Biochemistry **2008,** *47,* 9880–9887

Incorporation of Hydrostatic Pressure into Models of Hydrogen Tunneling Highlights a Role for Pressure-Modulated Promoting Vibrations[†]

Sam Hay* and Nigel S. Scrutton*

Manchester Interdisciplinary Biocentre and Faculty of Life Sciences, University of Manchester, 131 Princess Street, Manchester M1 7DN, U.K.

Received April 4, 2008; Revised Manuscript Received July 28, 2008

ABSTRACT: Hydrostatic pressure offers an alternative to temperature as an experimental probe of hydrogen-transfer reactions. H tunneling reactions have been shown to exhibit kinetic isotope effects (KIEs) that are sensitive to pressure, and environmentally coupled H tunneling reactions, those reactions in which H transfer is coupled to atomic fluctuations (a promoting vibration) along the reaction coordinate, often have quite temperature-dependent KIEs. We present here a theoretical treatment of the combined effect of temperature and pressure on environmentally coupled H tunneling reactions. We develop a generalized expression for the KIE, which can be used as a simple fitting function for combined experimental temperature- and pressure-dependent KIE data sets. With this expression, we are able to extract information about the pressure dependence of both the apparent tunneling distance and the frequency of the promoting vibration. The KIE expression is tested on two data sets {the reduction of chloranil by leuco crystal violet [Isaacs, N. S., Javaid, K., and Rannala, E. (1998) *J. Chem. Soc., Perkin Trans. 2*, 709−711] and the reduction of morphinone reductase by NADH [Hay, S., Sutcliffe, M. J., and Scrutton, N. S. (2007) *Proc. Natl. Acad. Sci. U.S.A. 104*, 507−512]} and suggests that hydrostatic pressure is a sensitive probe of nuclear quantum mechanical effects in H-transfer reactions.

Enzyme catalysis is a dynamic process, and the formation and breakage of bonds are often accompanied by atomic fluctuations along the reaction coordinate (*1, 2*). There is growing recognition of the importance of nonequilibrated fast (subpicosecond) dynamics, so-called promoting vibrations, that modify the reaction barrier and influence the rate of reaction (*3*−*12*). Experimentally, the identification of promoting vibrations is challenging, but hydrogen tunneling reactions are well-suited for the study of such dynamical processes. Promoting vibrations have been invoked in experimental studies of KIEs[1] (*6*−*8*) and "visualized" in computational simulations of enzyme-catalyzed H transfer (*4, 5, 13*). The first experimental evidence for a general role of protein motion during H tunneling reactions in enzymes (*14*−*16*) was inferred from observations of KIEs with unusual temperature dependencies, when $\Delta\Delta H^{\ddagger}$ (or ΔE_a) is either large or zero. These data were initially interpreted in terms of tunneling preceded by either (i) thermally equilibrated motion that is sufficient to bring the reactant and product wave functions to degeneracy or (ii) nonequilibrated motion along the reaction coordinate that brings the reactant and product states to a tunneling-competent conformation, yielding large values for $\Delta\Delta H^{\ddagger}$. It has recently become apparent that this criterion is an oversimplification and environmentally coupled H tunneling (ECHT) reactions can manifest KIEs with modest $\Delta\Delta H^{\ddagger}$ values (*7, 17*), which may not even be experimentally measurable (*7*). Hydrostatic pressure offers an alternative to temperature as an independent and sensitive experimental probe with which to study environmentally coupled H-transfer reactions (*6*), but to the best of our knowledge, there is no theoretical foundation with which to describe the pressure dependence of such reactions.

Semiclassical transition state theory (TST) dictates that pressure effects are associated with differences in the vibrational frequencies of the transferred atoms (*18*−*20*), and such stretching frequencies have been shown to be relatively insensitive to pressures of a few kilobars (*18*−*20*) (1 bar = 0.987 standard atmosphere or 100 kPa). Conversely, chemical systems with inflated isotope effects that cannot be explained by semiclassical TST [i.e., the reactions likely involve nuclear quantum mechanical (NQM) effects] have shown a significant pressure dependence of both the observed rate constants and the KIE (*21*). Thus, in principle, the pressure dependence of the KIE for an H-transfer reaction provides an excellent method of determining the contribution of NQM effects to the reaction, with a pressure-dependent KIE suggestive of a NQM tunneling reaction (*19, 22*).

PRESSURE DEPENDENCE OF THE REACTION RATE

Traditionally, the pressure (p) dependence of an observed rate constant has been described using absolute rate

---

[†] This work was funded by the UK Biotechnology and Biological Sciences Research Council (BBSRC). N.S.S. is a BBSRC Professorial Research Fellow.

* To whom correspondence should be addressed. E-mail: sam.hay@manchester.ac.uk and nigel.scrutton@manchester.ac.uk. Telephone: +44 161 306 5152. Fax: +44 161 306 8918.

[1] Abbreviations: ECHT, environmentally coupled H tunneling; ET, electron transfer; KIE, kinetic isotope effect; LCV, leuco crystal violet; MR, morphinone reductase; NADH, nicotinamide adenine dinucleotide; NQM, nuclear quantum mechanical; TST, transition state theory.

10.1021/bi8005972 CCC: $40.75 © 2008 American Chemical Society
Published on Web 08/22/2008

theory (*23, 24*) by assuming a quasi-equilibrium between the reactant and transition state:

$$k(p, T) = k_0 \exp(-\Delta V^{\ddagger} p/R_pT) \tag{1}$$

where R_p = 83.13 cm^3 mol^{-1} bar K^{-1}, k_0 is the observed rate constant extrapolated to 0 bar,[2] and $\Delta V^{\ddagger}$ is the apparent difference between the volume of the reactant state and the transition state. If the experimental data show significant curvature when plotted as ln k_{obs} versus p, then the norm is to fit the data to a quadratic function (*25*) such as

$$k(p, T) = k_0 \exp(-\Delta V^{\ddagger} p/R_pT) \exp(\Delta\beta^{\ddagger} p^2/2R_pT) \tag{2}$$

where $\Delta\beta^{\ddagger}$ is the compressibility of the transition state ($\Delta\beta^{\ddagger} = \mathrm{d}\Delta V^{\ddagger}/\mathrm{d}p$). The pressure dependence of the observed KIE can be treated in a similar manner:

$$\mathrm{KIE}(p, T) = \mathrm{KIE}_0 \exp(-\Delta\Delta V^{\ddagger} p/R_pT) \tag{3}$$

$$\mathrm{KIE}(p, T) = \mathrm{KIE}_0 \exp(-\Delta\Delta V^{\ddagger} p/R_pT) \exp(\Delta\Delta\beta^{\ddagger} p^2/2R_pT) \tag{4}$$

where KIE$_0$ is the observed KIE extrapolated to 0 bar, $\Delta\Delta V^{\ddagger} = \Delta V^{\ddagger H} - \Delta V^{\ddagger D}$, and $\Delta\Delta\beta^{\ddagger} = \Delta\beta^{\ddagger H} - \Delta\beta^{\ddagger D}$. However, no information regarding NQM effects is gained from this method as the magnitudes of observed activation volumes do not correlate with actual atomic volumes. Here, we explore alternate approaches to modeling the effect of pressure on NQM effects in H-transfer reactions. Our goal is to develop a simple model that can be used as a fitting function to extract NQM parameters from the temperature and pressure dependence of experimentally determined rate constants and/or KIEs.

