Internal Enzyme Motions as a Source of Catalytic Activity: Rate-Promoting Vibrations and Hydrogen Tunneling

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The standard view of the origin of the catalytic properties of enzymes focuses on the binding energy differences between the ground state and the transition state arising from the arrangement of residues in the active site (i.e., statics). There is an alternative view that suggests that protein motions (i.e., dynamics) might play a role in catalysis. Klinman and co-workers recently published (Kohen, A.; Cannio, R.; Bartolucci, S.; Klinman, J. *Nature* **1999**, *399*, 496−499) findings on rate measurements in thermophilic alcohol dehydrogonase (ADH). At lower temperatures (below 30 °C), this enzyme undergoes a transition to a more rigid structure, and it was found that the corresponding apparent activation energy increases and the primary kinetic isotope effect (KIE) increases and becomes temperature-dependent. Explaining these results presents a challenge to theory. We show that a model of the reaction coordinate for the rate-determining step, coupled to an enzymatic environment and a specific strongly coupled active complex mode, can simultaneously explain a tunneling-dominated mechanism and the experimental trends reported by Klinman and co-workers. We propose a specific protein internal motion for the rate-promoting vibration and discuss other systems in which such motions might be dominant.

I. Introduction

One widely accepted view in explaining enzymatic catalytic efficiency is the transition state binding concept of Pauling.[1] In this picture, as a chemical substance is being transformed from reactants to products, the species that binds most strongly to the enzyme is postulated to be at or near the top of the solution-phase (i.e., uncatalyzed) barrier to reaction. This preferential binding releases energy, thus stabilizing the transition state and lowering the barrier to reaction. This is a standard picture of nonbiological catalysis, and it also has significant experimental support, e.g., kinetic isotope effect methods that can probe the chemical structure of the transition state in the catalytic event. A second proposed mechanism, which might be viewed as the converse of transition state stabilization, is ground state destabilization. In this picture,[2] the role of the enzyme, rather than making the transition state more stable, is to make the reactants less stable thus lowering the energetic hill that must be climbed with thermal activation. Another mechanism, recently suggested by Bruice,[3] is that the dominant role of an enzyme is to position substrates in such a fashion that thermal fluctuations easily take them over the barrier to reaction. The set of positions that the enzyme encourages the substrates to take are known as near attack conformations or NACs.

There is another possibility for the action of enzymes in certain reactions: motions within the protein itself might actually speed the rate of a chemical reaction.[4] There is an obvious physical resemblance between this possibility and the NAC view of catalysis described above, but it must be stressed that the protein motion view is a dynamic one. In other words, it is not simply preparation of a reactive species but rather strong coupling of the reaction coordinate to *motions* of the protein. We and others[5,6] have suggested that such coupling could help account for signatures of hydrogen tunneling in enzymatic reactions. The characteristics of such reactions, in which specific vibrations couple strongly to a reaction coordinate, are moderate kinetic isotope effects as compared to the huge effects that would be expected for a one-dimensional tunneling reaction in a condensed phase. This concept was later used by other groups to explain hydride transfer kinetic isotope effects in methylamine dehydrogenase.[7]

Experimental evidence for the involvement of dynamic protein modes in catalysis is beginning to grow. Petsko and co-workers[8] have studied 3-isopropylmalate dehydrogenase from the thermophilic bacteria *Thermus thermophilus*, which hydrogen/deuterium exchange studies have shown to be significantly less flexible than the mesophile at room temperature. Another system that seems to demonstrate the potential for the involvement of protein modes in catalysis is dihydrofolate reductase. NMR data[9] show that regions of the protein are highly flexible and experience dynamic fluctuations on a time scale relevant to H-transfer dynamics. In addition, mutagenesis data[10] have shown that alteration of residues far from the active site can, at times, strongly influence the catalytic efficiency of the enzyme. Finally, the recent numerical work of Brooks and co-workers[11] showed that there are highly correlated motions in the protein with bound substrate, but that these motions disappear in product complexes that vary from substrate only in the placement of a hydrogen atom.

