How Important Are Quantum Mechanical Nuclear Motions in Enzyme Catalysis?

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Abstract: The role of quantum mechanical nuclear motions in enzymatic reactions is examined by realistic simulations that take into account the fluctuations of an entire enzyme−substrate complex. This is done by using the quantized classical path (QCP) approach which is based on Feynman's path integral formulation. The calculations evaluate the quantum mechanical activation free energy and deuterium isotope effect for the proton transfer step in the catalytic reaction of carbonic anhydrase. The calculated and observed isotope effects are in very good agreement, thus demonstrating the potential of our approach in extracting mechanistic information. Furthermore, the value of the calculated quantum mechanical rate constant is in a good agreement with the corresponding observed value. This is significant since the evaluation of the ratio between the quantum mechanical rate constants of the reaction in the protein and in aqueous solution does not involve any adjustable parameter. The reliability of our calculations is based on the use of the empirical valence bond (EVB) method. This method does not try to represent the potential surfaces of the reacting atoms by a first principle approach (this is easily done by fitting the EVB surface to experimental and theoretical results) but rather evaluates the effect of moving these atoms from solution to the enzyme active site. The possible catalytic advantage of quantum mechanical nuclear motions is examined by comparing these effects in the enzyme and in a reference solution reaction. It is found that while quantum mechanical corrections to activation free energies of enzymatic reactions can be quite large they are not drastically different than the corresponding corrections in solution. Apparently the largest catalytic effects are due to reduction in the reorganization energy and ΔG_0 by the electrostatic effects of the preorganized environment of the protein active site. Nevertheless, small but non-negligible catalytic contributions can be associated with quantum mechanical effects.

Introduction

Many enzymatic reactions and other fundamental biological processes involve transfer of protons or hydrides. Since these transferred ions are very light, it is reasonable to expect that their motion involves significant quantum mechanical effects. In fact, the existence of tunneling effects has been implicated in several enzymatic reactions (e.g., refs 1 and 2). Understanding the quantum mechanical nature of light-atom motion is crucial for a more complete description of enzyme catalysis. In particular, it is interesting to know whether the enzyme active site can "catalyze" reactions by enhancing quantum mechanical tunneling and other quantum mechanical effects. In exploring this issue it is important to find ways to deduce the effective height and width of the reaction barrier in the actual enzyme active site. It is also important to be able to examine the effect of enzyme fluctuations on the reaction barrier and on the corresponding quantum mechanical corrections of the barrier.

While structural, kinetic, and biochemical information are crucial for progress in this direction, it is also essential to find some quantitative way for calculating quantum mechanical rate constants in enzyme active sites. Such an approach should be important not only in trying to explore the possible existence of quantum mechanical catalytic effects but also, perhaps more importantly, in allowing one to correlate the observed isotope effect with the mechanism of action of the given enzyme.

Computer simulation approaches can provide, in principle, the rate constants of enzymatic reactions.[3] However, obtaining reliable quantum mechanical rate constants in condensed phases in general and in enzyme active sites in particular is a major challenge. Significant progress has been made in addressing the related problem of electron transfer (ET) reactions in solution and proteins.[4−9] However, proton transfer (PT) and hydride transfer (HT) reactions present a greater challenge since the coupling between the reactant and product electronic states is very large. In this limit, which is referred to as the *adiabatic* limit, one cannot exploit powerful tricks (e.g., the dispersed polaron approach[4] or the basically identical spin boson approach[6,8]), which are applicable in the weak coupling (*diabatic*) limit. Nevertheless, significant effort has been devoted to the search for effective methods for evaluation of quantum mechanical rate constants for adiabatic reactions in the condensed phase.[10−18] These studies include the work of ref 14b which

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provided what is to the best of our knowledge the first simulation of proton transfer reaction in solution and evaluated the corresponding activation free energy using a path integral formulation.

Recently we proposed a practical approach to calculate quantum mechanical rate constants.[14,15] This method, referred to as the quantized classical path (QCP) method, utilizes classical trajectories to obtain the quantum mechanical rate constant through a practical yet reliable approximation of the path integral centroid formulation.[17,18] The QCP method has been verified in studies of exactly solvable model systems[15] and was also used with the empirical valence bond (EVB) potential surfaces in studies of HT and PT in solutions.[14b,15,16] This method was also used in a preliminary computer simulation of an HT reaction in a protein.[14a] Similarly, the original centroid method[17,18] with the same type of EVB potential surfaces has also been used in studies of PT reactions in solution.[12] Both approaches provided encouraging results (see below), but their powerful potential has not been exploited in systematic simulations of enzymatic reactions. In this work, we employ the QCP method to evaluate the isotope effect on the proton-transfer step in the catalytic reaction of carbonic anhydrase.