BELL CORRECTION MODEL

Northrop has developed a model (*24*) for the pressure dependence of H-transfer reactions in solution based on the Bell correction (*26*) to account for small NQM effects:

$$\mathrm{KIE}(p, T) = \frac{k_{\mathrm{H}}}{k_{\mathrm{D}}} + \frac{k_{\mathrm{H}}}{k_{\mathrm{D}}}\left(\frac{Q_{\mathrm{H}}}{Q_{\mathrm{D}}} - 1\right) \exp\left(\frac{-\Delta V_{\mathrm{Q}} p}{R_pT}\right) \tag{5}$$

where $k_{\mathrm{H}}/k_{\mathrm{D}}$ is the purely semiclassical isotope effect, $Q_{\mathrm{H}}/Q_{\mathrm{D}}$ is the tunneling correction to the KIE, and ΔV_{Q} is the apparent volume difference (between H and D transfer) associated with the tunneling correction. This model assumes that there is both a semiclassical and tunneling contribution to the reaction [$k_{\mathrm{obs}} = k_{\mathrm{H}}Q_{\mathrm{H}}$ (*26*)] and that $k_{\mathrm{H}}/k_{\mathrm{D}}$ is invariant with pressure. If the reaction proceeds entirely by tunneling and we set $k_{\mathrm{H}}/k_{\mathrm{D}}$ equal to 1, then eq 5 simplifies to eq 3, where $Q_{\mathrm{H}}/Q_{\mathrm{D}}$ = KIE$_0$ and $\Delta V_{\mathrm{Q}} = \Delta\Delta V^{\ddagger}$. Equation 5 was initially used quite successfully by Northrop to validate the earlier experimental findings by Isaacs and co-workers that the large KIE of ~11 for the H transfer during the reduction of chloranil (2,3,5,6-tetrachloro-*p*-benzoquinone) by leuco crystal violet (LCV) is pressure-dependent (*18*−*20*). However, the KIE for this reaction has an apparent $\Delta\Delta H^{\ddagger}$ of 10−12 kJ mol^{-1} (Figure S1 of the Supporting Information) which is significantly larger than the upper limit of ~6 kJ mol^{-1} considered appropriate for the Bell correction (*26*).

Table 1: Pressure Dependence of Hydride Transfer during the Reduction of Chloranil by LCV and MR by NADH As Determined Using Transition State Theory (eqs 3 and 4) and a Bell Correction Model (eq 5)[a]

	TST (eq 4)		Bell correction (eq 5)		
T (K)	$\Delta\Delta V^{\ddagger}$ (cm^3 mol^{-1})	$\Delta\Delta\beta^{\ddagger}$ (cm^3 mol^{-1} kbar^{-1})	$k_{\mathrm{H}}/k_{\mathrm{D}}$	$Q_{\mathrm{H}}/Q_{\mathrm{D}}$	ΔV_{Q} (cm^3 mol^{-1})
		Chloranil/LCV			
294[b]	2.8 ± 0.2	0.6 ± 0.2	<5	>2.5	<6
302	9.3 ± 0.6	−5.2 ± 0.6	7.8 ± 0.1	1.44 ± 0.02	36.9 ± 3.5
313	2.9 ± 0.2	−0.4 ± 0.3	4.8 ± 1.5	1.9 ± 0.6	6.2 ± 2.5
		MR/NADH			
278	−10.2 ± 1.0	6.4 ± 0.9	8.6 ± 0.4	0.68 ± 0.03	29 ± 10
298	−8.8 ± 1.3	4.8 ± 1.1	5.8 ± 0.5	0.67 ± 0.05	20 ± 10
313	−14.4 ± 3.7	6.8 ± 3.2	5.2 ± 0.8	0.48 ± 0.06	20 ± 11

[a] This analysis is discussed in more detail in the Supporting Information. [b] The 294 K data did not converge when fit to eq 5.

Thus, it is probably not strictly correct to use a correction method and, thus, eq 5 to model this reaction. We have recently described the combined pressure and temperature dependence of the rate and primary H−D KIE for the reduction of morphinone reductase (MR) by nicotinamide adenine dinucleotide (NADH) (*6*), a putative enzyme-catalyzed ECHT reaction. In this previous work, we showed that the MR/NADH reaction is poorly described by eq 5. More recently, we have shown computationally that ~99% of H transfer during the MR/NADH reaction occurs by tunneling, thus further invalidating the use of such a model. For completeness, we have analyzed both the chloranil/LCV and MR/NADH reaction using eq 5 and compared the results to those obtained using eqs 3 and 4. For brevity, this analysis is discussed in the Supporting Information while the fit results are given in Table 1. Ultimately, as use of the Bell correction model (*26*) is often not appropriate and its use is also no longer in favor among many in the H-transfer community (*17, 27, 28*), an alternative approach to modeling pressure effects is desirable.

NONADIABATIC MODEL

There has been a recent trend toward describing H tunneling reactions, particularly in enzyme reactions, in terms of a Marcus-like nonadiabatic tunneling reaction (*7, 8, 11*), analogous to electron-transfer (ET) theory (*29, 30*). Obviously, this model was designed for ET reactions, and care must be taken when using this approach to model H-transfer reactions. For H transfer, these models are only valid for deep tunneling reactions, when the H tunnels far below the barrier. Therefore, to justify using nonadiabatic tunneling models, the reaction of interest should be initially shown to occur by a deep tunneling mechanism. In both the enzymes, aromatic amine dehydrogenase (*4*) and soybean lipoxygenase-1 (*31*), computational tunneling calculations have shown that 99% of H transfer occurs by tunneling; i.e., these are deep tunneling reactions. Both of these enzymes have very large primary H/D KIEs of >50, so from an experimentalist's standpoint, if the reaction of interest has a massive KIE, then the reaction can probably justifiably be treated using Marcus theory. As mentioned earlier, we have recently shown computationally that 99% of the H transfer in MR occurs by tunneling, despite the primary H/D KIE being normal (*32*), so the MR/NADH reaction can also be treated using

---

[2] For mathematical ease, we use 0 and 1 bar interchangeably in this study. Typical experimental studies extend over several kilobars, so the distinction is generally not important.

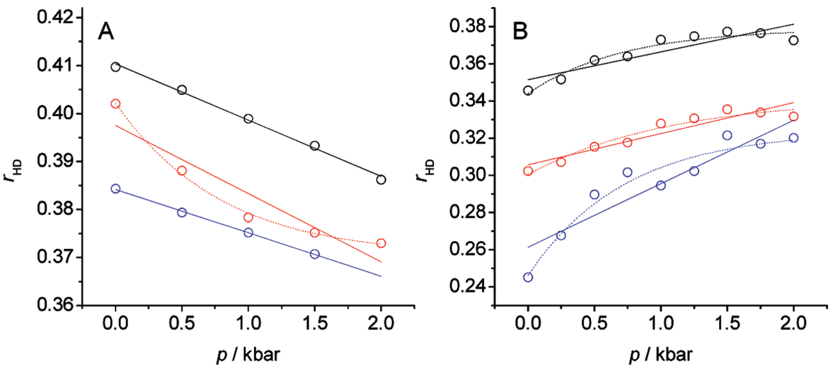

FIGURE 1: Apparent average H−D tunneling distances for (A) the reduction of chloranil by LCV at 294 (black), 302 (red), and 313 K (blue) and (B) the reduction of MR by NADH at 278 (black), 298 (red), and 313 K (blue). Both data sets were obtained by fitting the KIE values in panels A and B to eq 11, the rigid barrier model. The apparent r_{HD} values are fit to both linear (eq 12, solid lines) and exponential (eq 13, dashed lines) functions, with the resulting fitting parameters given in Table 2.

