *J. Am. Chem. Soc.* **1970**, *92*, 2891−2900.

Table 2. Solvation Energies[a] for the Enzyme and Water Reference Reaction in the Reactant State (RS) and Transition State (TS)

	water reference reaction		enzyme reaction	
	RS	TS	RS	TS
$\langle U_Q - U_0\rangle_Q$	−158.5	−114.2	−129.7	−102.4
$\langle U_Q - U_0\rangle_0$	2.9	4.4	−62.0	−59.1
ΔG_{solv}	−77.8[b]	−54.9	−95.9[b]	−80.8

[a] All energies are given in kcal/mol. [b] As can be seen from these results, the reacting fragments are solvated better in the enzyme than in the water reference reaction, which shows that the primary function of the enzyme is not to desolve the substrate. Instead, the transition state is better solvated in the enzyme than in the water reaction.

70 and 71. Using this cycle to evaluate the catalytic effect, we have

$$\Delta\Delta g^{\ddagger}_{w\rightarrow p} = \Delta g^{\ddagger}_{cat} - \Delta g^{\ddagger}_{w} \simeq (\Delta G^{p}_{solv})_{TS} - (\Delta G^{w}_{solv})_{TS} - (\Delta G^{p}_{solv})_{RS} + (\Delta G^{w}_{solv})_{RS} = (\Delta\Delta G^{\ddagger}_{solv})^{p} - (\Delta\Delta G^{\ddagger}_{solv})^{w} \quad (21)$$

For simplicity, we neglected the last terms in eq 20 since these terms were found to be small in ref 14 and do not change our conclusions. Obviously, this expression is independent of the gas phase barrier. Furthermore, we cannot determine this expression by only considering $(\Delta\Delta G^{\ddagger}_{solv})^{w}$, and the fact that $(\Delta\Delta G^{\ddagger}_{solv})^{w}$ is positive should not be confused with the desolvation proposal or with the overall solvation contribution to catalysis. Thus, to determine the catalytic effect, we must consider the solvation in the TS and RS both in the protein and in solution.

Regardless of the above consideration, it is clear that all the terms responsible for the solute−solvent interaction constitute the overall solvation effects. However, even the nature of this effect is not widely appreciated. That is, it is tempting to assume that the TSS effect is due to the existence of stronger electrostatic interaction between the charges of the TS and its surroundings in the enzyme than in solution. However, the average electrostatic interaction appears to be similar. Thus, we have to examine the overall energy of the solute−solvent system and its charge upon moving the TS from the gas phase to the given site (the $\Delta\Delta G_{solv}$ of eq 21). This free energy includes both the solute−solvent interaction at the TS and the energy associated with the reorganization of the environment. To asses this contribution we can use the LRA formulation of eq 8. The result of the LRA analyses is summarized in Table 2. As can be seen from Table 2, the difference between the solution and enzyme energetics is associated with the average over the configurations generated by the uncharged TS ($\langle U_Q - U_0\rangle_0$). This term, which is close to zero in the water solution, represent the crucial contribution of the preorganization of the active site.[72] This point will be discussed further below.

III.4. Catalytic Effect in Terms of the Solute and Solvent Coordinates. The discussion in the previous section makes it clear that in order to understand enzyme catalysis it is necessary to understand the role of the enzyme as a solvent. One of the best ways of understanding this is to describe the system in terms of its effective solute and solvent coordinates. Such an analysis has been used in early classifications of solvent effects[73] and in more systematic recent studies,[10] which will be used as a guide for the present study. As pointed out in section II, EVB provides a natural way of separating the system into solute and solvent coordinates. In other words, we can describe the two EVB states as[10]

$$\epsilon_1 \simeq \sum_i \frac{\hbar}{2}\omega^i_r(r_i + \delta^i_r/2)^2 + \sum_j \frac{\hbar}{2}\omega^j_q(q_j + \delta^j_q/2)^2 \simeq \frac{\hbar}{2}\omega^i_R(R + \delta_R/2)^2 + \frac{\hbar}{2}\omega^i_Q(Q + \delta_Q/2)^2$$

$$\epsilon_2 \simeq \sum_i \frac{\hbar}{2}\omega^i_r(r_i - \delta^i_r/2)^2 + \sum_j \frac{\hbar}{2}\omega^j_q(q_j - \delta^j_q/2)^2 + \Delta V_0 \simeq \frac{\hbar}{2}\omega^i_R(R - \delta_R/2)^2 + \frac{\hbar}{2}\omega^i_Q(Q - \delta_Q/2)^2 + \Delta V_0 \quad (22)$$