A third system in which such dynamic protein effects might be present is the thermophilic protein alcohol dehydrogonase from *Bacillus stearothermophilus*, studied recently by Klinman and co-workers,[12] that is the subject of the present paper. In this system, hydrogen transfer proceeds via quantum tunneling. Therefore the emphasis of the present paper is on the investigation of the effect of enzyme motions on the *quantum* dynamics

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TABLE 1: Experimental Results from Ref 12

	regime H	regime L
T (°C)	30−60	5−30
KIE	2.5	8.0−3.0
E_H (kcal/mol)	14.6	23.6

of the reaction coordinate. Because it is not feasible to exactly study quantum systems with many degrees of freedom for arbitrary intermolecular potentials, we must make some further assumptions about the effect of the enzymatic environment before we are able to perform quantum calculations.

II. Experimental Results and Their Interpretation

Thermophilic enzymes have been identified as a class of systems that seem to provide support for the dynamic view of catalysis. They show very low enzymatic activities at temperatures at which mesophilic enzymes of similar function are optimally active. Several plausible interpretations of this phenomenon can be excluded:[13] the suggestion that the rate-determining step is different at different temperatures cannot be reconciled with the conservation of the active site structure and chemical mechanisms, and crystal structure determinations have shown that thermophilic enzymes do not cold-denature.[14] One possible interpretation focuses on the temperature-dependent motions of proteins. This view has experimental support from measurements that show that the rigidity of thermophilic proteins is lower at high rather than low temperatures.[8]

Klinman and co-workers[12] studied hydrogen tunneling in alcohol dehydrogonase from *Bacillus stearothermophilus* (a thermophilic protein referred to as ADH-hT), that normally functions at 65 °C. Thermophilic enzymes often exhibit biphasic Arrhenius behavior: below some temperature, the activation energy increases, a fact frequently attributed to increased protein rigidity.[8] The Klinman group performed rate measurements in two different temperature regimes: H (high temperature) 30−65 °C and L (low temperature) 5−30 °C. We summarize their results in Table 1.

There are two striking features in these results: (i) The activation energy E_H is lower in regime H, which implies that tunneling is *enhanced* with increasing temperature. (ii) The primary KIE is small and temperature-independent in regime H but larger and temperature-dependent in regime L. In addition, similar tendencies are observed for the secondary KIE. We should also mention a study[7] of H transfer in methylamine dehydrogonase that reports results qualitatively similar to those of regime H of Table 1.

The fact that the activation energy is different in the two regimes is a strong indication that tunneling does not proceed through a one-dimensional rigid barrier. It is important to note that the activation energy switches to the higher value *at the same temperature for both* protonated and deuterated substrates, which is a strong indication that the chemical step is the same in both cases and that the origin of the phenomenon lies in an alteration of C−H bond activations. These results present a challenge to the standard theories of charge transfer in condensed phases, as we now explain.

There are several competing physical effects[15] in charge-transfer reactions in condensed phases. Which one will dominate depends on the values of several parameters, including temperature, potential barrier height, transfer distance, reactant well frequency, and strength of coupling to the environment. In different regions of this parameter space, different physical mechanisms dominate and, for historical reasons, distinct theoretical models have been used.

Region I: The dynamics occurs over the barrier (as described by TST) or just below the barrier (small quantum corrections).

Region II: The dynamics takes place by tunneling from excited energy states in the reactant well (moderate-to-large quantum effects).

Region III: The dynamics takes place by tunneling from the ground state in the reactant well (large quantum effects).

It should be emphasized that *the rate has Arrhenius form in all three regions*, but the activation energy has different meaning in each regime, as we describe later in this section.

As recently as 20 years ago, the only theoretical studies of tunneling reactions in condensed phases concerned regions I and III. The deep-tunneling limit (tunneling from the ground state of the reactant well) was studied using Marcus theory. The opposite limit (small tunneling corrections just below the transition state) was studied with the methods described by Bell.[16] Care must be taken in applying gas-phase approaches to condensed-phase reactions, as in that reference (e.g., in condensed-phase reactions, there is one additional and crucial time scale, the dephasing time of the wave function due to coupling with the environment).