Marcus theory. We are not aware of any computational studies on the tunneling prevalence in the choranil/LCV reaction, but we will model this reaction for completeness.

We have previously modeled the MR/NADH reaction (*6*) using the ECHT model of Kuznetsov and Ulstrup (*11*), a Marcus-like nonadiabatic model, and implemented this model in a manner similar to that used by Knapp and Klinman (*8, 17*). In the simplest case, where there is no promoting vibration and H transfer does *not* occur between vibrationally excited states (discussed below), the rate of a H tunneling reaction can be described by

$$k = (1/2\pi)\sqrt{4\pi^3/\lambda k_B T\hbar^2}\,|T_{DA}|^2(\mathrm{FC})\exp(-\Delta G^{\ddagger}/k_B T) \quad (6)$$

where T_{DA} is the electronic coupling and FC the Franck−Condon term describing the nuclear wave function overlap between the donor and acceptor states of the transferred H:

$$\mathrm{FC}_i = \exp(-\mu_i\omega_i r^2/2\hbar) \quad (7)$$

where μ_i is the average reduced mass and ω_i the angular frequency [typically taken to be the stretching frequency of the transferred H (*8*)] of the transferred isotope. r is the tunneling distance. The final exponential term in eq 6 describes the Marcus apparent activation energy, $\Delta G^{\ddagger}$, which confers the temperature dependence of the rate constant. If the FC term is excluded, eq 6 is equivalent to the typical Marcus ET equation.

A theoretical treatment of the effect of pressure on long-range ET in proteins has been described by Miyashita and Go (*33*). They concluded that, while volume changes associated with both the electronic coupling (T_{DA}) and the Marcus ($\Delta G^{\ddagger}$) terms contribute to the observed $\Delta V^{\ddagger}$ (typically −10 to −20 cm^3 mol^{-1}), unless $\Delta V^{\ddagger}$ is very small (1−2 cm^3 mol^{-1}), the contribution from T_{DA} can be ignored. The distance dependence of ET reactions is described by the electronic coupling:

$$|T_{DA}|^2 \sim \exp(-\alpha r) \quad (8)$$

where α is a decay factor with typical values of roughly 1 Å^{-1} (*34*). The distance dependence of a H tunneling reaction is described by both the T_{DA} and FC terms (eq 6), so we must consider the effect of pressure on effective coupling: $|T_{DA}|^2 \times$ (FC). The suggestion that T_{DA} contributes little to the pressure dependence of a protein ET reaction seems reasonable. For a typical ET over ∼10 Å, any pressure-induced changes in r will be small [perhaps ∼0.1−0.2 Å (*35*)] such that the relative change, $\Delta r(p)/r_0$, will be small (∼1%). However, r is much smaller (<1 Å) for a room-temperature H transfer, so $\Delta r(p)/r_0 > 10\%$. Consequently, we need to consider the effect of pressure on both the effective coupling ($|T_{DA}|^2 \times$ FC) and Marcus ($\Delta G^{\ddagger}$) terms.

The apparent activation energy ($\Delta G^{\ddagger}$) can be described using a modification of the usual Marcus form (*8*):

$$\Delta G^{\ddagger} = \frac{1}{4}\lambda[1 + (\Delta G^{\circ} + E_{vib})/\lambda]^2 \quad (9)$$

where ΔG° is the driving force of the reaction, λ is the reorganization energy (*29, 30*), and E_{vib} is the difference in vibrational energy between the donor (reactant) and acceptor (product) states (*8*). The pressure dependence of $\Delta G^{\ddagger}$ will be caused by changes to ΔG° and λ. As vibrational frequencies are insensitive to a few kilobars of pressure (*18−20*), there should be little effect on E_{vib}. However, E_{vib} is isotope-dependent, so there will be a significant isotope effect on $\Delta G^{\ddagger}$ if H transfer to or from vibrationally excited states is prevalent. Miyashita and Go suggest that the pressure dependence of λ is small for protein ET reactions (*33*), so the dominant effect of pressure on the Marcus term will likely be on ΔG°. For both ET and H transfer reactions in either solvent or solvent-exposed protein active sites, there may be a larger effect of pressure on λ, which is sensitive to solvent, as electrostriction of solvent around charged groups is accompanied by large negative volume changes (*25*). From ET theory (*30*), when $|\Delta G^{\circ}| \ll \lambda$, then $d(\ln k_{obs})/d(-\Delta G^{\circ}) \sim 0.5$, and k_{obs} will increase with an increase in driving force. Also, there is some experimental evidence that increasing ΔG° will *decrease* the primary KIE while *increasing* both the α–secondary and ^{12}C/^{13}C KIEs (*36, 37*). This effect has recently been discussed in terms of changes to the reaction symmetry, $|\Delta G^{\circ}|/\lambda$, by Marcus (*38*). One way to investigate the effect of pressure on the relative change in ΔG° may be to measure the pressure dependence of both the primary and either the α–secondary or ^{12}C/^{13}C KIE. Unfortunately, both the α–secondary and ^{12}C/^{13}C KIEs are small, and these experiments may often prove impractical. Nevertheless, they are not impossible, and Northrop has recently reported the pressure dependence of the ^{12}C/^{13}C KIE in yeast alcohol dehydrogenase (*39*). As a word of caution, it is not obvious what additional pressure effects would be on either α–secondary or ^{12}C/^{13}C KIE when $d\Delta G^{\circ}/dp$ is equal to 0.

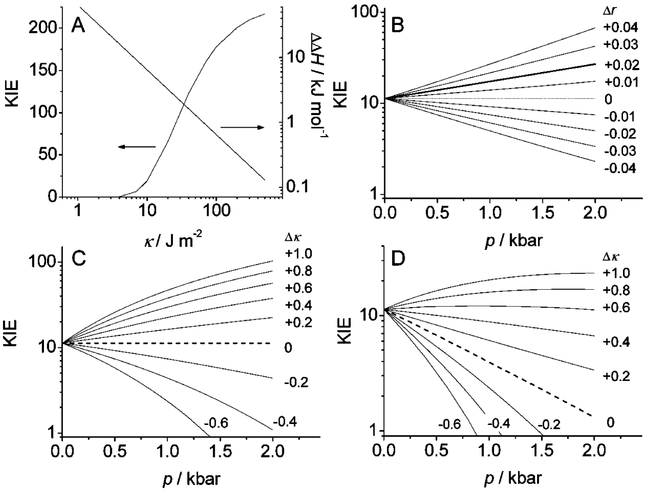

FIGURE 2: ECHT model incorporating hydrostatic pressure (eq 21). (A) Effect of the force constant of the promoting vibration (κ) on the magnitude and temperature dependence ($\Delta\Delta H^{\ddagger}$) of the KIE when $r_{HD} = 0.6$ Å and $\Delta r = \Delta\kappa = 0$. (B) Effect of Δr on the pressure dependence of the KIE when $\kappa = 2.5$ J m^{-2}, $r_{HD} = 0.70$ Å, and $\Delta\kappa = 0$. (C) Effect of $\Delta\kappa$ on the pressure dependence of the KIE when $\kappa = 2.5$ J m^{-2}, $r_{HD} = 0.70$ Å, and $\Delta r = 0$. (D) Like panel C but with $\Delta r = -0.03$ Å.