Simulation Methods

The starting point for the QCP approach is the observation that the quantum mechanical rate constant can be usually approximated by[17,18]

$$k_q = F\frac{k_B T}{h}\exp(-\beta\Delta g_q^{\ddagger}) \qquad (1)$$

When F is the transmission factor, k_B is the Boltzmann constant, T is the temperature, h is the Planck constant, and $\beta = 1/k_B T$. $\Delta g_q^{\ddagger}$ is the quantum mechanical activation free energy (q designates here "quantum mechanical"). The remarkable point about eq 1 is that the preexponential factor is approximately the same as in the classical rate constants. Thus the main quantum mechanical effects are associated with the probability factor $\exp(-\beta\Delta g_q^{\ddagger})$. Thus the task of evaluating the quantum mechanical rate constant is reduced to the evaluation of the quantum mechanical probability of being at the transition state. This probability factor can be evaluated by using Feynman's path integral approach where each quantum particle is represented by a "ring" of p quasiparticles, which are subjected to the effective "quantum mechanical" potential.

$$U_q = \sum_{k=1}^{p}\frac{1}{2p}M\Omega^2\Delta x_k^2 + \frac{1}{p}U(x_k) \qquad (2)$$

where $\Delta x_k = x_{k+1} - x_k$, $x_{p+1} = x_1$, $\Omega = p/\hbar\beta$, M is the mass of the particle, and U is the actual potential surface of the system (this potential is used in classical simulations). The quantum mechanical partition function can then be obtained by running classical trajectories where the quasiparticles experience the potential U_q. As pointed out by Gillan[17] and by Voth and coworkers,[18] it is possible to find the probability of being at different points along the reaction coordinate by evaluating the probability distribution for the center of mass of the quasiparticle ring, which is also referred to as the "centroid" of the system. The use of quasiparticles is a computational device whose relationship to quantum effects is not so straightforward (except, of course, that it works). Nevertheless, we provide in Figure 1 a qualitative rationalization for the fact that the path integral approach is able to evaluate quantum mechanical effects. The figure compares the classical (Figure 1a) and quantized (Figure 1b) description of a particle in a double well potential surface. As illustrated by the figure, a classical particle with a total energy $E < U^{\ddagger}$ cannot pass from the left to the right side of the potential since its energy is lower than the value of the potential at the transition state, $x^{\ddagger}$. On the other hand, a quantum mechanical particle can penetrate or "tunnel" through the barrier since each of the quasiparticles only experiences the potential $U(x_k)/p$ rather than $U(x_0)$. (Note, however, that when p increases we have on average less energy per particle). The only reason the tunneling does not occur so readily is the restoring force of the $M\Omega^2\Delta x_k^2/2p$ term that "connects" the quasiparticles to each other. This term keeps the quasiparticles close to each other at high temperature (small β), and when M is large, the system behaves classically. However, at low temperatures and when M is small, the quasiparticles can spread, and some of them can penetrate the barrier. The quantum mechanical probability that the system will reach $x^{\ddagger}$ is given by the chance that the center of mass of the quasiparticle ring will be at this point. Similarly the centroid path integral approach reproduces the quantum mechanical effect of the zero point energy. That is, in the classical limit at low temperature the particle will relax to x_0. On the other hand, in the quantum limit the systems will

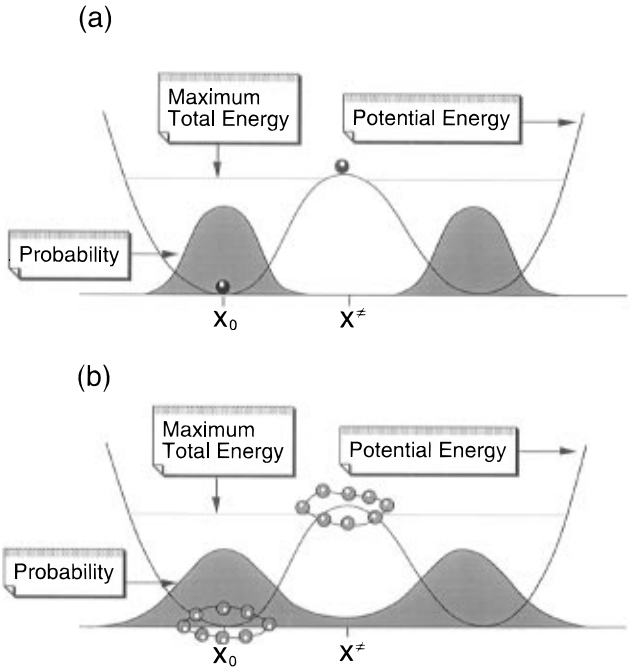

Figure 1. Schematic diagrams of the behavior of classical (a) and quantized (b) particles on a double-well potential surface. In order to make the discussion simple we only consider the contribution to the probability distribution from energies that are smaller than the barrier height, $\rho(x) = \int_0^{E_{max}} P(x,E)\exp(-\beta E)dE$ with $E_{max} < U(x^{\ddagger})$, where $P(x,E)$ is the probability to be at the given x with the designated energy (i.e., the probability obtained by running a trajectory with the given E). This probability distribution is represented by a shaded area. The upper figure (a) represents the classical particle by a single sphere. This particle has zero probability to be at $x^{\ddagger}$ for $E < U(x^{\ddagger})$. The lower figure (b) describes the quantum mechanical particle by a ring of beads. When the particle is at the transition state region, the beads can be at points with energy lower than $U(x^{\ddagger})$, and this allows the particle to be at $x^{\ddagger}$ and to tunnel through the barrier (in this case we have nonzero probability to be at $x^{\ddagger}$). When the particle is at the bottom of the potential well the beads can see points with potential higher than $U(x_0)$. This dispersion is reflected by the zero point energy.