The most interesting intermediate case (region II, tunneling from excited states in the reactant well) yielded its secrets only in the mid-1990s, when appropriate theoretical tools were developed. These results provided an answer to the following critical question: When does a system pass from region I to region II? That is, given some potential energy surface, when is classical dynamics expected to dominate (region I), and when is quantum tunneling from excited states (region II) expected to be large enough that the reaction has strongly quantum character? For example, it was rigorously proved[17] that, for a potential with a barrier height of 6 kcal/mol and a transfer distance of 2 Å that is immersed in a solvent, 50% of the proton-transfer rate can be due to tunneling *even at room temperature.* In other words, we now know that many systems that 10 years ago we would have intuitively expected to be classical, instead have substantial quantum character. We now briefly discuss the theoretical models that have been used in the three regions described above.

Region I: Small Quantum Corrections. This limit has been successfully studied using semiclassical theory,[16,18] which is applicable when quantum corrections are small compared to the transition state theory rate, i.e., tunneling takes place close to the barrier top. It predicts a transfer rate of

$$k = A_H e^{-\beta(V-\omega_H/2)} \tag{1}$$

where A_H is the Arrhenius prefactor, $\beta = 1/k_B T$, V is the chemical barrier height, and ω_H is the C−H bond frequency. Equation 1 predicts a KIE (assuming $A_H = A_D$) of

$$\ln \frac{k_H}{k_D} = \frac{\beta}{2}(\omega_H - \omega_D) = \frac{\beta}{2}\left(1 - \sqrt{\frac{m_H}{m_D}}\right)\omega_H \tag{2}$$

For C−H bond cleavage, eq 2 predicts a KIE equal to $k_H/k_D \approx 7$ at room temperature. It cannot explain why a change in rigidity effects a very small primary KIE in regime H. In the limit where the semiclassical theory is valid, it is often useful to study the Schaad−Swain exponent

$$\frac{\ln(k_H/k_T)}{\ln(k_D/k_T)} = \frac{\ln(A_H/A_T) + (\omega_H - \omega_T)\beta/2}{\ln(A_D/A_T) + (\omega_D - \omega_T)\beta/2} \quad (3)$$

In the special case that the pre-Arrhenius factor A_L is the same for all isotopes, then eq 3 predicts a value 3.26 for this exponent. Larger observed values are often interpreted as signs of increased tunneling. Note that, according to eq 3, in tunneling reactions where the ratio $A_H/A_D \neq 1$, the semiclassical theory predicts an exponent that is *not* equal to 3.26 and is temperature-dependent.

Region III. Large Quantum Effects. When the barrier height is very high or the transfer distance is large, tunneling takes place from the ground state. In this limit, the Marcus−Levich−Dogonadze theory[19] has been used with great success. This theory has been successful in the study of electron transfer in solution and biomolecules. It uses the fact that, in the deep-tunneling limit, one can use the tunneling matrix element Δ as a small parameter in a perturbative approach and find a transfer rate equal to

$$k \approx \Delta^2 e^{-\beta(E_r+\epsilon)^2/4E_r} \quad (4)$$

where E_r is the reorganization energy of the *solvent*, ϵ is the exothermicity, and Δ is the tunneling matrix element. Note that the quantum result in eq 4 predicts an Arrhenius form for the rate, similarly to the TST result. In this theory, the KIE is equal to $k_H/k_D \approx \Delta_H^2/\Delta_D^2$, which in the deep-tunneling limit has a value on the order of 10^3-10^4. The Marcus theory cannot explain the small KIE in regime H and the dependence of KIE on temperature in regime L. It is worth pointing out that Marcus−Levich−Dogonadze theory is valid only when the temperature is high enough that most environmental modes are excited. If the temperature is low enough that these modes are frozen, then a plot of the rate vs temperature shows a plateau.