To circumvent many of the complications arising from the pressure dependence of the Marcus activation term, if one only examines the pressure dependence of the KIE, the effect of both $d\lambda/dp$ and $d\Delta G°/dp$ can be largely ignored (*6, 24*). Further, in the absence of vibrationally excited H transfer (discussed below), $\Delta G^{\ddagger H}/\Delta G^{\ddagger D} \sim 1$ and the Marcus term can be omitted entirely. In this case, which is reasonable for "deep" tunneling reactions, the pressure dependence of the KIE will then be described only by changes in the effective coupling:

$$k_H/k_D = \mathrm{KIE}_0 \exp[(\mu_D\omega_D {r_D}^2 - \mu_H\omega_H {r_H}^2)/2\hbar] \qquad (10)$$

where KIE_0 is essentially the difference in electronic wave function overlap between H and D donor and acceptor states. As the wavelength of an electron is at least 1 order of magnitude larger than that of H or D, we will take KIE_0 to approximate 1. If we consider the average tunneling distance of H and D, r_{HD}, then eq 10 becomes useful:

$$k_H/k_D = \mathrm{KIE}_0 \exp[(\mu_D\omega_D - \mu_H\omega_H){r_{HD}}^2/2\hbar] \qquad (11)$$

For reference, typically C−H stretching frequencies of ~3000 and 2200 cm^{-1}are used to describe the H and D nuclear wave function overlap, respectively (*7, 8, 11*), such that for a deuterium KIE, $(\mu_D\omega_D - \mu_H\omega_H)/2\hbar \sim 15.0$ Å^{-2}. Little difference is found if the donor/acceptor heavy atom is nitrogen or oxygen versus carbon.

MODELING PRESSURE

Both the chloranil/LCV and MR/NADH KIE data sets were fit to eq 11 (Figure 1) to extract the pressure dependence of r_{HD}. The pressure dependence of the observed KIEs was then analyzed using eq 11 by substituting either

$$r_{HD}(p) = r_0 + \Delta r_{lin}\, p \qquad (12)$$

or

$$r_{HD}(p) = (r_0 + \Delta r_{exp}) - \Delta r_{exp} \exp(-P_{1/2}\, p) \qquad (13)$$

where eqs 12 and 13 describe a linear and exponential change in r_{HD} with pressure, respectively. Here r_0 is the r_{HD} value at 0 or 1 bar (the difference is not significant), and $\Delta r_{lin} = dr/dp$. For an exponential dependence, Δr_{exp} is the extrapolated total change in r with a half-pressure of $P_{1/2}$. Note that in both cases, a negative value of Δr reflects a decrease in r_{HD} with increasing pressure, i.e., barrier compression. In all cases, the data were reasonably described by both methods, and the resulting r_0, Δr, and $P_{1/2}$ values are listed in Table 2. As for the earlier analysis using eq 5, the chloranil/LCV data at 294 K did not converge using eq 13 due to the lack of curvature in the data. However, by fixing the $P_{1/2}$ value as significantly greater than the maximum experimental pressure, we were able to estimate reasonable r_0 and Δr_{exp} values. Fairly similar values of r_0 and Δr were estimated using eqs 12 and 13 for both reactions (Table 2), so we will use eq 12 for the remainder of this study due to its simpler form and less adjustable parameters. It seems reasonable to model a linear pressure dependence of the transfer distance as this distance should be only weakly dependent on pressure over a few kilobars, i.e., the pressure range typically employed by the experimentalist. The exponential dependence of r_{HD} on p makes more sense for much higher ranges of pressure, where compression will become more difficult at higher pressures due to steric clashes, etc. Of concern is the fact that the combination of eq 11 with eq 12 or 13 predicts very short tunneling distances (0.3−0.4 Å) for both the chloranil/LCV and MR/NADH reactions. Also, due to the temperature dependence of the KIEs, these distances significantly decrease with an increase in temperature. A change in r_{HD} with temperature is expected; however, this change should be very small over the temperature range accessible to solution studies (e.g., 0 to ~60 °C), and we

Table 2: Pressure Dependence of Hydride Transfer during the Reduction of Chloranil by LCV and MR by NADH As Determined Using eq 11 with either a Linear (eq 12) or Exponential (eq 13) Compression of the Tunneling Distance with Pressure

	linear compression (eq 12)		exponential compression (eq 13)		
T (K)	r_0 (Å)	Δr_{lin} (Å kbar^{-1})	r_0 (Å)	Δr_{exp} (Å)	$P_{1/2}$ (kbar)
			Chloranil/LCV		
294[a]	0.410 ± 0.001	−0.012 ± 0.001	<0.41	<−0.06	>5
302	0.398 ± 0.003	−0.015 ± 0.003	0.401 ± 0.001	−0.032 ± 0.002	0.8 ± 0.1
313	0.384 ± 0.001	−0.009 ± 0.001	0.384 ± 0.001	−0.085 ± 0.067	8.7 ± 7.4
			MR/NADH		
278	0.353 ± 0.004	0.013 ± 0.003	0.342 ± 0.004	0.035 ± 0.004	0.6 ± 0.1
298	0.307 ± 0.003	0.016 ± 0.03	0.299 ± 0.004	0.040 ± 0.005	0.9 ± 0.3
313	0.267 ± 0.007	0.029 ± 0.005	0.248 ± 0.011	0.079 ± 0.011	0.8 ± 0.3

[a] The 294 K data did not converge when fit with eq 15. See the text for details.

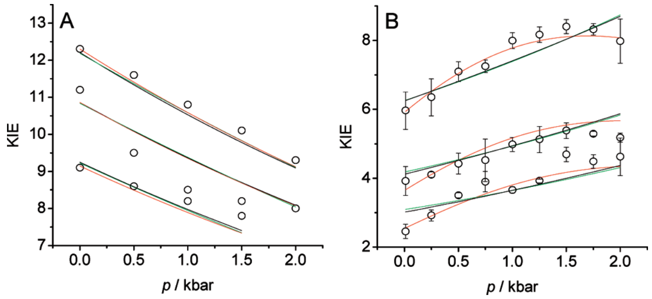

FIGURE 3: Observed KIE values for (A) the reduction of chloranil by LCV and (B) the reduction of MR by NADH. Both data sets are fit to eq 21 with either (i) no fixed parameters (red lines), (ii) $\Delta r = 0$ (green lines), or (iii) $\Delta\kappa = 0$ (black lines). Fitting parameters are listed in Table 3.

will assume, to a first approximation, that r_{HD} is invariant with temperature in this study. Further, these models predict that in the MR/NADH reaction the tunneling distance increases with pressure, despite the large rate enhancement observed with an increase in pressure (Figure 3B and Supporting Information).