where R and Q are the effective dimensionless coordinates for the solute and solvent, respectively. The effective frequencies ω_Q and ω_R are evaluated by $\omega = \int\omega P(\omega)d\omega$ in which $P(\omega)$ is the normalized power spectrum of the corresponding contribution to $(\epsilon_2 - \epsilon_1)$. The R is related to the regular reaction coordinate $R' = (b_1 - b_2)$ by $R = R'(\omega_R\mu_R/\hbar)^{1/2}$, where μ_R is the reduced mass for the normal mode that is the compression of b_1 and extension of b_2. The reaction coordinate Q is defined in terms of the electrostatic contribution $(\epsilon_2 - \epsilon_1)_{el}$ to the total $(\epsilon_2 - \epsilon_1)$. Thus, we have $Q = -(\epsilon_2 - \epsilon_1)_{el}/(\hbar\omega_Q\delta_Q)$, which is also related to the regular solvent coordinate, Q', by $Q = Q'(\omega_Q m_Q/\hbar)^{1/2}$. ΔV_0 is the difference between the minima of ϵ_2 and ϵ_1. Here we replace the contribution from each set of coordinates by one effective coordinate. The displacements δ's are related to the so-called reorganization energy, λ, given by

$$\lambda = \lambda_R + \lambda_Q = \sum_i \frac{\hbar\omega^i_r}{2}(\delta^i_r)^2 + \sum_j \frac{\hbar\omega^j_q}{2}(\delta^j_q)^2 \simeq \frac{\hbar\omega_R}{2}\delta_R{}^2 + \frac{\hbar\omega_Q}{2}\delta_Q{}^2 \quad (23)$$

An equivalent and more familiar definition of the solvent coordinate can be obtained in terms of the macroscopic reaction field (ξ_R) at the solute cavity, i.e., taking Q to be proportional to ξ_R we obtain

$$(\epsilon_2 - \epsilon_1)_{el} = (\mu_1 - \mu_2)\xi_R = CQ(\mu_1 - \mu_2) \quad (24)$$

where μ_1 and μ_2 are the dipole moments of the solute for the corresponding diabatic states, and $Q = \xi_{||}/C$ (here $\xi_{||}$ is the projection of ξ on $(\mu_1 - \mu_2)$).

With the above definition we can describe the energetics of the S_N2 reaction in DhlA in the solute−solvent coordinate system. Such a description is given in Figure 4, which was obtained by evaluating the solute and the solvent reorganization energies. Figure 4a shows the total adiabatic reaction profile for the enzyme and water reaction (red and blue lines respec-

(70) Guimaraes, C. R. W.; Repasky, M. P.; Chandrasekhar, J.; Tirado-Rives, J.; Jorgensen, W. L. Contributions of conformational compression and preferential transition state stabilization to the rate enhancement by chorismate mutase. *J. Am. Chem. Soc.* **2003**, *125*, 6892−6899.
(71) Marti, S.; Andres, J.; Moliner, V.; Silla, E.; Tunon, I.; Bertran, J. Transition structure selectivity in enzyme catalysis: a QM/MM study of chorismate mutase. *Theor. Chem. Acc.* **2001**, *105*, 207−212.
(72) Warshel, A. Electrostatic origin of the catalytic power of enzymes and the role of preorganized active sites. *J. Biol. Chem.* **1998**, *273*, 27035−27038.
(73) Kurz, J. L.; Kurz, L. C. Anomalous Selectivities in Methyl Transfers to Water − an Explanation Using Free-Energy Surfaces Which Model the Effects of Nonequilibrium Solvation. *Isr. J. Chem.* **1985**, *26*, 339−348.

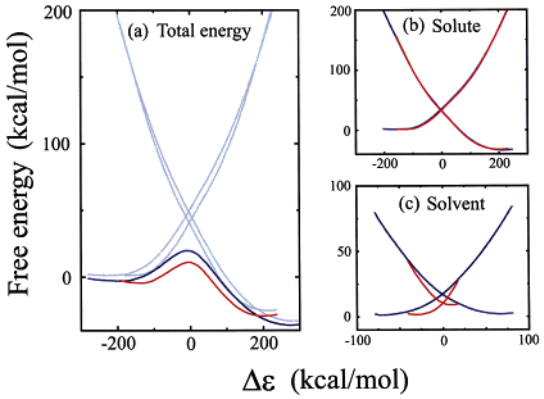

Figure 4. Description of the free energy surface of the S_N2 reaction step in DhlA (red lines) and in water (blue lines) in terms of generalized solute and solvent coordinates: (a) total free energy function for the enzyme and water system (red and blue, respectively); (b) solute and (c) solvent components are depicted to the right. As seen from the figure, the difference between free energy surfaces of the enzyme and water reaction is due to the difference along the solvent coordinate (which reflects the change in λ_Q).

