Simulations of Quantum Mechanical Corrections for Rate Constants of Hydride-Transfer Reactions in Enzymes and Solutions

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A practical approach for simulating quantum mechanical corrections for rate constants of enzymatic reactions is presented. This approach is based on a combination of the dispersed polaron model and a centroid path integral treatment. The quantized activation free energy is conveniently obtained by propagating classical trajectories on the classical potential surface while using the free particle distribution to evaluate the relevant quantum correction. The method is used in a preliminary study of the hydride-transfer step in the catalytic reaction of lactate dehydrogenase (LDH). The isotope effect for this step is evaluated and the relationship between the quantum corrections for the reaction in the enzyme and in a reference solvent cage is examined.

1. Introduction

Proton-transfer (PT) and hydride-transfer (HT) reactions play an important role in many chemical and biological processes.[1] Some aspects of the catalysis and control of such processes are understood on a reasonable qualitative level. It is clear, for example, that the rate of PT reactions is correlated with the difference between the pK_a's of the donor and acceptor group[2] (the ΔpK_a). It is also clear that the pK_a can be controlled by the local polarity at the donor and acceptor site[2] and by other factors such as electrostatic interactions with metal ions.[3] The correlation between the ΔpK_a and the corresponding rate constant seems to follow reasonably well conventional linear free energy relationship even in protein sites.[4b-6] However, the possible rule of nuclear tunneling effect in biological PT and HT reactions is not fully understood and the relationship between such effect and the actual structure of the protein active site is not yet established.

In recent years there has been a considerably progress in theoretical studies of PT and HT reactions in polar solvents and in proteins.[2-5,8-12] The studies of PT and HT in enzymes have included such systems as lysozyme,[4] trypsin,[5,7,8a] subtilisin,[7] Snase,[3] LDH,[13] DHFR,[11] Papain,[9] and TIM.[8b] Yet none of these studies explored nuclear tunneling effects, although a preliminary simulation study of tunneling effects in PT in solution has been reported.[14] Interesting phenomenological studies of proton tunneling in proteins have been reported (e.g. ref 15) but the parameters used are not directly related to the microscopic situation in the given sites. There are also interesting studies that correlated observed isotope effects with different feasible mechanisms, (e.g. ref 16) but despite the progress associated with these approaches they are not based on microscopic models.

This work develops a practical approach for simulating quantum mechanical nuclear effects (i.e., tunneling and zero point energy contributions) in enzymatic reactions. The potential surfaces of the enzyme substrate complex is evaluated by the empirical valence bond (EVB) method that allows one to simulate a wide class of chemical reactions in proteins by molecular dynamics (MD) or related approaches. The effect of nuclear tunneling is incorporated in the EVB simulations by a combination of the dispersed polaron (DP) model[17] and a path integral approach.[14,18,19] Preliminary calculations of the quantum corrections for the rate constants of the hydride-transfer step in the catalytic reaction of LDH and the corresponding reaction in solution are presented.

2. Theoretical Approach

In order to calculate quantum mechanical corrections to rate constants of enzymatic reactions one has to address the following two problems: (i) the evaluation of the electronic energy as a function of the positions of the nuclear coordinates [the Born-Oppenheimer (BO) surface] and (ii) the nuclear motion on the reaction BO surface. The first issue has been addressed repeatedly in our previous studies that examined different approaches for evaluating the BO potential surfaces for enzyme-substrate complexes, ranging from a combined quantum mechanical classical SCF-MO approaches[1,4a] to the EVB method.[1,7] Here we use the EVB method which represents the reacting system by a Hamiltonian whose diagonal elements, H_{ii}, are the energies of the differnt resonance structures of the reacting system in the solvated enzyme. These energies are represented by semiempirical force fields which are calibrated by experimental and theoretical informations about the reaction in solution. The off-diagonal elements are calibrated by using information from gas phase ab-initio calculations and/or solution experiments.[7] The diagonalization of the EVB Hamiltonian by the eigenvectors C provides the ground-state potential surface and its analytical derivatives.

$$V_g = \mathbf{C}'_g \mathbf{H} \mathbf{C}_g \tag{1}$$

$$\partial V_g/\partial X_k = \mathbf{C}'_g \left(\frac{\partial \mathbf{H}}{\partial X_k} \right) \mathbf{C}_g$$

The detailed description of the EVB surface is given elsewhere[7]

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and is not the subject of this work.