Region II: Moderate-to-Large Quantum Effects. This is the case in which tunneling takes place from excited states that are not close to the barrier top, and it has eluded solution for decades because there is no small parameter available (as in the previous two cases). Finally, this problem was numerically solved[17] in 1994, and the solution was analytically verified with a different method by our group.[20] This class of problems is referred to as the quantum Kramers problem and is the model that we use in this paper and develop in the next section.

It is instructive to have a feeling for when and how the semiclassical theory fails. In Table 3, we compare the exact results[17,20] with the predictions of the semiclassical model (eq 1). The potential barrier height was 6 kcal/mol, the transfer distance 2 Å, and the reactant barrier frequency was 700 cm^{-1}. In the third column, we show the ratio of the exact quantum rate, $k_{quantum}$, to the TST rate, k_{TST}, and in the last column, we show the semiclassical prediction for this ratio. In the second column, we show the ratio of the reactant well frequency ω_R to the thermal energy (this ratio is a measure of the quantum character of the reaction, because the energy states in the reactant well are separated by ω_R). The exact calculations were performed for various values of friction (for the meaning of friction, see the next section, eq 8). As expected, the semiclassical model fails when quantum corrections are large. Also, it cannot account for the strong dependence of the rate on friction. For larger barrier heights or higher barrier frequencies, the semiclassical model fails at higher temperature. This is certainly the case for enzymatic hydride transfer (for C−H cleavage at room temperature, the ratio $\omega_R/k_BT \approx 13$).

Two-Dimensional Potential Energy Surfaces. All of the theories mentioned so far assume a one-dimensional potential energy surface. Hynes[5] was the first to emphasize the importance of rate-promoting vibrations for hydrogen transfer. Because of the larger mass of the proton, the tunneling probability is extremely sensitive to the tunneling distance, and as a consequence, any vibration that modulates this distance will strongly affect the rate. In the framework of the Marcus model (i.e., in the deep-tunneling limit, region III), he was able to derive the expression for the rate

$$k \approx e^{-\beta E_r/4} e^{2\alpha^2/(\beta M_Q \Omega_Q^2)} \quad (5)$$

where M_Q and Ω_Q are the mass and frequency, respectively, of the rate-promoting vibration and the parameter α modulates the tunneling matrix element Δ and varies with the isotope mass m_L roughly as $\alpha \approx \sqrt{m_L}$; a typical value for hydrogen transfer is $\alpha \approx 30$ $Å^{-1}$ (in contrast, for electron transfer, it is only $\alpha \approx 1$ $Å^{-1}$.) This model cannot explain the dependence of the KIE on the rigidity of the enzyme.

The models used for regions I and III and the model of eq 5 have significant limitations when applied to the problem at hand: the solvent is described by a classical one-dimensional coordinate, or tunneling takes place only from the ground state. Because of the large proton mass, we expect that contributions from excited states (region II) play an important role. This issue has been addressed in recent years by us[20] and several other groups, but these works do not include the effect of rate-promoting vibrations, which has been correctly captured in eq 5, that are always[21] important in proton-transfer reactions. It is this lacuna in theory and the startling experimental results of the Klinman group that the present paper addresses.

TABLE 2: Theoretical Predictions for Our Model System

	regime H	regime L
T (°C)	30−60	5−30
E_r (kcal/mol)	3.2	32
c	0.35−0.38	0.170−0.185
KIE	3.4−3.2	9.2−3.6
E_H (kcal/mol)	2.4	12.2

TABLE 3: $k_{quantum}/k_{TST}$ Ratio

T (K)	ω_R/k_BT	exact	semiclassical
100	9.6	30−1000	12.9
200	4.8	2.0−3.8	2.3
300	3.2	0.8−1.8	1.5

III. Quantum Theory

As we mentioned earlier, the unavailability of methods of numerical solution (except for cases where tunneling corrections are small) for quantum systems with many degrees of freedom (where the destruction of quantum coherence is physically important, rather than the arrangement of atoms, as in classical charge transfer) forces us to use a simplified model for the enzymatic environment. However, we believe that the following discussion captures the essential physics.