TEMPERATURE- AND PRESSURE-DEPENDENT KIES

The most obvious problem with eq 11 is that there is no temperature term in the expression, causing unrealistically large decreases in r_{HD} with an increase in temperature. It would be convenient to analyze both the pressure and temperature dependence of the KIE using a singular approach. One approach is to extend eq 6 to describe H transfer between the vibrationally excited donor (v) and acceptor (w) states:

$$k_{\text{tun}} = \sum_{v} \left\{ \left[\exp(-E_v/k_BT) \sum_{w} k_{v,w} \right] \Big/ \sum_{v} \exp(-E_v/k_BT) \right\} \tag{14}$$

$$k_{v,w} = (1/2\pi)\sqrt{4\pi^3/\lambda k_BT\hbar^2}\,|T_{\text{DA}}|^2(\text{FC}_{\text{gating}}) \exp\{-[\Delta G^\circ + E_{v(i)} + \lambda]^2/4\lambda k_BT\}$$

where E_v is the (isotope-dependent) vibrational energy of the vth excited reactant state. Obviously, eq 14 is not suited for use as a routine fitting function. At room temperature, the majority of H transfer will occur from the ground state as $E_{1,\text{H}} \sim 36$ kJ mol^{-1} $\gg k_BT \sim 2.5$ kJ mol^{-1}. Therefore, a reasonable simplification is to omit the contribution to k from excited states. Further, the temperature dependence of k or the KIE arising from the partition function in eq 14 should, in most cases, be significantly smaller than that arising from excitation of a promoting vibration. To incorporate a promoting vibration or gating, to account for very temperature-dependent KIEs, the Franck−Condon term is modified:

$$\text{FC}_{\text{gating}0,0(i)} = \int_0^{r_0} [\exp(-\mu_i\omega_i\Delta r^2/2\hbar)] \exp(-E_X/k_BT)\,\text{d}X \tag{15}$$

where $\Delta r = (r_0 - r_X)$ is now the tunneling distance reduced from an equilibrium separation, r_0, by the distance of gating, r_X. The promoting vibration is treated classically as a harmonic oscillator with an isotope-independent gating energy, $E_X = (1/2)\hbar\omega_X{}^2$, where X is the reduced tunneling coordinate [$X = r_X(m_X\omega_X/\hbar)^{1/2}$] and m_X and ω_X are the reduced mass and frequency of the promoting vibration, respectively. This model has been successfully extended to more accurately describe the wave function overlap in terms of a Morse potential (*40*), but for the purposes of this study, we use the harmonic oscillator approach. Note that eq 15 gives the FC term for ground state tunneling. Vibrationally excited states are incorporated into this model by using modified FC terms (*8, 41*). All other terms in eqs 14 and 15 have their usual meaning and are defined in refs *7* and *8*. To describe the pressure dependence of a temperature-dependent KIE, the simplest model (i.e., excluding contribution from excited states) would then be

$$\frac{k_{\text{H}}}{k_{\text{D}}}(p,T) = \text{KIE}_0 \frac{\int_0^{r_0} \{\exp[-\mu_{\text{H}}\omega_{\text{H}}\Delta r(p)^2/2\hbar]\} \exp[-E_X(p)/k_BT]\,\text{d}X}{\int_0^{r_0} \{\exp[-\mu_{\text{D}}\omega_{\text{D}}\Delta r(p)^2/2\hbar]\} \exp[-E_X(p)/k_BT]\,\text{d}X} \tag{16}$$

The effect of pressure is (i) to change Δr by increasing or reducing the equilibrium separation, r_0, and/or the distance of gating, r_X, and/or (ii) to change the gating energy E_X by altering the frequency of the promoting vibration and/or r_X. We will assume that the tunneling distance does not significantly change with temperature (see above). Clearly, eq 16 will never be appropriate as a simple fitting function, but it can be used to model experimental data quite successfully (*6, 7, 11, 17, 40, 42*). Ultimately, it would be useful to have an ECHT model which *can* be used as a fitting function, preferably where the tunneling distance and the gating frequency can be independently varied.

As an alternative to eqs 14 and 15, a rate expression for an environmentally coupled H transfer has been described by Borgis and Hynes (*12*) and extended by Antoniou and Schwartz (*3*), and a similar approach to modeling the promoting vibration has also been described by Bruno and Bialek (*10*) and by Hammes-Schiffer (*43*). The general form of this rate equation is similar to the model of Kuznetzov and Ulstrup (*11*), but the $\text{FC}_i = \exp(-\mu_i\omega_i r^2/2\hbar)$ element of the Franck−Condon term [as called by Knapp and Klinman and ourselves (*7, 8*)] is essentially buried within the electronic coupling term, Δ:

$$k \sim \Delta^2 \exp(-\Delta G^\ddagger/k_BT) \exp(4E_{\text{sub}}k_BT/\hbar^2\omega_{\text{sub}}{}^2) \tag{17}$$

$$E_{\text{sub}} = \alpha^2\hbar^2/2m_{\text{sub}}$$

where $\Delta \sim \exp(-\alpha r)$ and is analogous to eq 8 and m_{sub} and ω_{sub} are the reduced mass and frequency, respectively, of an oscillating mode (promoting vibration) that modulates tunneling probability. Clearly, eq 16 has a non-Arrhenius temperature dependence, but this expression is useful (i.e., essentially linear) over modest changes in temperature either side of room temperature. Typical values of α for proton transfer have been suggested to be 20−35 Å^{-1} (*3, 12, 43*), which can be compared to an equivalent value of ∼1 Å^{-1} for ET reactions in solution (see above). α is analogous to $\mu_i\omega_i/2\hbar$ from eq 7, both describing the distance decay of the donor/acceptor wave function overlap. We will continue to use the wave function overlap of Kuznetsov and Ulstrup (eq 7) in preference to Δ in eq 17, but either approach will work as there is an isotope effect on α of $\alpha_{\text{D}} \sim \sqrt{2} \times \alpha_{\text{H}}$ (for

Table 3: Pressure Dependence of Hydride Transfer during the Reduction of Chloranil by LCV and MR by NADH As Determined using eq 21

	two-step fitting[a]		simultaneous fitting[b]	
	chloranil/LCV	MR/NADH	chloranil/LCV	MR/NADH
r_0 (Å)	0.69 ± 0.03	0.75 ± 0.03	0.69 ± 0.04	0.75 ± 0.01
Δr (Å kbar^{-1})	0 ± 4	-0.033 ± 0.004	-0.01 ± 0.04	-0.034 ± 0.005
κ_0 (J m^{-2})	2.6 ± 0.3	1.7 ± 0.2	2.6 ± 0.4	1.71 ± 0.06
$\Delta\kappa_0$ (J m^{-2} kbar^{-1})	0 ± 46	0.29 ± 0.03	0.1 ± 0.5	0.31 ± 0.04

[a] Parameters are taken from the fits in Figures S4 and S5 of the Supporting Information. [b] Parameters are taken from the fits in Figure 3.