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always have nonzero potential energy when the centroid position is at x_0, since some of the quasiparticles will be at $x_k \neq x_0$. Thus, at low temperature the beads can be at points whose potential energy is larger than $U(x_0)$ and will have larger average potential energy than the corresponding classical particle. This dispersion is reflected by the zero point energy.

Actual calculations of centroid probabilities in the condensed phase reactions are very challenging and may involve major convergence problems. The QCP approach offers an effective and rather simple way for evaluating this probability without significantly changing the simulation program. This is done by propagating classical trajectories on the classical potential surface of the reacting system and using the positions of the atom of the system to generate the centroid position for the quantum mechanical partition function. This treatment is based on the finding that the quantum mechanical partition function can be expressed as[15,19]

$$Z_q(\bar{x}) = Z_{cl}(\bar{x}) \langle\langle \exp\{-(\beta/p) \sum_k U(x_k) - U(\bar{x})\}\rangle_{fp}\rangle_U \quad (3)$$

where $\bar{x}$ is the centroid position, $\langle\rangle_{fp}$ designates an average over the free particle quantum mechanical distribution obtained with the implicit constraint that $\bar{x}$ coincides with the current position of the corresponding classical particle, and $\langle\rangle_U$ designates an average over the potential U. Using eq 3 we can obtain the quantum mechanical free energy surface by evaluating the corresponding probability by the same combined free energy perturbation umbrella−sampling approach that has been repeatedly applied in our classical simulations (see ref 3) and also in our quantum mechanical simulations (e.g. ref 14b), but now we use the double average of eq 3, rather than an average over a regular classical potential. The actual equations used in our free energy perturbation (FEP) umbrella sampling calculations are given elsewhere,[15] but the main point of the QCP is that one can evaluate the quantum mechanical free energy function by a centroid approach which is constrained to move on the classical potential. This provides stable and relatively fast converging results which have been shown to be quite accurate in studies of well defined test potentials (where the exact quantum mechanical results are known[15]).

Equation 3 provides what is formally the rigorous centroid path integral result, although the simple form of this equation led some to believe that this is an approximated expression such as the effective potential of Feynman and Hibbs.[21] It should also be noted that the main idea behind our approach is not the derivation of eq 3 but the use of classical MD simulation over x_p to obtain the quantum free energy by performing fp average. This practical idea whose simplicity might be confused with triviality provides a practical way for efficient calculations of quantum mechanical activation free energies using standard simulation programs (e.g., ENZYMIX[33]) without changes except the addition of a single subroutine.

With the Z_q of eq 3 we can calculate the activation free energy of eq 1 using

$$g_q(\bar{x}) = \beta^{-1} \ln Z_q(\bar{x})$$

$$\Delta g_q^{\ddagger} = g_q(\bar{x}^{\ddagger}) - g_q(\bar{x}_a) \quad (4)$$

where $\bar{x}_a$ is the position of the minimum of the g_q curve. This provides a direct way for estimating quantum mechanical rate constants in solution and proteins.

The reliability of the QCP approach and related path integral calculations of the proton transfer reactions in solution have been established to a reasonable level of confidence (see footnote 22).

Simulating the Proton Transfer Step in the Reaction of Carbonic Anhydrase

In order to demonstrate and test our approach we chose the catalytic reaction of carbonic anhydrase (CA). This enzyme catalyzes the reaction

$$CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+ \quad (5)$$

Extensive biochemical (e.g., refs 23−25), structural (e.g. refs 25−27), and theoretical studies (e.g., refs 28−31) help to clarify the reaction mechanism. It is very likely that the rate limiting step in the "hydration" reaction is a proton transfer from a zinc bound water to His 64 through one or more water molecules.[23] According to our pK_a calculations (which are quite reliable as is demonstrated elsewhere, e.g., ref 33) the highest barrier in this process involves a PT between the zinc bound water (W_1) to its neighboring water (W_2) molecule ($\Delta pK_a \sim 7$), while the PT between the second water and His 64 is exothermic ($\Delta pK_a \sim -6$). This situation is illustrated in Figure 3 of ref 28a. The actual mechanism might involve a stepwise process of PT between the two water molecules (W_1 and W_2) and then PT from W_2 to His 64 or a more concerted process (see related proposal for the back-reaction in ref 23b). Since the present work does not try to determine the ultimate mechanism of CA we focus on the stepwise mechanism (see more discussion below) and consider the step with the highest barrier, which can be written formally as

$$(2H_2O)^{\text{active site}} \rightleftharpoons (OH^- + H_3O^+)^{\text{active site}} \quad (6)$$