Our starting point is the stochastic description of the transfer process provided by the Langevin equation[22,23]

$$m\ddot{s} = -\frac{\partial V(s)}{\partial s} + \int_0^t dt'\, \gamma(t-t')\dot{s} + F_{env}(t) \quad (6)$$

where $V(s)$ is the potential energy surface (PES). The influence of the enzymatic environment in eq 6 is represented by the random force $F_{env}(t)$, which is related to the friction $\gamma(t)$ through the fluctuation−dissipation theorem.[22] From the previous discussion, it is clear that eq 6 will be useful for the present problem

because it explicitly includes the effect of protein rigidity on the reaction coordinate through the friction function $\gamma(t)$.

A milestone result in rate theory was the observation by Zwanzig[24] that the classical dynamics of the Hamiltonian

$$H = \frac{p_s^2}{2m_s} + V(s) + \sum_k \frac{p_k^2}{2m_k} + \sum_k \frac{1}{2} m_k \omega_k^2 \left(q_k - \frac{c_k s}{m_k \omega_k^2} \right)^2 \quad (7)$$

is described by eq 6. The potential in eq 7 is the sum of the one-dimensional potential energy surface $V(s)$ of eq 6 and a potential that describes interactions of the reaction coordinate s with the environment. Zwanzig proved that this latter potential can be written as a sum of independent harmonic potentials of oscillators q_k with mass and frequency m_k and ω_k, respectively, that have time-dependent centers of oscillation at $c_k s/m_k \omega_k^2$. (This last term represents the coupling of the environment to the reaction coordinate.)

We should point out that the Marcus−Levich−Dogonadze result in eq 4 is the solution of the Hamiltonian in eq 7 in the deep-tunneling limit. In addition, the solution of the Hamiltonian in eq 7 in the classical limit reproduces the transition state theory result, corrected for recrossings of the barrier and for memory effects.[23] These results mean that the Zwanzig Hamiltonian provides a unified description of proton-transfer reactions in all three parameter regions defined in the previous section.

The critical point is that these harmonic oscillators q_k represent a *hypothetical* environment (the real one is anharmonic) that generates a friction

$$\gamma(t) = \sum_k \frac{c_k^2}{m_k \omega_k^2} \cos(\omega_k t) \quad (8)$$

that is equal to the friction that enters the phenomenological Langevin equation (eq 6). Thus, the hypothetical harmonic environment (eq 7) was shown by Zwanzig to generate the *same dynamics* for the reaction coordinate as the real anharmonic environment. The cosine Fourier transform of eq 8 is called the spectral density $J(\omega)$ and is a quantity that can be measured experimentally or calculated from a molecular dynamics simulation. The reorganization energy of the environment that appears in Marcus-type theories is proportional to

$$E_r \approx \int d\omega \, \frac{J(\omega)}{\omega} \quad (9)$$

We must now express the "rate-promoting" vibration idea of Hynes in the Zwanzig formalism. An obvious approach is to select one enzymatic vibration and assume that it is very strongly coupled to the reaction coordinate, but we shall shortly see that this natural idea fails. Instead, we follow an idea by Benderskii[21] that he introduced in his studies of gas-phase reactions: we assume that there is an environment vibration Q that is *symmetrically* coupled to the reaction coordinate through a term cs^2Q. Note that what is different from the other environment modes is not the strength but the *symmetry* of the coupling. It is easy to verify that, for example, for a quartic double well PES, such a term reduces both the barrier height and the transfer distance, as we expect a rate-promoting vibration to do.

Even without the complication of the rate-promoting Q vibration, the numerical solution of the quantum Hamiltonian in eq 7 is a difficult task. Here, we briefly outline a method that we developed elsewhere.[25,26] (i) Find the environment coordinates that symmetrize the PES in eq 7 (e.g., $q_k = 0$ for a symmetric PES). (ii) Group the quadratic counterterm (which is obtained when we expand the harmonic potential energy in eq 7) with the reaction coordinate s. (iii) Solve for the dynamics of the Hamiltonian in eq 7 in the adiabatic limit (which is appropriate in the current case[26]). (iv) Finally, calculate the quantum rate using the flux−flux correlation formalism.[27]