more detail, see, for example, ref *44*). We can modify eqs 6 and 7 by adding the substrate vibration term from eq 17 and substituting $\alpha^2 = \mu_i\omega_i/2\hbar$:

$$k_i \sim |T_{\mathrm{DA}}|^2 \exp\left(-\frac{\mu_i\omega_i r^2}{2\hbar}\right) \exp\left(\frac{\mu_i\omega_i}{\hbar}\frac{k_{\mathrm{B}}T}{\kappa_{\mathrm{sub}}}\right) \exp\left(-\frac{\Delta G^{\ddagger}}{k_{\mathrm{B}}T}\right) \tag{18}$$

where the wave function overlap (cf., the tunneling distance) is now directly modulated by the promoting vibration, thus decoupling the distance and temperature dependence of the reaction. We have used the force constant of the gating mode in preference to the reduced mass and frequency with $\kappa_{\mathrm{sub}} = m_{\mathrm{sub}}\omega_{\mathrm{sub}}^2$. Note that the shape of the wave function overlap in eq 17 differs from that used by Kuznetsov and Ulstrup (eq 6; see Figure S3 of the Supporting Information for more details). As a result, $(\mu_i\omega_i/2\hbar)$ has units of Å^{-2} while α has units of Å^{-1}, so we must substitute $\mu_i\omega_i/2\hbar = \alpha^2$, cf., α, into eq 18. This is quite an important point, as $\mu_i\omega_i/2\hbar < \alpha^2$ for α values of greater than ~7 (i.e., typical literature values). This means that estimated values of κ_{sub} determined using eqs 17 and 18 will not be equivalent even when $\alpha^2 = \mu_i\omega_i/2\hbar$. However, values suggested for α are somewhat variable (*3, 12, 43*), and this parameter has been previously used as a fitting function (e.g., ref *44*). Consequently, κ_{sub} values should be treated qualitatively, and if it is important to compare such values with previous literature values of α and/or κ_{sub}, then eq 17 can be used in preference to eq 18 with the following substitutions made accordingly.

By using the same arguments that are used in eq 15, and assuming that κ_{sub} is isotope-independent, we arrive at

$$k_{\mathrm{H}}/k_{\mathrm{D}}(p,T) \sim \mathrm{KIE}_0 \exp[(\mu_{\mathrm{D}}\omega_{\mathrm{D}} - \mu_{\mathrm{H}}\omega_{\mathrm{H}}) r_{\mathrm{HD}}^{\;2}(p)/2\hbar] \exp[-(\mu_{\mathrm{D}}\omega_{\mathrm{D}} - \mu_{\mathrm{H}}\omega_{\mathrm{H}}) k_{\mathrm{B}}T/\hbar\kappa_{\mathrm{sub}}(p)] \tag{19}$$

At small (physically unrealistic) values of both r and ω_{sub}, the KIE will become inverse. We will continue to assume that $\mathrm{KIE}_0 \sim 1$, but this term could be used as a factor to calibrate eq 19 against experimental data if they become available. Now, the effect of pressure is to change (i) the tunneling distance r_{HD} and/or (ii) the frequency of the promoting vibration (κ_{sub}). Unlike eq 15, these two parameters are partly independent of each other with the temperature dependence of the KIE dictated solely by κ_{sub} and the magnitude of the KIE dictated by both r_{HD} and κ_{sub}.

While bond stretching frequencies have generally been taken to be insensitive to pressures of a few kilobars (*18*−*20*), there are often small but significant (i.e., a few wavenumbers) changes in frequency over kilobar changes in pressure. These are caused by changes in both the harmonic force constant and the anharmonicity of the oscillator (see, for example, refs *45* and *46*). As a first approximation, we can treat the effect of pressure on the promoting vibration force constant in a similar way, as a linear function (see, for example, ref *47*):

$$\kappa_{\mathrm{sub}}(p) = \kappa_0 + \Delta\kappa p \tag{20}$$

We stress that this is obviously a gross simplification, and if experimental or computational insight into the pressure dependence of the promoting vibration becomes available, eq 20 can be amended accordingly. In eq 20, a positive value of $\Delta\kappa$ denotes an increase in the frequency of the promoting vibration. By substituting eqs 12 and 20 into our new KIE expression from eq 19, we arrive at

$$k_{\mathrm{H}}/k_{\mathrm{D}}(p,T) \sim \mathrm{KIE}_0 \exp[(\mu_{\mathrm{D}}\omega_{\mathrm{D}} - \mu_{\mathrm{H}}\omega_{\mathrm{H}})(r_0 + \Delta rp)^2/2\hbar] \times \exp[-(\mu_{\mathrm{D}}\omega_{\mathrm{D}} - \mu_{\mathrm{H}}\omega_{\mathrm{H}})k_{\mathrm{B}}T/\hbar(\kappa_0 + \Delta\kappa p)] \tag{21}$$

As discussed, we could use a more elaborate function to describe the pressure dependence of r_{HD} (e.g., eq 14) and/or κ_{sub}, but we feel this is unnecessary at this stage. Using simple linear pressure dependencies, there are only five adjustable parameters in our KIE expression (eq 21): KIE_0, r_0, Δr, κ, and $\Delta\kappa$. However, as discussed above, we will fix KIE_0 to 1. The influence of the four remaining parameters on the apparent pressure dependence of the KIE is modeled in Figure 2. To the best of our knowledge, this is the first treatment of a nonadiabatic tunneling expression where hydrostatic pressure can be explicitly modeled.