The quantized treatment of the nuclear motion is the primary issue of the present study. Our previous studies of enzymatic reactions have evaluated the rate constant for the motion of the nuclei from the reactant to the product state by the widely used classical approximation

$$k_{cl} = F(k_B T/h) \exp(-\beta \Delta g^{\ddagger}_{cl}) \quad (2)$$

where $\beta = 1/k_B T$ with k_B designating the Boltzmann constant. F is the transmission factor and $\Delta g^{\ddagger}_{cl}$ is the classical activation free energy. This classical approximation gives major insight about the factors that determine the rate constant but does not take into account quantum mechanical aspects of the nuclear motion. To examine these aspects we exploit the hypothesis[14,18,19] that the quantum mechanical correction of the rate constant can be attributed primarily to the corresponding correction of the activation free energy, so that the quantum mechanical rate constant can be written as

$$k_{qu} \cong k_{cl} \exp\{-\beta(\Delta g^{\ddagger}_{qu} - \Delta g^{\ddagger}_{cl})\} \quad (3)$$

where qu and cl designate respectively quantum and classical approaches. Thus our problem is reduced to the evaluation of $\Delta g^{\ddagger}_{qu} - \Delta g^{\ddagger}_{cl}$.

In order to estimate the quantum mechanical correction to the $\Delta g^{\ddagger}$ of an enzymatic reaction one needs some way to quantize the motion of the entire enzyme–substrate complex. This can be accomplished, at least in principle, by path integral treatments that involve all the protein atoms. However, the convergence of such an approach might be rather slow. Instead one may exploit an idea introduced in our earlier work[14] and replace the actual dependence of the diagonal EVB matrix elements on the nuclear coordinates of the actual multidimensional system by the quasiharmonic approximation

$$H_{ii} = V_i \cong \tfrac{1}{2}\hbar(\mathbf{q}-\boldsymbol{\lambda}_i)\,\boldsymbol{\omega}(\mathbf{q}-\boldsymbol{\lambda}_i) + \Delta V_i^0$$

$$H_{ij} \cong H_{ij}^0 \exp(-\Delta\mathbf{q}_{ij}\boldsymbol{\mu}_{ij}\Delta\mathbf{q}_{ij})$$

$$V_g = \mathbf{C}'_g \mathbf{H} \mathbf{C}_g \quad (4)$$

where the components of the vectors $\mathbf{q}$ and $\boldsymbol{\lambda}$ are respectively the normal modes and origin shifts of the system and $\boldsymbol{\omega}$ is a diagonal matrix whose diagonal elements are the frequencies of the system. Here $\Delta\mathbf{q}_{ij} = \mathbf{q} - \boldsymbol{\lambda}_{ij}$ and $\boldsymbol{\lambda}_{ij} = (\boldsymbol{\lambda}_i + \boldsymbol{\lambda}_j)/2$. The key trick is to obtain the ω's and λ's for the entire enzyme–substrate complex. This is done here by the dispersed polaron (DP) method that was used previously in studies of electron transfer in solutions[17a] and proteins[17b] (see ref 20 for a related approach). The DP method uses the same classical MD simulation involved in the evaluation of $\Delta g^{\ddagger}_{cl}$ to obtain the time-dependent energy gaps between the ith and jth diabatic states, $\Delta V(t) = V_i(t) - V_j(t)$. Expressing the fluctuations of $V(t)$ by $u(t) = \Delta V(t) - \langle \Delta V(t) \rangle$ one can evaluate the power spectrum $A(\omega)$ by[17a]

$$A(\omega) = \int_{-\infty}^{\infty} \langle u(0)\, u(t) \rangle e^{i\omega t}\, dt \quad (5)$$