Obviously as always we treat this in the presence of all its zinc and all the rest of the enzyme and solvent molecules. Steiner et al.[24] have observed an isotope effect of 3.8 on k_{cat} of human carbonic anhydrase II. This provides further support to the idea that the rate limiting step is a PT reaction. Here we start with the crystal structure of CA and examine the performance of

(19) Equation 3 is closely related to an expression developed by Doll and Myers.[20] However the elegant proposal of ref 20 was developed for ground state partition functions and not for calculations of rate constants by centroid approaches. Furthermore, our approach and the work described in ref 14b have introduced the use of FEP/umbrella sampling methods to path integral centroid calculations of rate constants. We consider this to be quite useful and like to point out that many works in this field developed effective simulation approaches for implementation of the original idea of Feynman, rather than invented a new quantum mechanical concept.

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(22) The use of the QCP approach and the EVB potential surfaces[3,32] has reproduced the experimentally observed dependence of isotope effects on pK_a.[15] This method also reproduced the quantum mechanical temperature dependence of well defined test cases where the corresponding exact results are known.[15] Furthermore, related centroid studies of Voth and co-workers[12] that used the EVB potential surfaces were applied successfully to studies of PT reactions in solutions. As to the EVB potential itself; it has been used extensively in studies of proton transfer reactions in solution and proteins and reproduces the observed rate constants,[32] with the implicit assumption that the quantum mechanical corrections are similar in the active site and in the corresponding reference solvent case. Since some readers might be concerned about the "empirical" nature of the EVB approach, it is important to note recent demonstrations[40] that the EVB potential surface correctly reproduces the corresponding ab initio surfaces.

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our approaches by simulating the reaction and calculating the corresponding isotope effect.

In order to model the energetics of our enzymatic reaction, we used the empirical valence bond (EVB) approach. This powerful approach which is described in detail elsewhere (e.g. refs 3 and 32) might look to some as too simple to be reliable. We find it useful to point out that the close relationship between the EVB and the corresponding ab initio surfaces has been established elsewhere (e.g., ref 40). Some might still wonder how we can describe an enzyme by such an approach when other methods have difficulties in obtaining reliable potential surfaces for triatomic molecules. However, the EVB does not attempt to evaluate potential surfaces of reacting fragments but instead fits them to known theoretical or experimental results. Then the method focuses on the real issue, which is the effect of changing environment on the quantum mechanical region. Perhaps more convincing for the skeptics and critics could be the fact that this approach has now been adopted by several research groups (e.g., refs 12 and 45−47).

The EVB treatment of the PT reaction of eq 6 included in the quantum mechanical active space the two reacting water molecules, which were represented by two resonance structures[32]

$$\psi_1 = [H_2O \qquad H_2O]$$

$$\psi_2 = [OH^- \quad H_3O^+] \tag{7}$$

while treating the rest of the system classically. It has been shown before that other valence bond structures corresponding to the higher energy configurations could be effectively incorporated into these structures.[3] The diabatic potential function of the *i*th structure was expressed in the following form[28]

$$\epsilon_{cl}^{i} = \sum_j \Delta M_j^{(i)}(b_j^{(i)}) + \sum_j \xi_j^{(i)} K_j^{(i)}(\theta_j - \theta_{0,j}^{(i)})^2 + V_{SS}^{(i)} + V_{Ss}^{(i)} + V_s + \alpha^{(i)} \tag{8}$$

where b and θ designate bond length and bond angle, respectively, while S and s designate solute and solvent, respectively. Here, the first term $\Delta M_j^{(i)}$ denotes the Morse potential corresponding to the jth bond in the ith valence bond structure, the second term describes the bond angle bending interactions. The factor $\xi_j^{(i)}$ in the second term is a coupling between bonds that are being broken or formed and those angles depending on those bonds. The third and fourth terms represent the solute−solute and solute−solvent nonbonded interactions, while the fifth term represents the solvent−solvent interaction potentials. The $\alpha^{(i)}$ term accounts for energy difference between ψ_1 and ψ_2 with the reacting fragments at an infinite separation in the gas phase.[3] These parameters were adjusted so that they could reproduce the solvation free energies of the reacting species, i.e., H_2O, H_3O^+, and OH^-. Molecular dynamics (MD) simulations were performed by a version of the program ENZYMIX[33] which included the QCP option. The reaction regions, i.e., the two water molecules, were described by a closed string of 20 beads for each atom, and the surrounding environments were treated classically. The parameters of the reacting species, i.e., H_2O, H_3O^+, and OH^- , were those used in the standard EVB library of ENZYMIX. The Zn(II) metal at the active site was treated with the octahedral 1 + 6 center model.[34] The crystal structure of carbonic anhydrase I[26] was equilibrated for 20 ps and then used as the starting configuration for the simulations. The EVB region was completed to a 16 Å sphere of SCAAS water molecules,[33] surrounded by a 18 Å spherical grid of Langevin dipoles. Solvation outside the Langevin grid was treated by a continuum model.[33] Long range electrostatic interactions were treated by the local reaction field (LRF) method.[35] All calculations were done at a temperature of 300 K and a step-size of 2 fs. A typical trajectory time for the simulation of each mapping state was around 8 ps, and the total trajectory time for one complete run was about 80 ps. The final results were the average of those of forward and backward mapping. The calculations were performed on IBM RISC/6000 3BT and 590. The convergence and stability of our approach is discussed in ref 36.