An advantage of using the Zwanzig formalism for quantum rates is that one can perform a classical molecular dynamics simulation to obtain the dynamical friction $\gamma(t)$, which is then used as the only input to the quantum calculation. This computational scheme has also been used by other groups for the calculation of quantum dynamics in enzymes.[28,29]

To generalize this method for the case when a rate-promoting vibration Q is present, we make the substitution[30]

$$V(s) \rightarrow V(s) + \frac{1}{2} M_Q \Omega_Q^2 + cs^2 Q \quad (10)$$

to obtain a two-dimensional PES that will be immersed in an environment. Computationally, this means that we use the new potential of eq 10 in the calculation of the rate and integrate out the Q degree of freedom in the final result (details of the calculation can be found in ref 30.)

It is this two-dimensional PES that is appropriate for our study of hydrogen transfer in alcohol dehydrogonase, e.g., it can explain the observed moderate values of KIE even for extreme proton tunneling. It is worth clarifying the difference between the dynamic mechanism that we are describing in this section and the static view, where some structural change brings donor and acceptor closer and enhances tunneling: In the static view, tunneling takes place in a one-dimensional PES and leads to a high quantum rate and a large KIE. In the rate-promoting-vibration mechanism, tunneling takes place in a two-dimensional PES and leads to a high quantum rate but a moderate KIE.

IV. Results and Discussion

We have performed a molecular dynamics simulation of the transfer of a hydride from alcohol to NAD^+ in a solution of 255 acetonitrile molecules. (We used acetonitrile, which is a common choice for the simulation of a polar enzymatic environment, because there are no crystallographic structures of thermophilic alcohol dehydrogonase available.) For the reaction coordinate, we used a PES obtained in a simulation in ref 31 (the barrier height is equal to 15 kcal/mol). For the rest of the molecular and solvent system we have used the function developed by Hurley and Hammes-Schiffer.[32] (This function has the desirable property that the structure of the substrate changes as the hydride moves along the reaction coordinate.) This hybrid potential was needed because the calculations of ref 32 did not include a metal ion. It is known that the barrier for hydride transfer is much lower in the alcoholate than in the neutral species without the metal ion; it is probably this lower barrier for the alcoholate that represents the actual situation in the enzyme.

The potential energy surface that we used from ref 32 defines a reactant and a product configuration for the alcohol substrate and the cofactor and algebraically interpolates between these two for intermediate configurations. In other words, this potential does not yield energy as a function of all atomic coordinates, and as a result, we are not able to study the unusual secondary kinetic isotope effects that have been observed in this system.

For the MD simulation, we used the algorithm (modified RATTLE) introduced by Hurley and Hammes-Schiffer.[32] The molecular dynamics simulation provides the dynamical friction $\gamma(t)$, and after a Fourier transform, one obtains the spectral

density $J(\omega)$. Then, one can proceed with the computational scheme outlined in the previous section.

Our simulation gave a renormalization energy (calculated from eq 9) equal to 3.2 kcal/mol. We can reproduce the experimental trends of ref 12 with the following physical description:

(i) In regime L, the enzyme undergoes a transition to a phase with substantial rigidity. We simulated this effect by using a reorganization energy E_r equal to the value obtained by the molecular dynamics in regime H and 10 times larger than that obtained in regime L. (We note that this assumption is supported by recent experimental results[33] of Kohen and Klinman. which show that thermophilic ADH is significantly more rigid at 25 °C than at the optimal temperature.)

(ii) However, because some protein motions freeze out in regime L, we expect the rate-promoting vibration to have a smaller effect; in addition, because the motion of the promoting vibration is temperature-dependent, we allowed the coupling constant c to vary by 0.03 over a temperature range of 30 °C. To simulate these effects, we used a value of the coupling constant c (which appears in the coupling term cs^2Q in eq 10) equal to 0.35−0.38 au in regime H and a value half as large in regime L.

(iii) For the rate-promoting vibration Q, we chose reasonable values for the bound NAD^+ motion relative to the alcoholate: a mass equal to 15 C atom masses and a frequency $\Omega_Q = 200$ cm^{-1}.