With the V's of eq 4 we can express $A(\omega)$ as

$$A(\omega) = \frac{2\pi}{\beta} \sum_{k=1} \tfrac{1}{2}\hbar\omega_k \lambda_k^2 \delta(\omega - \omega_k) \quad (6)$$

This expression is normalized by the classical "solvent reorganization energy", α, using the relationship

$$\int_{-\infty}^{\infty} A(\omega)\, d\omega = 2\pi\alpha/\beta$$

$$\alpha = \Delta g_j(X_i^0) - \Delta g_i(X_i^0) - \Delta G_{i\to j} \quad (7)$$

where X_i^0 is the minimum of the Δg_i curve of the ith diabatic state (the Δg associated with V_i) and $\Delta G_{i\to j}$ is the difference in free energies between the i and j states.

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Now with the λ's and ω's of eq 6 and with eq 4 we have a rather simple analytical surface where the entire spectrum of the λ's can be represented by a limited number of modes and our problem is reduced to the evaluation of $\Delta g^{\ddagger}_{qu} - \Delta g^{\ddagger}_{cl}$ for V_g.

When one deals with the common case of only two diabatic surfaces, it is convenient to use the approach of ref 14, obtaining the quantized diabatic free energy through the corresponding analytical expression[14] and then evaluating the difference between the diabatic and adiabatic $\Delta g^{\ddagger}_{qu}$ by a path integral formulation. However, more general studies of concerted mechanisms may involve three diabatic states. Thus we explore below an alternative method that exploits the simplicity of eq 4 and uses a variant of the "centroid" path integral method[14,18,19] to evaluate $\Delta g^{\ddagger}_{qu} - \Delta g^{\ddagger}_{cl}$.

In computer simulations of path integrals it is convenient to exploit the isomorphism between the Feynman's path integral formulation[22] and the classical average over the phase space of the corresponding ring of quasiparticle.[23] This is done by representing each quantum particle by p quasiparticles which are connected by the harmonic potential $^1/_2 M\Omega^2(x_{k+1} - x_k)^2$, where M is the mass of the particle, $\Omega = p/\hbar\beta$, and x_k is the coordinate of the kth quasiparticles. The quantum mechanical partition function for such system is then evaluated by

$$Q^{qu} = \int d\bar{x} \left\{ \int \mathcal{D}_x \exp(-\beta V^{qu}) \right\} \quad (8)$$

where

$$\mathcal{D}_x \equiv (1/\Lambda) \prod_k^p dx_k/\Lambda$$

$$\Lambda = [2\pi\hbar^2\beta/pM]^{1/2}$$

$$V^{qu} = \sum_k^p \left\{ \frac{1}{2p} M\Omega^2 \Delta x_k^2 + \frac{1}{p} V(x_k) \right\} \quad (9)$$

where we replaced the regular Boltzmann average over the potential energy of the system by an average over the effective potential V^{qu} and where $\Delta x_k \equiv x_{k+1} - x_k$, with the understanding that $x_{p+1} = x_1$. Here each classical particle experiences $1/p$ of the actual potential energy $V(x_k)$ of the system as well as the harmonic forces with its $k-1$ and $k+1$ neighbors. In eq 9 and in the rest of the paper we restrict the formulation to the one dimensional case for the sake of clarity (the multidimensional extension is quite simple).

While the use of path integral formulations in calculations of free energy differences is rather straightforward, the proper treatment for evaluation of activation free energies of multidimensional systems is not completely clear despite recent progress[14,18,19] (see also ref 24 for a related approach for treating reactions in the diabatic limit). Our present approach for evaluation of quantized activation free energies is based on the previously derived expression[14]

$$\exp[-\beta\Delta g_{qu}(X)] = \exp[-\beta\Delta G_{qu}(\mathbf{m})] \langle \delta(X - \Delta V^c) \exp(-\beta(V_g^{qu} - V_{\mathbf{m}}^{qu})) \rangle_{V_{\mathbf{m}}^{qu}} \quad (10)$$