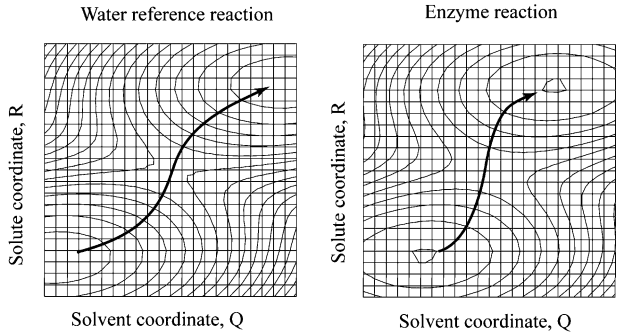

Figure 5. Showing the solute−solvent surface for the enzyme and water reaction. The dynamic behavior is similar for the two systems and the difference in solvent coordinate reflects smaller reorganization energy for the enzyme (whereas the solute coordinate is identical). The arrows indicate a schematic reactive trajectory for each case.

tively; the diabatic curves are depicted in gray). As seen from Figure 4b,c, the solute reorganization energy is almost identical in the two cases, whereas the solvent reorganization energy in the enzyme is considerably smaller than in the solution, 22 and 69 kcal/mol, respectively. The effect of this reduction can be better approximated by reactions with $\Delta G^\circ \approx 0$ and is given by

$$\Delta g^{\ddagger} \simeq \frac{\lambda}{4} - H_{12} \qquad (25)$$

This means that simply considering the solvent reorganization energy gives a reduction of about 12 kcal/mol, which is close to the catalytic effect of DhlA. Of course this effect is directly related to the LRA results of Table 2. The similarity of the solute reorganization energy in the protein and in the solution is a general feature of enzyme active sites and has also been found in other systems. Though the RS geometry is frequently different in the two cases, the solute reorganization energy is often very similar. This point can also be understood by considering the fact that the NAC energy is small as pointed out in ref 14.

Another way to look at the free energy surface in terms of its solute and solvent coordinates is provided by Figure 5, which demonstrates that the main difference between the enzyme and solution surfaces is associated with the corresponding reorganization energies (which are related to the Q coordinate).

The finding that the catalysis involves reduction of the reorganization energy is consistent with the idea that the enzyme is preorganized with its permanent dipoles already partially pointing toward the transition-state charge distribution.[4] In other words, the reaction in water involves a relatively large penalty since the solvent have to reorient itself during the change of the substrate charge from its reactant to product distributions. It is important to recognize, however, that the reduction of λ is associated with a fixed polar environment rather than with a nonpolar environment, which instead would lead to reactant state destabilization.

IV. Analyzing the Dynamical Effects in DhlA and Solution

With the solute−solvent coordinate concept, we can now start to analyze the dynamical behavior of the system. This will be done here by examining the dynamics according to different feasible definitions.

IV.1. Defining Dynamical Effects. As discussed in section II, some of the most rigorous treatments of rate theories assigns all the dynamical effects to the transmission factor, e.g., refs 55 and 59. Comparative studies of the magnitude of the transmission factors in enzymes and the corresponding reactions in solutions indicate that these transmission coefficients are similar within a factor of 2.[3,32,34,35] Thus, by this definition dynamical effects do not contribute to catalysis in a significant way (catalytic effects involves changes of sometimes more than 10 orders of magnitude).[2,3,32] Nevertheless, it is useful to be more open minded in defining dynamical effects and look for some less restrictive definitions. Perhaps the most obvious definition of dynamical effects is to see if the rate constant is associated with coherent vibrations that do not obey the Boltzmann distribution. Inversely, it is reasonable to assume that the rate constant does not involve any dynamical effects if we can evaluate it by simply using a Monte Carlo (MC) procedure to calculate the activation free energy (the MC does not require any MD simulation). Other definitions of dynamical effects are much "softer". For example, reactions in solution and in enzymes could involve qualitatively different mixtures of solute and solvent coordinates. The solvent coordinates could be more (or less) frozen in a protein than in solution, and one may then argue that this situation cannot be analyzed simply in terms of the overall activation free energy. In fact, it has been implied that nonequilibrium solvation effects are associated with dynamical effects. This proposal will be considered briefly in section IV. Other proposals implied that the reorganization relaxation is a dynamical effect that plays an important role in catalysis. This will be considered in section V.