In addition to simulating the reaction in the protein active site we also simulate a reference reaction of the two water molecules of eq 6 with a zinc ion in a solvent cage. To clarify a common confusion it is important to realize that this reference reaction is *not* the actual noncatalyzed reaction in water. Our reference reaction is (and always has been) a hypothetical reaction where the same mechanism assumed for the enzyme is considered with the active site replaced by a solvent cage. The thermodynamic cycle that compares the enzymatic reaction and the corresponding reference reaction allows us to eliminate the somewhat trivial and unnecessary confusing issue of the relationship between concentration to the probability of being in the solvent cage (see problem 5.1 in ref 3). Moreover it provides a unique way of defining catalytic effects without resorting to the concept of "effective concentration." Furthermore since we can almost always use experimental thermodynamic information (e.g., pK_as in water) to determine the "corners" of the free energy surface in the reference solution reaction (see refs 3 and 28), we have a unique way of calibrating the potential surface in the enzyme using experimental observations. Thus our calculations of the catalytic effects are reduced to evaluations of the difference between the same reaction in enzyme and in solution, and we basically avoid the enormous challenge of calculating accurately bond energies and other contributions by a first principle quantum mechanics approach; the quantum mechanical energy of the reacting fragments is canceled out, and we can focus on reliable calculations of environmental effects.

All the details about the rather unique experimental informa-

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(35) Lee, F. S.; Warshel, A. *J. Chem. Phys*, **1992**, *97*, 3100.
(36) The reliability and convergence of FEP calculation in proteins is a rather complex issue. Usual convergence tests, such as forward and backward integration, are not always informative or even appropriate as far as overall accuracy is concerned. An important point to remember in the error analysis is to be consistent with the methodology used to parametrize the model. We and others have invested considerable effort addressing these issues (e.g. refs 33 and 35) and concluded that a useful estimate of the actual error range can be obtained by running a set of simulations with different initial conditions. By this definition we have an error range of about 2 kcal/mol in the absolute value of the activation free energy. The error in the activation free energy difference between D and H transfer reactions is around 0.20 kcal/mol which corresponds to an error range of about ±1.0 in the corresponding isotope effect.