where X is the reaction coordinate, ΔV^c designates the hypersurface of constant energy gap between the diabatic potential surfaces, i.e. $\Delta V^c \equiv V_i(\bar{x}) - V_j(\bar{x}) =$ constant, where $\bar{x}$ is the "centroid" of the system, which is the average position of our quasiparticles ($\bar{x} = \sum_k x_k/p$), δ is the delta function, $V_{\mathbf{m}}$ is a mapping potential that is taken as a linear combination of the diabatic states,[5,7,14] i.e. $V_{\mathbf{m}} = \sum_i m_i V_i$ and the V_g^{qu}, $V_{\mathbf{m}}^{qu}$ are the quantum effective potentials for the ground-state and the mapping potential, respectively. The average $\langle \ldots \rangle_{V_{\mathbf{m}}^{qu}}$ designates a Boltzmann average over the partition

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function of $V_{\mathbf{m}}^{qu}$ which can be obtained by Monte Carlo (MC) or MD simulations. The function $\Delta G(\mathbf{m})$ represents the free energy associated with changing the mapping parameter $\mathbf{m}$ from its initial value to its current value and is obtained by standard free energy perturbation approaches but with $V_{\mathbf{m}}^{qu}$ rather than $V_{\mathbf{m}}$ (see eq 13 of ref 14).

In this work, we try to bring eq 10 to a somewhat more convenient form that should allow one to effectively use the results of classical MD in quantum simulations of many dimensional systems. We start our derivation by noting that the classical partition function can be expressed (see ref 22 for a related expression) as

$$Q^{cl} = \int d\bar{x} \left\{ \int \mathcal{D}_x \exp(-\beta V^{sc}) \right\} \tag{11}$$

$$V^{sc}(\mathbf{x};\bar{x}) = \frac{1}{p}\sum_k^p [\tfrac{1}{2}M\Omega^2 \Delta x_k^2 + V(\bar{x})] \tag{12}$$

This effective "semiclassical" potential V^{sc} retains the quantized part of the "constrained" free particle system but uses the potential $V(\bar{x})$ of the system at the centroid location rather than the quantized $(1/p)\sum_k V(x_k)$. Note that the integration inside the brace is under the constraint that $(1/p)\sum_k x_k$ must be equal to the given $\bar{x}$. With eqs 8 and 11 we can write

$$\frac{Q^{qu}}{Q^{cl}} = \frac{\int d\bar{x} \int \mathcal{D}_x \exp(-\beta(V^{qu} - V^{sc})) \exp(-\beta V^{sc})}{\int d\bar{x} \int \mathcal{D}_x \exp(-\beta V^{sc})}$$

$$= \langle \exp\{-(\beta/p)\sum_k \Delta V_k\} \rangle_{V^{sc}} \tag{13}$$

where $\Delta V_k = V(x_k) - V(\bar{x})$. Now, we can further simplify eq 13 by noting that the average over V^{sc} can be performed separately with regard to $\bar{x}$ and the positions of the quasiparticles (for the given value of $\bar{x}$). That is, we can write eq 13 as

$$\langle \exp\{-(\beta/p)\sum_k \Delta V_k\} \rangle_{V^{sc}} = \frac{\int d\bar{x} \exp(-\beta V(\bar{x})) f(\bar{x})}{\int d\bar{x} \exp(-\beta V(\bar{x}))} \tag{14}$$

$$f(\bar{x}) = \frac{\int \mathcal{D}_x \exp\{-(\beta/2p)\sum_k M\Omega^2 \Delta x_k^2\} \exp\{-(\beta/p)\sum_k \Delta V_k\}}{\int \mathcal{D}_x \exp\{-(\beta/2p)\sum_k M\Omega^2 \Delta x_k^2\}}$$

$$= \langle \exp\{-(\beta/p)\sum_k \Delta V_k\} \rangle_{fp,\bar{x}}$$

where fp designates "free particle" and $\langle\ \rangle_{fp,\bar{x}}$ indicates an average on the free particle distribution constrained at $\bar{x}$. This equation can be written as

$$\langle \exp\{-(\beta/p)\sum_k \Delta V_k\} \rangle_{V^{sc}} = \langle \langle \exp\{-(\beta/p)\sum_k \Delta V_k\} \rangle_{fp,\bar{x}} \rangle_V \tag{15}$$