Although we will examine the above ideas, we would like to clarify that even though enzymatic reactions unavoidably involves atomic motion, it cannot be considered as a dynamical effect since such motion is involved in all chemical reactions at room temperature. Thus, we require that the motion is different in the enzyme and solution reaction and that this motion is not simply associated with the Boltzmann probability of the activation barrier.

IV.2. Autocorrelation of the Energy Gap. The EVB approach provides a powerful description of enzymatic reactions

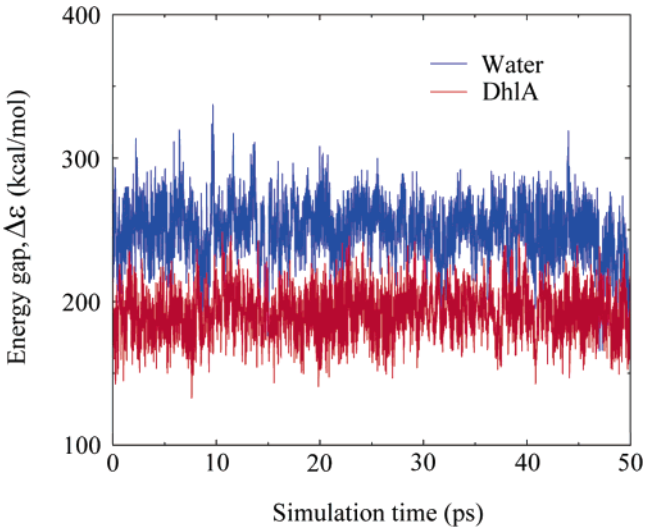

Figure 6. Fluctuations of the energy gap for the S_N2 step of the DhlA reaction evaluated for trajectories at the RS. The figure gives the gap for the reacting system in the enzyme (red) and in water (blue).

in terms of the fluctuating energy gap. When the solvent or the protein fluctuates it can stabilize or destabilize the product state (relative to the reactant state) and, thus, modulate the chance that the solute will move to the product state.[33] The calculated time dependence of the energy gap is depicted in Figure 6 for the protein and water systems, respectively, in the RS region. As seen from Figure 6, the fluctuations of the solvent coordinates in both the enzyme and solution reactions look quite similar. However, to quantify the similarity between the two sets of fluctuations it is essential to examine the autocorrelation of the energy gap, $C(t)$. This autocorrelation has been introduced in our early works as a tool for getting a qualitative estimate of the transmission factor (see section II). Furthermore, the use of this autocorrelation is also well-known in solvation dynamics.[63,74−77] Our analysis of $C(t)$ will be described below.

Before we compare $C(t)$ in DhlA and in solution it is useful to consider a related recent work of Nam et al.[36] This study used a QM/MM molecular orbital approach, which does not provide a diabatic energy gap or $C(t)$. Instead, the force autocorrelation was evaluated, $C(t)_F$, which is a valid but somewhat less direct measure of the solvation dynamics than $C(t)$. It was found that $C(t)_F$ relaxes more slowly in water than in DhlA. Furthermore, the $C(t)_F$ of the enzyme also showed some oscillations. The finding that $C(t)$ can be somewhat different in the enzyme and in water is not new and has been reported by Villa and Warshel.[3] Unfortunately, the study of ref 36 did not provide a separate analysis for the solute and solvent coordinate. Such an analysis is quite challenging when using standard QM/MM studies. In particular, attempts to get the solute contribution by omitting the electrostatic contribution in the QM/MM Hamiltonian is not so useful since it gives the gas-phase results, which are very different than the proper behavior of the solute in solution (see discussion of a similar problem in ref 10). The EVB, on the other hand, provides a simple way of separating the fluctuations of the energy gap into its solute and solvent components and of evaluating the corresponding autocorrelation functions. The analysis of the EVB energy gap is provided in Figure 7 for the reaction of DhlA (red lines) and for the corresponding water reference reaction (blue lines) both at the RS and TS regions (parts a and b of Figure 7, respectively). Figure 7 provides in each case the autocorrelation function of the total energy gap, $C(t)$, and the autocorrelation of the electrostatic contribution, or solvent coordinate, $C(t)_{el}$ (upper and lower graphs, respectively). It turns out that the result is rather sensitive to small changes in parameters and depends on initial conditions even though the system has been equilibrated thoroughly before running the trajectories for the autocorrelation and the trajectories are simulated for a long time with small time step. Therefore, we have depicted the autocorrelation function for two trajectories for each protein and water case that is representative to what we obtain. As seen from Figure 7, the $C(t)$ of both systems have in general an almost identical decay. However, the water system has a somewhat slower second component in one of the runs when considering the total energy at the RS region (the upper graph in Figure 7a). This is quite similar to what was reported by Nam et al. for the autocorrelation of the force at the transition state.[36] The difference between the slow components becomes much smaller when one examines the autocorrelation of the solvent coordinate. Thus, the slow component primarily reflects the solute motion. It should also be pointed out that our analysis of trajectories in the transition state region and the electrostatic component of either region are much more stable and show much smaller differences between the water and DhlA system. Furthermore, the total $C(t)$ show some larger solute oscillations in the case of the enzyme. The difference in the Franck−Condon factors of the solute in the enzyme and water systems may reflect the confinement effect of the enzyme. However, in a previous study[14] it was found that the corresponding catalytic effect is rather small.