We have used the observed KIE values for the chloranil/LCV and MR/NADH reactions to test eq 21 in two ways. First, for each reaction, the three data sets at different temperatures were simultaneously fit with all four of the fitting parameters shared, yielding single values of r_0, Δr, κ, and $\Delta\kappa$ for each reaction. This method is not the only option available, and to reduce the number of variables in the fitting, it is possible to initially fit a KIE versus temperature data set measured at atmospheric pressure to eq 21 with both Δr and $\Delta\kappa$ fixed to zero such that there are only two adjustable parameters (akin to the Arrhenius equation; see Figure S4 of the Supporting Information). This method requires fixing p to 0, cf., 1 bar. However, this is a reasonable simplification as the difference between 0 and 1 bar can be ignored if pressure changes over several kilobars are measured. Once reasonable estimates of r_0 and k_0 have been determined in this manner, the pressure dependence of the KIE can be fit with r_0 and/or κ_0 restrained to the values determined previously. Further, this method allows one to use eq 21 to fit the pressure dependence of a KIE at a single temperature. Simultaneous fits of both the chloranil/LCV and MR/NADH data sets to eq 21 are shown in Figure 3, and values of the four fitting parameters are listed in Table 3. Also given in Table 3 are the equivalent parameters determined from a single temperature and pressure experiment (see the Supporting Information and Table 3 for more details and these additional fits). While the simultaneous fit to the chloranil/LCV data is reasonable, the fit to the 302 K data is not particularly good. While eq 21 predicts a more reasonable tunneling distance for the chloranil/LCV reaction than eqs 11−13 [0.69 vs ~0.40 Å (Table 2)], significant values of all four fitting parameters were only obtained if either Δr or $\Delta\kappa$ were fixed to zero (see Figure 3, black and green fits, respectively). This becomes worse if only one pressure dependence is fit (Table 3 and Table S2 of the Supporting Information). However, we feel that the poor fit in Figure 3A is not so much the result of too many adjustable parameters in eq 21 but rather the consequence of the fact that there is markedly different curvature in this data set (see also the $\Delta\Delta\beta^{\ddagger}$ values in Table 1), perhaps due to experimental error and/or the relatively small number of data points measured. Both the simultaneous and individual fits of eq 21 to the MR/NADH data are excellent (Figure 3B and the Supporting Information), predicting a *decrease* in tunneling distance and an *increase* in κ (and thus the frequency of the promoting vibration) with an increase in pressure. Note this is the prediction we made previously (*6*) on the basis of the Kuznetsov and Ulstrup model (*11*), where we were able to qualitatively explain the combined pressure and temperature dependence of the MR/NADH KIE if r_0 decreases while ω_X increases with pressure. If we take the reduced mass of the gating mode in MR to be 100 Da, we predict from eq 21 and Table 3 that $\omega_0 \sim 17$ cm^{-1} (hence the strongly temperature-dependent KIE) and $\Delta\omega_{\mathrm{sub}} \sim 2$ cm^{-1} kbar^{-1}. Such a small change in ω_{sub} is in line with the very small changes in bond vibrational frequencies typically observed with kilobar changes in pressure (*45−47*). An increase in κ with pressure will cause the apparent temperature dependence of the KIE ($\Delta\Delta H^{\ddagger}$) to *decrease* with an *increase* in pressure. We were unable to experimentally measure a significant change in $\Delta\Delta H^{\ddagger}$ with pressure for the MR/NADH reaction (*6*) which would further suggest that, if eq 21 is realistic, $\Delta\kappa$ must be small. To describe the curvature in the MR/NADH data, the first exponential term in eq 21 must cause a decrease in the KIE with p as $\Delta r < 0$ (Figure 2B). However, the second (gating) exponential term causes an increase in KIE with p as $\Delta\kappa > 0$ (Figure 2C). These two terms oppose each other and cause the curvature in the KIE versus p plot (Figure 2D). Consequently, the usefulness of eq 21 as a fitting function to extract reasonable values of Δr and $\Delta\kappa$ will hinge on good quality data with sufficient data points to allow fitting of this curvature.

The nonlinearity of the pressure dependence of the KIE (the curvature described above) is described using TST by the magnitude of the compressibility of the transition state, $\Delta\beta^{\ddagger}$, from eq 4. Interestingly, adiabatic compressibility, β_{T}, has been previously suggested to be a descriptor of protein conformational dynamics (*48, 49*), as the mean square volume fluctuations, $\langle\delta V\rangle^2$, of a protein of volume V can be described by the relationship $\langle\delta V\rangle^2 = k_{\mathrm{B}}T\beta_T V$ (*49*). Changes in the frequency of any promoting motion will be crudely manifest as a change in $\langle\delta V\rangle^2$ and thus as changes in the apparent compressibility, i.e., $\Delta\beta^{\ddagger}$.

Finally, while we cannot model the effect of pressure on the rate of H transfer due principally to uncertainly in $\mathrm{d}\Delta G^{\ddagger}/\mathrm{d}p$, we can check whether the fitted parameters from Figure 3 will give reasonable pressure dependencies of k_{H} by substituting them into eq 18 and assuming $\mathrm{d}\Delta G^{\ddagger}/\mathrm{d}p = 0$. While there is poor agreement between the modeled and experimental data, both the MR/NADH and chloranil/LCV data showed ln k versus p to be fairly linear (Figure 4). This suggests that linear changes in r and κ with pressure (eqs 12 and 20) are appropriate, and while the magnitude of the apparent $\Delta V^{\ddagger}$ values differs from the observed values, they are of the correct order of magnitude and, more importantly, both predict an increase in k_{obs} with pressure.

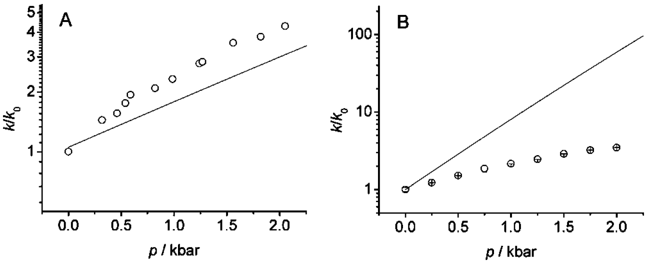

FIGURE 4: Modeled rate (cf., KIE) of hydride transfer for (A) the reduction of chloranil by LCV at 302 K and (B) the reduction of MR by NADH at 298 K. The rate constants were modeled (solid lines) using the r, κ, r_0, and $\Delta\kappa$ values given in Table 3 (simultaneous fits) and with a $\Delta G^{\ddagger}$ of 0. The experimental data are shown as empty circles. Note that the rate constants are plotted on a log scale.

CONCLUSIONS

We describe various approaches for modeling the effect of hydrostatic pressure on H tunneling reactions in solution and/or in enzymes. One such model, which is based on the Bell correction (*26*), has been described previously by Northrop (*24*). We show that this model does not describe the environmentally coupled MR/NADH reaction (*6*) in a physically realistic manner. Instead, we have developed a nonadiabatic deep tunneling model (eq 21) that can describe the combined pressure and temperature dependencies of the KIEs for the MR/NADH and chloranil/LCV data sets. In our model, hydrostatic pressure modifies the reaction barrier by compressing the tunneling distance (barrier width) and/or altering the frequency of a promoting vibration which is coupled to the reaction coordinate. This model can be used as a fitting function for experimental data, with only four adjustable parameters, all directly reporting on NQM effects. Thus, studies of pressure effects on KIEs are important new probes of tunneling reactions. The analysis presented in this paper provides a theoretical framework for interpretation of these effects for nonadiabatic deep ECHT reactions.

SUPPORTING INFORMATION AVAILABLE

Plot of the temperature dependence of KIE for the chloranil/LCV reaction at different pressures; plots of the pressure dependence of rate constants and KIEs for chloranil/LCV and MR/NADH systems and associated parameters from data fitting to transition state theory and Bell correction models; plots of hydrogenic wave function overlap for the Kuznetsov and Ulstrup model and the Bruno and Bialek model; plots of the temperature and pressure dependence of the observed KIE for chloranil/LCV and MR/NADH systems analyzed using eq 21 and associated parameters derived from model fitting. This material is available free of charge via the Internet at http://pubs.acs.org.