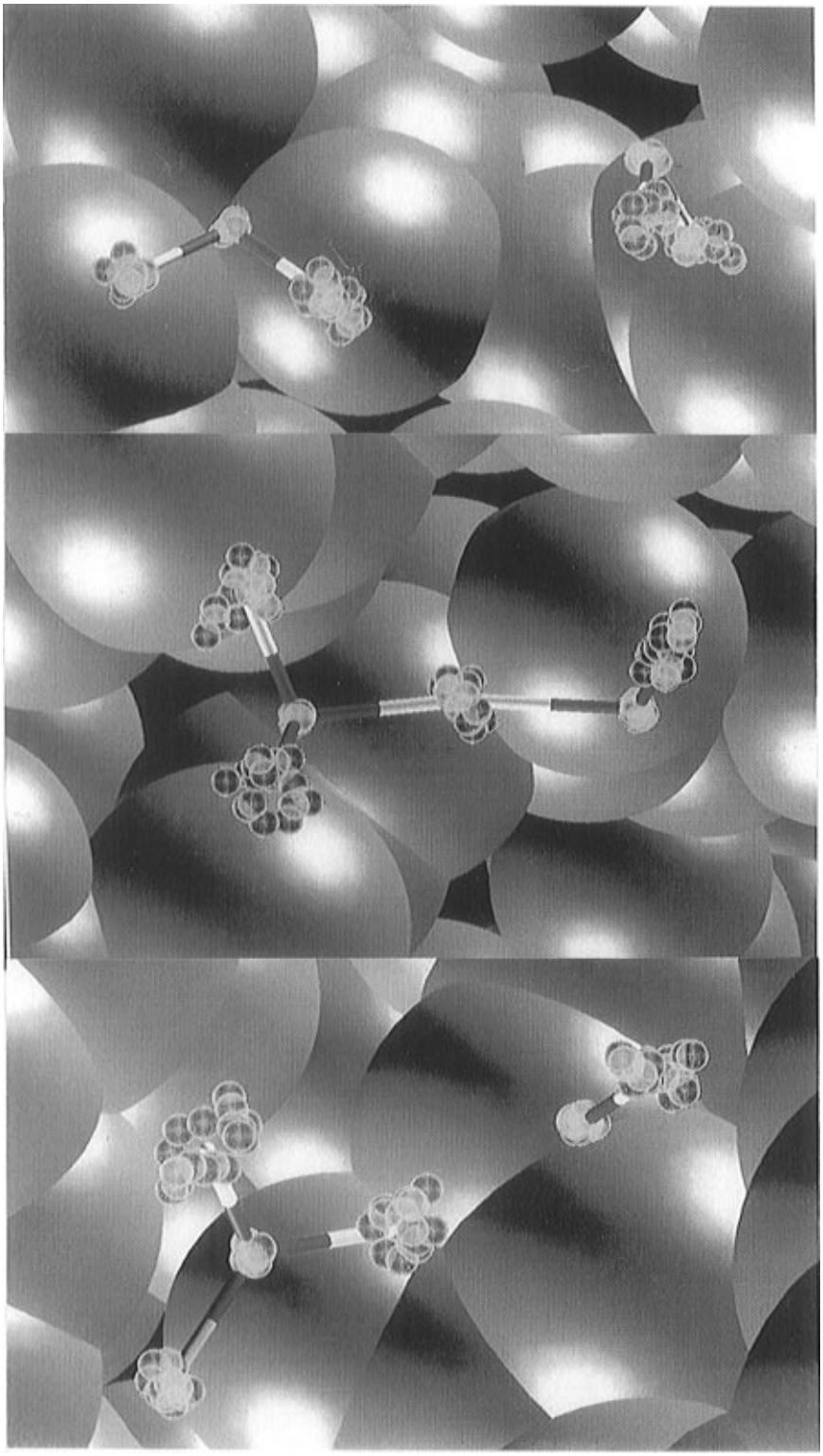

Figure 2. Snapshots of our simulations of the proton transfer reaction in carbonic anhydrase, showing three different states of the reaction: the reactant state, the transition state, and the product state. The figure shows the quantum beads of the reacting water molecules (dotted yellow spheres) and the surrounding protein and solvent regions (blue space-filling spheres).

tion used to describe the reference reaction has been given elsewhere (e.g., refs 3 and 28 and even as early as in ref 41). The experimental information for the reference reaction in the presence of a zinc ion is analyzed in ref 28.

Figure 2 presents snapshots of the simulated system in the reactant, transition state, and product regions. The figure emphasizes the quasiparticles that correspond to each atom and illustrates their spread during the simulation. The centroids of the quasiparticles were used to determine the quantum mechanical free energy functions through the use of eq 4. The corresponding free energy functions for proton transfer (PT) and deuterium transfer (DT) in the first step of our reaction are

(37) Some readers might confuse the many force field parameters of eq 8 with empirical parameters that allow one to adjust the calculated rate constant to any desirable value. However, while our approach refines parameters by reproducing properties of molecules in solution (as is done by all current approaches), it does not allow any of these parameters to change when calculating the difference between the energy of the given reaction in the protein active site and in water.

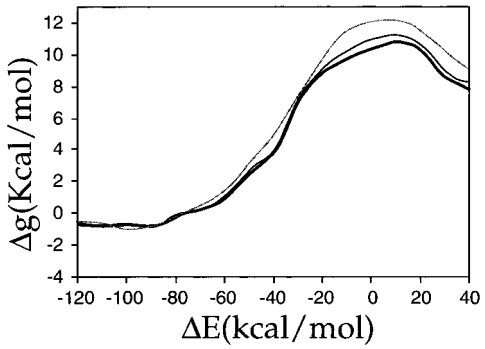

Figure 3. The free energy profile of PT and DT reactions in carbonic anhydrase. The classical result is shown in gray line. The quantum mechanical result involving proton transfer is in thick black line, while that of deuterium transfer is in thin black line.