Thus, we obtain the following useful conclusion: The average can be performed by running classical simulations on V while evaluating the average of $\exp\{-(\beta/p)\sum_k \Delta V_k\}$. Thus, the evaluation of eq 13 can be achieved by classical simulation on V rather than on V^{qu}. Next, we can rederive eq 10 using $V_{\mathbf{m}}^{sc}$ rather than $V_{\mathbf{m}}^{qu}$. This gives

$$\exp[-\beta \Delta g_{qu}(X)] \sim \exp[-\beta \Delta G_{qu}(\mathbf{m})] \times \langle \delta(X - \Delta V^c) \exp\{-(\beta/p)\sum_k [V_g(x_k) - V_{\mathbf{m}}(\bar{x})]\} \rangle_{V_{\mathbf{m}}^{sc}} \tag{16}$$

using the approach of eq 15, we obtain

$$\exp[-\beta \Delta g_{qu}(X)] = \exp[-\beta \Delta G_{qu}(\mathbf{m})] \times \langle \langle \delta(X - \Delta V^c) \exp\{-(\beta/p)\sum_k [V_g(x_k) - V_{\mathbf{m}}(\bar{x})]\} \rangle_{fp,\bar{x}} \rangle_{V_{\mathbf{m}}} \tag{17}$$

Finally, we obtain the desired quantum correction by

$$\exp\{-\beta[\Delta g_{qu}(X) - \Delta g_{cl}(X)]\} \approx \frac{\langle \langle \delta(X - \Delta V^c) \exp\{-(\beta/p)\sum_k [V_g(x_k) - V_{\mathbf{m}}(\bar{x})]\} \rangle_{fp,\bar{x}} \rangle_{V_{\mathbf{m}}}}{\langle \langle \delta(X - \Delta V^c) \exp\{-\beta[V_g(\bar{x}) - V_{\mathbf{m}}(\bar{x})]\} \rangle_{fp,\bar{x}} \rangle_{V_{\mathbf{m}}}} \tag{18}$$

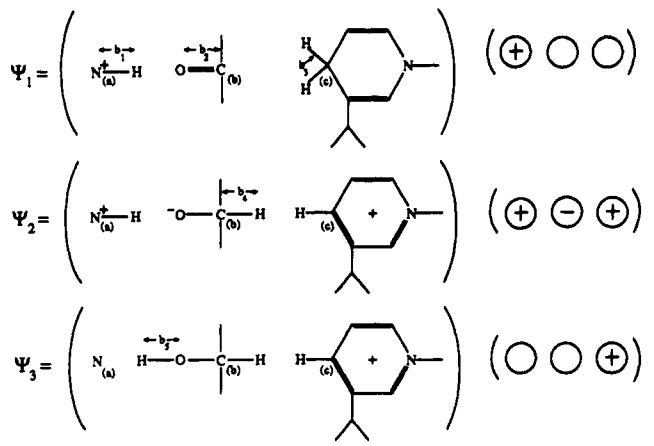

Figure 1. The EVB resonance structures for the catalytic reaction of LDH. $N_{(a)}$, $C_{(b)}$, and $C_{(c)}$ designate respectively the histidine nitrogen, the carbon of the pyruvate, and the NADH carbon (see ref 13 for more details). The present simulation only considers the transfer from ψ_1 to ψ_2, and did not include the histidine in the "solute" surface.

where we assume that $(Q_{\mathbf{m}}^{qu}/Q_{\mathbf{m}}^{cl})/(Q_0^{qu}/Q_0^{cl}) \approx 1$, which is a reasonable approximation when V_0 and $V_{\mathbf{m}}$ have similar curvatures.