Our results are shown in Table 2. In regime H, the KIE is practically temperature-independent and equal to 3.2−3.4. As we pass to regime L, we see two changes. The activation energy increases significantly, and the KIE becomes larger and temperature-dependent, taking values of 3.6−9.2 in the temperature range 5−30 °C. Comparing these results with Table 1, we see that we have reproduced the trend observed in the experiment. (To reproduce the experimental activation energies it would be necessary to know the exact spectral density of the enzyme.)

A few remarks about the dependence of these results on the choice of values for the unknown quantities should be made. The mass (15 C atom masses) and frequency (200 cm^{-1}) of the rate-promoting vibration are fairly typical values for a realistic system. The value of 0.35 for the coupling constant was selected so that there was a 30% reduction of the barrier height along the one-dimensional "sudden-trajectory"[30] barrier. In the high-rigidity regime, we used half that value, which is probably reasonable. The value for the reorganization energy is a guess, as we used acetonitrile in the simulation. If we had multiplied this value by 5 in the high-rigidity regime, we would have obtained qualitatively similar answers, but we chose a value of 10 to have better quantitative agreement with the experimental values of the KIE. The purpose of this paper is not to determine the correct values for these parameters, but rather to determine whether the proposed mechanism reproduces qualitatively the experimental trends with a choice of typical values for these parameters.

Discussion. The results presented in this paper suggest future directions for both theoretical and experimental work.

(i) We must find a way to rigorously identify the rate-promoting vibration in real systems. There is evidence from both experiment[34] and molecular dynamic simulations[3] that the residue Val^{203} (which is next to the hydride-receiving cofactor, distal to the donating alcoholate) forces the NAD^+ ring into closer proximity to the alcohol, strongly suggesting that internal motions of this residue are central to the rate-promoting vibration. What would be a clear signature of such a vibration in a molecular dynamics simulation? One possibility, suggested in ref 35, is that the spectral density would exhibit a peak at the frequency of the strongly coupled vibration. We are currently working on determining the signature of a rate-promoting vibration in a spectral density determined by molecular dynamics. Preliminary results[36] do, in fact, show a peak at the spectral density in model systems in which the intermolecular potential is chosen to create a rate-promoting vibration, but this peak is displaced with respect to the frequency of the rate-promoting vibration.

(ii) There is the intriguing possibility that it might be feasible to modulate the transfer rate by modifying some parameter (e.g., by isotopic substitution or by a change in the size of the residue) of the rate-promoting vibration. Such possibilities have been investigated in ref 34 for various mutants of horse liver alcohol dehydrogenase. A specific mutation, $Val^{203} \rightarrow Ala$, that significantly affects enzyme kinetics has been identified. Modification of this residue to the smaller alanine both significantly lowers the catalytic efficiency of the enzyme as compared to that of the wild type and significantly lowers indicators of hydrogen tunneling. Bruice's recent molecular dynamics calculations[3] produce results consonant with the concept that mutation of the valine changes protein dynamics (in his language, it lowers the production of near attack conformations.)

(iii) Our two-dimensional model shows that some care must be exercised in the interpretation of Arrhenius plots. When there is a mechanism that enhances the transfer rate (like the coupling to the rate-promoting vibration Q) and that becomes more efficient with increasing temperature, then this mechanism manifests itself in an Arrhenius plot of log(rate) vs inverse temperature as a *convex* plot. This should be contrasted with tunneling through one-dimensional barriers, where large slopes in an Arrhenius plot mean large activation energies and low transfer rates. Therefore, whereas it was suggested in ref 12 (from an analysis of the Arrhenius plots) that there is more tunneling in the high-temperature regime, the present analysis (based on a two-dimensional PES) offers an alternative interpretation.

The startling, but chemically and physically plausible, conclusion is that nature might well have evolved this class of enzymes to speed reactions by changing barrier geometry, not just barrier heights. The only way to analyze and understand such a situation is with a dynamic model of protein action in an enzyme.

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