shown in Figure 3. Using the difference between the activation barriers for PT and DT in the protein active site we obtained an isotope effect of 3.9 ± 1.0, which is in good agreement with the observed isotope effect[24] of 3.8. The convergence of the calculations is discussed in footnote 36. Since it is not entirely clear that the rate limiting step involves the PT of eq 6, we also simulated the second step in the "hydration" of the reaction that involves the nucleophilic attack of the OH^- ion on the CO_2 molecule. This simulation produced a smaller isotope effect ($k_H/k_D \approx 1.48$). Thus we can conclude, based on comparing the calculated and observed isotope effects, that the hydration step is probably not rate limiting. It is still possible that the rate limiting step involves a PT from the H_3O^+ ion to His 64. However, this step has a negative ΔG_0 (our protein dipoles Langevin dipoles PDLP unpublished calculation gave pK_as of $\sim$1 and $\sim$7, respectively, for the H_3O^+ and His 64 in the presence of the Zn^{2+}, the OH^-, and the rest of the protein), and the corresponding isotope effect is expected to be larger than that observed experimentally. It is also possible that the actual mechanism involves a concerted process with a simultaneous transfer of a proton from W_1 to W_2 and a transfer from W_2 to His 64 (see the free energy map Figure 3 of ref 28a). Our previous experiences with modeling activation barriers in solution indicated that the concerted path usually has a similar barrier to that of the stepwise path. We also note qualitative arguments that support the stepwise mechanism at the end of ref 23d. Furthermore, our experience indicated that such a Grotthuss-type mechanism (where the participants have different pK_as and the path is not fully concerted) will have similar isotope effect to that of the stepwise mechanism. Thus, at the present stage, we do not think that calculations of the isotope effect of the concerted mechanism (which are clearly feasible with our approach) will allow one to discriminate between the stepwise and concerted mechanism. In our opinion the best way to obtain more conclusive mechanistic information is to use simulations of the type described here in studies of mutation experiments where His 64 is replaced by other bases (see Figure 2 of ref 23d). Hopefully the calculated results of one of the two feasible mechanisms will not agree with the observed trend. Such a project is in progress in our group but it is not the subject of the present work. We basically view our study as a demonstration of the general potential of simulation methods in extracting mechanistic information from experimentally observed isotope effects rather than a specific elucidation of the mechanism of CA. Furthermore, we consider the examination of the quantum mechanical nuclear effects in the reaction of eq 6 as a reasonable test case for the importance of such effects in enzyme catalysis which is the primary subject of this work.

The present approach evaluated not only the isotope effect but also the actual quantum mechanical rate constant for the rate determining step in the catalytic reaction of CA. The rate constant obtained from eq 1 is $3.4 \times 10^5\ s^{-1}$ with an error range of a factor of 10. This compares reasonably well to the observed value[23] of k_{cat} ($2 \times 10^5\ s^{-1}$ in isosyme I and $1.4 \times 10^6\ s^{-1}$ in isosyme II). What is much more significant, however, is the fact that the calculated rate enhancement by the enzyme is about 10^8 relative to the reference reaction in water discussed above, and that this reproduces the corresponding observed effect to within a factor of 10. The same order of magnitude of catalysis is expected for the more concerted pathway considering our repeated experience that the catalytic effects for the concerted mechanism is strongly correlated with that of the stepwise mechanism.[3,42] Even if our quantitative success is coincidental it is encouraging to see that the enormous catalytic effect of the enzyme (around 11 kcal/mol) is reproduced without using adjustable parameters.[37] This ability to reproduce the overall observed effect of the enzyme, without first assuming it, allows us to probe questions which are difficult to determine by direct experiments. In particular, molecular modeling may start to be used in addressing the long standing question about the possible catalytic role of quantum mechanical nuclear effects. In order to examine this possibility it is essential to compare the enzymatic reaction to a reference reaction in solution. This is done in Figure 4 where the activation barrier, $\Delta g^{\ddagger}$, of the reaction in the enzyme and of the reference reaction in the water cage is correlated with the corresponding reaction free energy ΔG_0. These linear free energy relationships (LFER) are obtained by changing the gas phase proton affinity of the proton donor in a parametric way (to simulate the effect of different metals). This analysis (see ref 38 for a related study) allows us to compare the rates in the enzyme and in solution for the same ΔG_0, thus separating the electrostatic effect of changing the pK_a of the proton donor and changing the ΔG_0 from other catalytic effects. As seen from Figure 4 we have, for the same ΔG_0 (or ΔpK_a), much smaller classical activation barrier in the enzyme active site than in the reference solvent cage. This effect is due to a major electrostatic effect; the reduction of the so-called "solvent reorganization energy" by the enzyme. That is, one can express the classical activation barrier by the modified Marcus relationship of Warshel and co-workers (see for example refs 3, 18 and 38)

$$\Delta g^{\ddagger} \approx \frac{(\Delta G_0 + \lambda)^2}{4\lambda} - H_{12} + \frac{H_{12}^2}{(\lambda + \Delta G_0)} \qquad (9)$$

where λ is the reorganization energy. When, for example, $\Delta G_0 \approx 0$ we have $\Delta g^{\ddagger} = \lambda/4 - H_{12}$ and the smaller λ the smaller is $\Delta g^{\ddagger}$. Enzymes with their preoriented polar environments lead to small λ's and reduce the corresponding $\Delta g^{\ddagger}$'s (see refs 3, 38 for discussion and ref 44 for the original proposal of preoriented dipoles). It is important to note that recent attempts to fit enzymatic reaction rate to Marcus formula (e.g., refs 23c and

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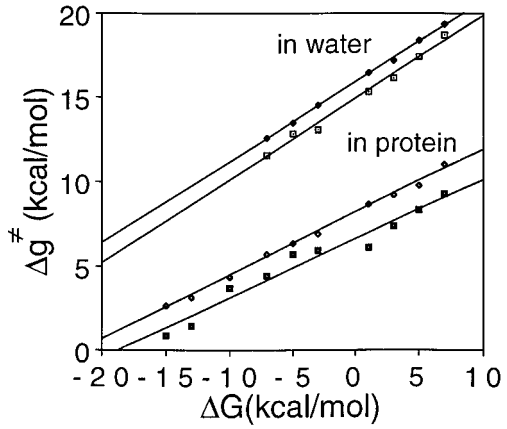