Considering the above results we may clarify several misunderstandings in ref 36. (i) As much as the solvent coordinate is concerned, the enzyme and the solution dynamics are quite similar. (ii) The assumption that the slower relaxation in the water case contributes significantly to catalysis is problematic. First, using eqs 13 and 14 one finds that the difference between the characteristic downhill times of the reaction in the enzyme and solution has a trivial effect on the rate constants (relative to the effect of the activation barriers). Incidentally, all workers in the dynamics community, e.g., ref 76, expect that the autocorrelation in the enzyme will have a slower decay than in solutions, and water is known to be a fast solvent rather than a slow solvent.

IV.3. Spectral Distribution Analysis Using the DP Model. After analyzing the $C(t)$ of the solvent coordinate we can upgrade our analysis and examine the actual spectral distributions of the enzyme and solution reactions. This is done here by the dispersed polaron (spin-boson) analysis described in section II, and the corresponding results are summarized in Figure 8, which gives the projection of the vibrations of the system along its reaction coordinate and thus tells us what vibrations have to be "excited" during the reactive event.

(74) Maroncelli, M.; Fleming, G. R. Computer-Simulation of the Dynamics of Aqueous Solvation. *J. Chem. Phys.* **1988**, *89*, 5044−5069.
(75) Fleming, G. R.; Wolynes, P. G. Chemical-Dynamics in Solution. *Phys. Today* **1990**, *43*, 36−43.
(76) Nandi, N.; Bhattacharyya, K.; Bagchi, B. Dielectric relaxation and solvation dynamics of water in complex chemical and biological systems. *Chem. Rev.* **2000**, *100*, 2013−2045.
(77) Pal, S. K.; Peon, J.; Zewail, A. H. Ultrafast surface hydration dynamics and expression of protein functionality: alpha-Chymotrypsin. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, **99**, 15297−15302.