3. Results and Discussion

In order to examine the performance of our approach we considered the hydride-transfer step in the catalytic reaction of lactate dehydrogenase (LDH). This reaction might involve a concerted mechanism,[13] and the hydride-transfer step may not be the rate-limiting step. Yet, the hydride-transfer step is identified in related solution reactions[25] and the corresponding step in the enzyme does provide a useful model for general studies of tunneling effects in proteins. The EVB surface of our system is described in detailed in ref 13 and is based on the three resonance structures of Figure 1. The present study considers the quantum mechanical correction for the free energy function of the transfer from ψ_1 to ψ_2. In deriving the DP model for this system, we introduced a convenient approximation by evaluating the diabatic expansion of eq 4 separately for the reacting fragments ("the solute") and the rest of the system ("the solvent"). The diabatic "origin shifts" (the λ's) of the solute were determined by using the QCFF/PI[26] approach. This was done by considering the solute in its protein active site, constraining $C_{(b)}$ and $C_{(c)}$ to be 3.0 Å apart, evaluating the minimized structures and normal modes of ψ_1 and ψ_2 and then using our general method for calculating Franck–Condon factors[27] to evaluate the λ's. This calculations were performed using the QCFF/SOL[28] version of the QCFF/PI program that allows one to consider the vibrations of substrates and cofactors in the protein active sites. The QCFF/PI parameters for the $C_{(b)}$–H and $C_{(c)}$–H stretching potentials were adjusted to reproduce the corresponding diabatic EVB potentials. The origin shifts for the protein vibrations were then determined by fixing the solute in the protein active site with $C_{(b)}\cdots C_{(c)}$ at 3 Å, running a MD trajectory with $V_{\mathbf{m}} = 0.5V_1 + 0.5V_2$, while collecting the time dependent energy gap $V_2(t) - V_1(t)$ and using the power spectra and eqs 5 and 6 to obtain the frequency dependent $\lambda(\omega)$ shown in Figure 2. The MD simulation was done using the program ENZYMIX[28a] starting with 20 ps equilibration, followed by 20 ps collection of the time-de-

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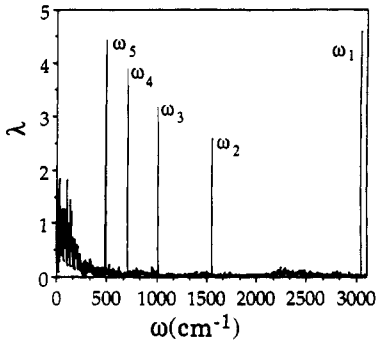

Figure 2. The $\lambda(\omega)$ of the DP model for the hydride-transfer step of the catalytic reaction of LDH. The five sharp lines correspond to the effective modes of the solute.

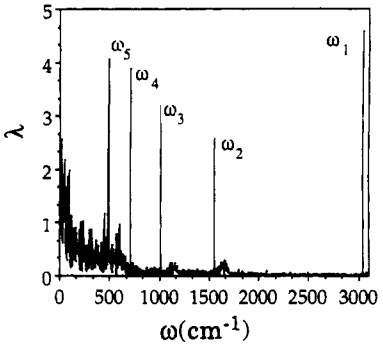

Figure 3. The $\lambda(\omega)$ of the DP model for the hydride-transfer step for the reference reaction in solution.

pendent energy gap with step size of 0.5 fs.

The vibronic expansion of the solute modes was grouped into five effective modes (Q_1, Q_2, ..., Q_5) with λ's of 4.57, 2.59, 3.20, 3.88, and 4.42 for ω's 3045, 1554, 1012, 713, and 494 cm^{-1}, respectively. These modes are also included in Figure 2. The corresponding modes of the system where the hydride is deuterated are represented by λ's 4.52, 3.41, 3.48, 5.49, and 4.23 and ω's 2323, 1123, 1002, 770, and 493 cm^{-1}. In treating the solvent modes we follow a similar procedure grouping the λ's of the protein into 10 equally spaced effective modes at the region between 0 and 750 cm^{-1}. The μ_{ij}'s of the H_{12} of eq 4 were obtained by fitting eq 4 to the actual EVB surface using the recently proposed EVB variant of Chang and Miller.[30] This fitting procedure was based on using H_{ij}^0 = 32.5 kcal/mol and adiabatic barrier of 5 kcal/mol. The adiabatic barrier frequencies were taken directly from the first guess of the EVB potential with $H_{ij} = H_{ij}^0$, except that ω_1^* was taken as $1000i$ cm^{-1} following a recent related study[25c] (a more systematic fitting procedure should use an ab initio estimate for the frequencies).