Figure 4. The dependence of the quantum (□, ⊡) and classical (◇, ◆) $\Delta g^{\ddagger}$ on ΔG_0 for PT and DT in the active site of carbonic anhydrase and in a solvent cage. In order to focus on the effect of the reorganization of the solvent we included the Zn^{2+} ion in the solvent cage in addition to the two reacting water molecules (our regular reference reaction does not include the Zn^{2+} in the reference reaction). This procedure is justified since we are interested in quantum mechanical catalytic effects for the hypothetical case where ΔG_0 is the same in the enzyme and water reactions. As the figure shows, the activation barriers are much larger in aqueous solution than in the enzyme site, and the quantum corrections are larger in the enzyme than in solution. Also note that the main reason for the difference in $\Delta g^{\ddagger}$ is the reorganization energies and not the quantum corrections (for $\Delta G_0 = 0$ the difference between the classical $\Delta g^{\ddagger}$ is given by the difference in the corresponding values of $\lambda/4$).

43) used the original Marcus equation which corresponds to the case when $H_{12} \rightarrow 0$. This equation does not give the correct absolute value of $\Delta g^{\ddagger}$'s for adiabatic reactions (e.g., PT and other bond-breaking-bond-making reactions), and the reorganization energies deduced are underestimated (see the analysis in ref 16). At any rate, our consistent evaluation of λ from first principle simulations is around 24 and 60 kcal/mol for the reaction in the enzyme and the reference reaction, respectively. Thus our calculations reproduce the reduction in reorganization energy and the corresponding catalytic effect.

While the classical effects that reduced $\Delta g^{\ddagger}$ were studied and discussed before (e.g., refs 3, 38 and 44), the calculations presented in Figure 4 point out toward a new and interesting quantum mechanical effect. That is, when we compare the enzyme and solution reactions for the same ΔG_0, it is apparent that the quantum mechanical corrections are larger in the enzyme than in solution. It is tempting to attribute this effect to the reduction of λ that is associated with a reduction in the amplitude of the enzyme fluctuations. However, the analytical dependence of the quantum mechanical activation barrier in simple model potentials (e.g., ref 39) does not support such a proposal, and studies with more realistic model potentials are needed in order to see how the change in the reorganization energy should be reflected in the quantum mechanical rate constant. It is also possible that the enzyme active site increases the effective ground state frequency relative to the corresponding value in the reference reaction in solution and thus increases the zero point energy and reduces the barrier. Regardless of the exact reason for the calculated effect (which will be the subject of further studies) it seems to us that this effect does not reflect numerical artifacts. Thus we believe that while the quantum mechanical contribution to the difference between the activation barrier in the enzyme and in solution is not very large, some difference does exist.

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Concluding Remarks

This work examines the potential use of simulation methods in exploring quantum mechanical effects in enzymatic reactions. It is demonstrated that the QCP approach can provide reasonable estimates of quantum mechanical rate constants for fluctuating enzyme−substrate complexes. This allows one to progress beyond classical transition rate theory and to explore the role of nuclear tunneling and zero point energy in modifying the classical rate constants. The fact that our calculations reproduce the observed rate constant without using adjustable parameters (in comparing the reaction in the enzyme to the corresponding reaction in aqueous solution) indicates that the simulations can be quite useful in elucidating mechanistic issues. That is, the simulations can be used in estimating the rate constant and isotope effects for different assumed reaction mechanisms. This should allow one to discriminate between different mechanistic options. For example, the fact that our calculated isotope effect is in excellent agreement with the corresponding observed values supports our assumed mechanism. The same method should be quite useful in other cases including the extraction of mechanistic information of solvent isotope effects. Using the QCP method in comparative studies of reactions in the enzyme active sites and in the corresponding solvent cage allows us to explore the role of quantum mechanical nuclear effects in enzyme catalysis. It is found that quantum mechanical contributions can lead to small but non-negligible catalytic effects. It is possible that this effect can be enhanced at low temperatures and although such an effect is not directly relevant to physiological processes, it might help in more fundamental understanding of enzymatic reactions.

Although quantum mechanical effects are important it should be pointed out that classical calculations are very useful for studies of enzyme catalysis. That is, as shown above, although the quantum corrections can be quite large, their magnitude is similar in enzymes and the corresponding reference reactions in solution. Since enzyme catalysis is determined by the *difference* between the activation barrier in the enzyme and in solution, $\Delta g^{\ddagger}_{\text{enzyme}} - \Delta g^{\ddagger}_{\text{water}}$, the quantum correction is canceled out to a large extent, and the corresponding classical result provides a very useful estimate.

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