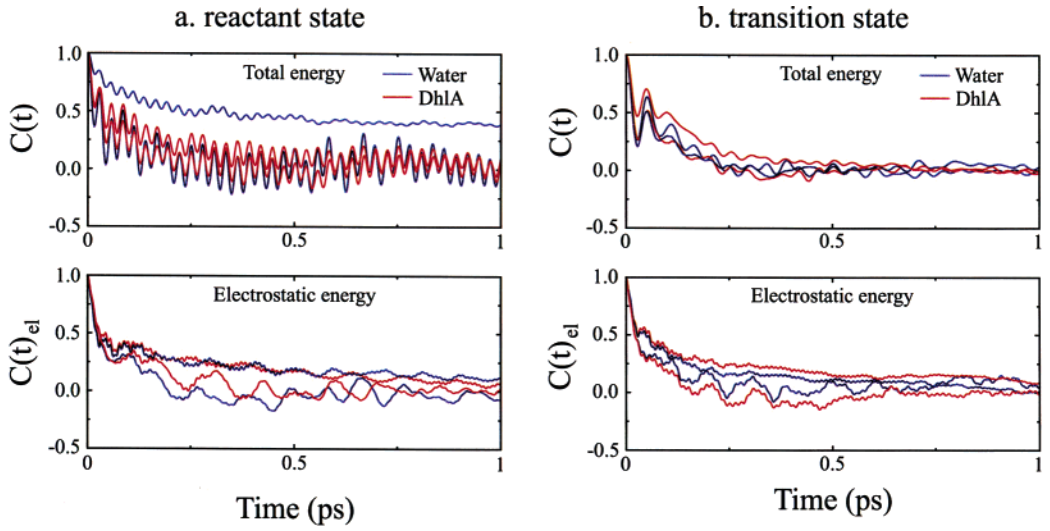

Figure 7. Autocorrelation of the energy gap for the reaction of DhlA and the reference reaction. The figure provides the analysis for the total energy gap, $\epsilon_1 - \epsilon_2$ (top) and for the electrostatic contribution to the gap (bottom) at the reactant state (a) and the transition state (b). Each graph shows two trajectories for each system to show the stability of the different systems.

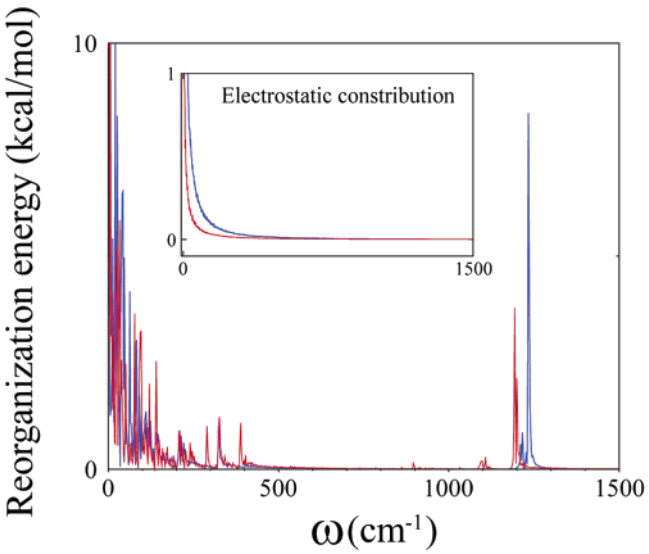

Figure 8. Dispersed polaron spectral distribution analysis of the reaction of DhlA (red spectrum) and the solution reference reaction (blue spectrum). The analysis is given both for the full solute−solvent system and for the solvent coordinate (inset).

Overall, and in agreement with our previous finding,[3] we see that the solute coordinate has more high-frequency reactive vibrations in the enzyme than in the solution reaction. This represents some confinement effects that will be discussed below. However, the most relevant comparison should be done in terms of the solvent coordinate. Here we find that the spectral distribution is similar in the enzyme and solution reaction with the exception of a few modes that may be due to the vibration of Trp175 and Trp125 (note that we can determine the nature of the relevant vibration,[78] but this is out of the scope of the current work). The main point, which will also be illustrated below, is that the spectral distribution of the solvent coordinate is overall quite similar in the protein and solution reactions. The fundamental difference is not in the detailed distribution but in its integrated effect, which is exactly the solvent reorganization energy (see eq 19).

IV.4. Downhill Dynamics. After considering all the above indirect definitions of the dynamical effects it is useful to move to the most direct definition, which is obtained by simply monitoring the dynamics of the reactive trajectories on the solute solvent coordinate system. This is done here by propagating downhill trajectories from the TS. The time reversal of these trajectories gives exactly the reproductive trajectories that goes through the TS and reacts. Thirty such trajectories are depicted for the water and enzyme reaction in Figure 9 by running 200 fs directly on the adiabatic surface (starting from the TS, where $\Delta\epsilon \sim 0$, half of the trajectories will go to the reactant state and half to the product state). This picture reflects non coherent dynamics and shows similar behavior for the water and enzyme reactions. Note that the different scale of the *x*-axis reflects the different solvent reorganization energy, whereas the solute reorganization energy is similar in the two cases.

The fact that the solute reorganization energy does not change significantly between the enzyme and solution reaction is not a special feature of the EVB model (although it might be difficult to separate the reorganization energy into solute and solvent contributions with other approaches). The fact that the position of the solute TS in the enzyme and in solution is similar is confirmed by results obtained by other approaches.[70,71] The structure in the ground state is different in the enzyme and in solution but the energy contribution associated with this difference has been shown to be small in analysis of the NAC effect.[14]

At this point it might be useful to comment on the proposal that nonequilibrium solvation effects are different in enzyme and in solution and that this can lead to dynamical contributions to catalysis.[34] This issue has been analyzed in great length, e.g., refs 3 and 60, and it has been clarified that the so-called nonequilibrium solvation (NEQS) effects are actually probabilistic equilibrium effects. The special name reflects the fact that the activation barrier involves rearrangement of the solvent coordinate that is not captured by calculations that only use the solute coordinate in evaluating the potential of mean force (PMF). On the other hand, the activation free energy evaluated by consistent calculations using eq 6 includes the nonequilibrium

(78) Warshel, A.; Chu, Z. T.; Parson, W. W. Dispersed Polaron Simulations of Electron-Transfer in Photosynthetic Reaction Centers. *Science* **1989**, *246*, 112−116.