The set of parameters described above and the corresponding V_g were then used with eq 18 to obtain the quantum corrections to the activation free energy of our system. The actual evaluation of eq 18 involved the use of a stochastic Langevin equation for the corresponding potentials. The numerical algorithm used to solve the Langevin equation is taken from ref 31. The preliminary convergence tests showed that the number of the pseudoparticles equal to 80 was more than sufficient. The final results are within statistical uncertainties of less than 1%.

The resulting classical and quantum mechanical free energies are shown in Figure 4. The calculated quantum mechanical correction for the reaction in the protein active site are summarized

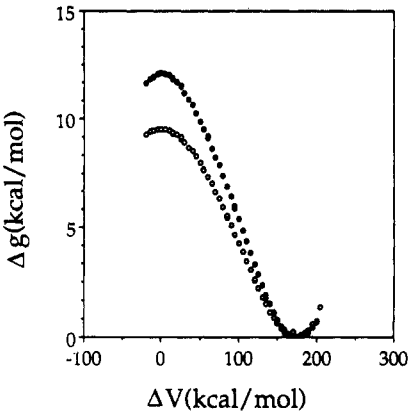

Figure 4. The classical free energy function, $\Delta g_{cl}(X)$ (filled circles), and the corresponding quantum mechanical function, $\Delta g_{qu}(X)$ (open circles), for the hydride-transfer step in the catalytic reaction of LDH.

TABLE I: Calculated Quantum Corrections for the Catalytic Reaction of LDH and the Reference Reaction in Aqueous Solution[a]

system[b]	η^*	η^R	$\eta^*_H\eta^R_D/\eta^*_D\eta^R_H$
protein (H)	2.33×10^{-3}	2.85×10^{-5}	
protein (D)	3.15×10^{-3}	1.97×10^{-4}	5.0
aqueous (H)	3.29×10^{-3}	2.65×10^{-5}	
aqueous (D)	4.27×10^{-3}	1.91×10^{-4}	5.6

[a] The quantum corrections are expressed in terms of the corresponding contributions for the reactant (R) and the transition state (η^R and η^*), so that $\exp[-\beta(\Delta g^*_{qu} - \Delta g^*_{cl}] = \eta^*/\eta^R$. [b] H and D designate the reaction with H^- and D^- for the hydride ion, respectively.

in Table I. The table also includes quantum corrections for the enzymatic reaction with a deuteriated hydride. As seen from the table, the calculated isotope effect is ~5. This value should not yet be compared directly to the observed[29] isotope effect in LDH (k_H/k_D between 3 and 2) since the calculations might not correspond to the actual rate-limiting step and can only be considered as a preliminary feasibility study. It is interesting, however, to note that we obtain a value much smaller than the regular value reported from reactions with $\Delta G \sim 0$; such a small value is also observed in hydride-transfer reactions in solutions.

One of the interesting issues that can be examined by the present approach is the possible role of nuclear tunneling in enzyme catalysis. To explore this point we performed the simulations for the reference reaction in solution (the corresponding DP results are given in Figure 3) and examined the difference between the results for the protein and the solution reactions. The calculated $\Delta\Delta g^*_{cl\to qu}$ are 2.6 and 2.9 kcal/mol in the protein and in solution, respectively. While the difference between these two cases is not zero, it is much smaller than the absolute difference between the Δg^*'s of the enzyme and the solution reaction (12.1 and 18.1 kcal/mol, respectively) which is largely due to the corresponding difference in reorganization energies[13] (note that ref 13 only considered the solvent contribution to Δg^*). While the quantum mechanical contributions to catalysis seem rather small, the corresponding calculations may be very useful, however, for diagnostic purposes such as the correlation between the observed isotope effect and the calculated reaction pathway. It is also clear that more studies should be conducted to verify our preliminary results and to examine the validity of different methods for obtaining quantum mechanical rate constants for adiabatic reactions in proteins.

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