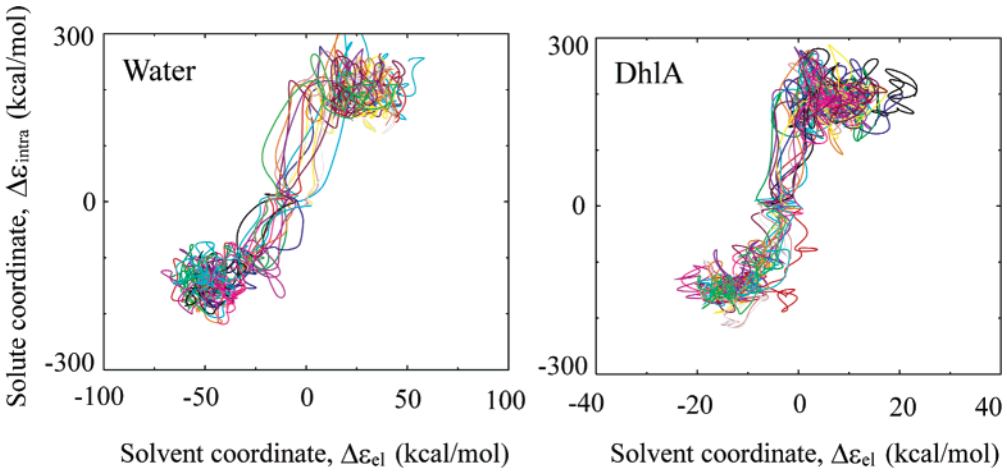

Figure 9. Behavior of downhill trajectories running for 200 fs on the ground-state EVB surface for the DhlA system (b) and the water reference system (a). The figure shows the trajectories separated into solvent and intramolecular solute components. The time reversal of these trajectories corresponds to the actual reactive trajectories.

solvation effect. Now, some workers assume that the PMF obtained while forcing only the solute coordinates to react is the activation barrier that should be used in the TST equation (e.g., ref 34). This makes the nonequilibrium solvation effect a part of the transmission factor and thus a dynamical effect. This is simply the result of an inconsistent analysis since the NEQM effect is an integral part of $\Delta g^{\ddagger}$. The contribution of the NEQS effects is thus a well-defined nondynamical free energy contribution (for example in the case of electron transfer reactions where it is the Marcus activation free energy) and its difference between the solution and enzyme reactions simply reflects the difference in the corresponding reorganization energy (for further discussion, we again refer the reader to ref 3).

Basically, the rate constant depends exponentially on the activation energy and linearly on dynamical effects. Thus, it is much harder for evolution to catalyze reactions by changing dynamical effects rather than by changing the activation barrier. Here, one may argue that there are more reactive trajectories that reach the TS in the enzyme than in solution and that this is a dynamical effect, but the number of reactive trajectories is determined uniquely by the activation free energy (unless there is coherent dynamics) and the catalysis is due to the change in activation barrier.

V. Concluding Remarks

This work examines the nature of enzyme catalysis in terms of a solute solvent coordinate, focusing on the S_N2 reaction of DhlA. The first question that we have addressed is how the enzyme can catalyze such a reaction. This issue is not so trivial since the charge distribution of the substrate is more localized in the RS than in the TS (see Figures 1 and 2). Thus, it is hard for the enzyme to stabilize the TS more than the RS, relative to the corresponding gas-phase reaction (which is, however, not the correct reference state as much as catalysis is concerned). Here, the use of the desolvation idea where the enzyme is supposed to destabilize the ground state by desolving it (e.g., ref 15) does not help. First, it does not help in reducing k_{cat}/K_M, which reflects the binding energy of the TS (see ref 2) and, thus, contradicts the experimentally observed reduction. Second, the actual calculations do not show any RSD effect. Thus, it is important to find out which effect is really used by the enzyme. Our analysis of this key issue established that the enzyme work by solvating the RS and the TS by a similar amount, thus providing a much lower barrier than water where the RS is solvated much more than the TS (see Figure 3). This clearly means that the enzyme operates by controlling the solvation effects, TSS rather than RSD, since the TS is much more stable in the enzyme than in water. This type of TSS appears to be the general way for enzymes in accelerating S_N2 type reactions.

Apparently, ref 20 overlooked the fact that the generalized "solvation" includes all the electrostatic effects and suggests that "Shurki et al. found that there is a greater solvation effect of 6.1 kcal/mol in water than in the enzyme". In fact, ref 14 suggested that the enzyme solvates the TS more, rather than less, than water does and this point is established by the LRA calculations of the present work, Figure 3. Unfortunately, the problem here is more serious than overlooking the statement of ref 14 that the solvation effect include all the protein−solute electrostatic interactions. That is, ref 20 supported explicitly the desolvation mechanism proposed by Bruice and co-workers[13] (who had in mind the correctly defined desolvation proposal) but then evaluated 8 kcal/mol contribution of $\Delta g^{\ddagger}_w - \Delta g^{\ddagger}_g$, which they identified as a key contribution to catalysis. The focus on $\Delta g^{\ddagger}_w - \Delta g^{\ddagger}_g$ is problematic since this quantity has little to do with the catalytic effect that is given by $\Delta g^{\ddagger}_{cat} - \Delta g^{\ddagger}_w$. In other words, since the catalytic effect is due to the difference between the solvation in the protein and solution it is not dependent on the gas-phase energy (which is the same in both cases). Thus, in contrast to the conclusion of ref 20 the catalysis is not related to the desolvation in water or to the desolvation hypothesis. Basically the desolvation of the TS relative to the RS is *smaller* in the protein than in water. Furthermore, as clarified in section III, the desolvation hypothesis has been defined as RSD.[17,69] More importantly, with a correct cycle (Figure 3) it is easy to see that the enzyme indeed solvates the TS more than water does. Finally, the analysis of the type provided in reference 20 is simply unable to address the desolvation hypothesis since this requires reliable calculations of the solvation energy of both the RS and TS in the enzyme and in water. The calculations in the enzyme amount to calculations of the electrostatic contribution to the binding energy (as is done by our LRA treatment).

The next major issue addressed here is the nature of the dynamical effects in the enzyme and solution reactions. Here we have shown that the dynamics does not contribute in a major way to catalysis regardless of the definition used for defining dynamical effects. We believe that the same remain true for other reasonable definitions.

We also demonstrated that τ_Q and the related transmission factor are similar in enzyme active sites and in solutions. Furthermore, we clarified that analysis of enzyme catalysis must consider the role of the enzyme as a special solvent and that this should also be applied to studies of dynamical contribution to catalysis. Overlooking this issue has probably led to the conclusions of ref 36. More specifically, Nam et al. did not separate the reaction coordinate to solute and solvent coordinate and thus did not realized that the dynamics of the effective "solvent" coordinate is similar in the enzyme and in water and that both environments should be considered as solvents. In fact, the dynamics of the solvent coordinate in both enzymes and in the corresponding water reactions involve a very fast initial relaxation (about 30 fs) and sometimes a slower component of a few picoseconds. The "slow" relaxation has, however, little to do with catalysis. That is, the reaction rate is determined mainly by the time to generate a reactive trajectory which is then scaled by the time it takes the reactive trajectory to reach the barrier. The first factor (which is determined by the reorganization energy rather than the solvent relaxation) can vary by many orders of magnitude between the water and enzyme reaction. The second factor is in the picoseconds range in both enzymes and solutions and thus does not affect the catalysis.

In summary, the dynamics of the reaction appear to be similar for the enzyme and solution reaction. The speed of the downhill relaxation is similar for both systems and the main difference is the "solvent" reorganization energy and the corresponding $\Delta g^{\ddagger}$s. Another way to look at this finding is to realize that the electrostatic coupling between the environment and the solute has a similar nature and similar overall spectral distribution but the main difference is in the amplitudes of these modes and the corresponding reorganization energy. It should also be noted that the rate constant depends exponentially on the activation energy and linearly on dynamical effects. Thus, it is much harder for evolution to modify enzyme catalysis of reactions by changing dynamical effects than by changing the activation barrier. Again, even though the number of reactive trajectories that reach the TS is larger in the enzyme than in solution, it is not an effect of protein dynamics, but determined uniquely by the lower activation free energy.

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