REFERENCES

1. Yon, J. M., Perahia, D., and Ghelis, C. (1998) Conformational dynamics and enzyme activity. *Biochimie 80*, 33–42.
2. Henzler-Wildman, K., and Kern, D. (2007) Dynamic personalities of proteins. *Nature 450*, 964–972.
3. Antoniou, D., and Schwartz, S. D. (1997) Large kinetic isotope effects in enzymatic proton transfer and the role of substrate oscillations. *Proc. Natl. Acad. Sci. U.S.A. 94*, 12360–12365.
4. Masgrau, L., Roujeinikova, A., Johannissen, L. O., Hothi, P., Basran, J., Ranaghan, K. E., Mulholland, A. J., Sutcliffe, M. J., Scrutton, N. S., and Leys, D. (2006) Atomic description of an enzyme reaction dominated by proton tunneling. *Science 312*, 237–241.
5. Caratzoulas, S., Mincer, J. S., and Schwartz, S. D. (2002) Identification of a protein-promoting vibration in the reaction catalyzed by horse liver alcohol dehydrogenase. *J. Am. Chem. Soc. 124*, 3270–3276.
6. Hay, S., Sutcliffe, M. J., and Scrutton, N. S. (2007) Promoting motions in enzyme catalysis probed by pressure studies of kinetic isotope effects. *Proc. Natl. Acad. Sci. U.S.A. 104*, 507–512.
7. Johannissen, L. O., Hay, S., Scrutton, N. S., and Sutcliffe, M. J. (2007) Proton tunneling in aromatic amine dehydrogenase is driven by a short-range sub-picosecond promoting vibration: Consistency of simulation and theory with experiment. *J. Phys. Chem. B 111*, 2631–2638.
8. Knapp, M. J., Rickert, K., and Klinman, J. P. (2002) Temperature-dependent isotope effects in soybean lipoxygenase-1: Correlating hydrogen tunneling with protein dynamics. *J. Am. Chem. Soc. 124*, 3865–3874.
9. Antoniou, D., and Schwartz, S. D. (2001) Internal enzyme motions as a source of catalytic activity: Rate-promoting vibrations and hydrogen tunneling. *J. Phys. Chem. B 105*, 5553–5558.
10. Bruno, W. J., and Bialek, W. (1992) Vibrationally Enhanced Tunneling as a Mechanism for Enzymatic Hydrogen Transfer. *Biophys. J. 63*, 689–699.
11. Kuznetsov, A. M., and Ulstrup, J. (1999) Proton and hydrogen atom tunnelling in hydrolytic and redox enzyme catalysis. *Can. J. Chem. 77*, 1085–1096.
12. Borgis, D., and Hynes, J. T. (1996) Curve crossing formulation for proton transfer reactions in solution. *J. Phys. Chem. 100*, 1118–1128.
13. Mincer, J. S., and Schwartz, S. D. (2004) Rate-promoting vibrations and coupled hydrogen-electron transfer reactions in the condensed phase: A model for enzymatic catalysis. *J. Chem. Phys. 120*, 7755–7760.
14. Basran, J., Sutcliffe, M. J., and Scrutton, N. S. (1999) Enzymatic H-transfer requires vibration-driven extreme tunneling. *Biochemistry 38*, 3218–3222.
15. Kohen, A., Cannio, R., Bartolucci, S., and Klinman, J. P. (1999) Enzyme dynamics and hydrogen tunnelling in a thermophilic alcohol dehydrogenase. *Nature 399*, 496–499.
16. Maglia, G., and Allemann, R. K. (2003) Evidence for environmentally coupled hydrogen tunneling during dihydrofolate reductase catalysis. *J. Am. Chem. Soc. 125*, 13372–13373.
17. Knapp, M. J., and Klinman, J. P. (2002) Environmentally coupled hydrogen tunneling: Linking catalysis to dynamics. *Eur. J. Biochem. 269*, 3113–3121.
18. Isaacs, N. S., Javaid, K., and Rannala, E. (1978) Reactions at High-Pressure. 5. Effect of Pressure on Some Primary Kinetic Isotope Effects. *J. Chem. Soc., Perkin Trans. 2*, 709–711.
19. Isaacs, N. S. (1984) in *Isotope Effects in Organic Chemistry* (Buncel, E., and Lee, C. C. Eds.) pp 67−105, Elsevier, London.
20. Isaacs, N. S., Javaid, K., and Rannala, E. (1977) Pressure Effects on Proton Tunnelling. *Nature 268*, 372–372.
21. Northrop, D. B. (2006) Unusual origins of isotope effects in enzyme-catalysed reactions. *Philos. Trans. R. Soc. London, Ser. B 361*, 1341–1349.
22. Northrop, D. B. (2002) Effects of high pressure on enzymatic activity. *Biochim. Biophys. Acta 1595*, 71–79.
23. Gladstone, S., Laidler, K. J., and Eyring, H. (1941) *The theory of rate processes*, McGraw-Hill, New York.
24. Northrop, D. B. (1999) Effects of high pressure on isotope effects and hydrogen tunneling. *J. Am. Chem. Soc. 121*, 3521–3524.
25. Masson, P., and Balny, C. (2005) Linear and non-linear pressure dependence of enzyme catalytic parameters. *Biochim. Biophys. Acta 1724*, 440–450.
26. Bell, R. (1980) The Tunnel Effect in Chemistry, Chapman % Hall, London.
27. Masgrau, L., Basran, J., Hothi, P., Sutcliffe, M. J., and Scrutton, N. S. (2004) Hydrogen tunneling in quinoproteins. *Arch. Biochem. Biophys. 428*, 41–51.
28. Dutton, P. L., Munro, A. W., Scrutton, N. S., and Sutcliffe, M. J. (2006) Introduction. Quantum catalysis in enzymes: Beyond the transition state theory paradigm. *Philos. Trans. R. Soc. London, Ser. B 361*, 1293–1294.
29. Marcus, R. A. (1956) Theory of Oxidation-Reduction Reactions Involving Electron Transfer. *J. Chem. Phys. 24*, 966–978.
30. Marcus, R. A., and Sutin, N. (1985) Electron Transfers in Chemistry and Biology. *Biochim. Biophys. Acta 811*, 265–322.
31. Tejero, I., Garcia-Viloca, M., Gonzalez-Lafont, A., Lluch, J. M., and York, D. M. (2006) Enzyme dynamics and tunneling enhanced by compression in the hydrogen abstraction catalyzed by soybean lipoxygenase-1. *J. Phys. Chem. B 110*, 24708–24719.
32. Pang, J., Hay, S., Scrutton, N. S., and Sutcliffe, M. J. (2008) Deep tunneling dominates the biologically important hydride transfer reaction from NADH to FMN in morphinone reductase. *J. Am. Chem. Soc. 130*, 7092–7097.
33. Miyashita, O., and Go, N. (1999) Pressure dependence of protein electron transfer reactions: Theory and simulation. *J. Phys. Chem. B 103*, 562–571.
34. Moser, C. C., Keske, J. M., Warncke, K., Farid, R. S., and Dutton, P. L. (1992) Nature of Biological Electron Transfer. *Nature 355*, 796–802.
35. Kundrot, C. E., and Richards, F. M. (1987) Crystal Structure of Hen Egg-White Lysozyme at a Hydrostatic Pressure of 1000 atm. *J. Mol. Biol. 193*, 157–170.
36. Hermes, J. D., Morrical, S. W., Oleary, M. H., and Cleland, W. W. (1984) Variation of Transition-State Structure as a Function of the Nucleotide in Reactions Catalyzed by Dehydrogenases. 2. Formate Dehydrogenase. *Biochemistry 23*, 5479–5488.
37. Scharschmidt, M., Fisher, M. A., and Cleland, W. W. (1984) Variation of Transition-State Structure as a Function of the Nucleotide in Reactions Catalyzed by Dehydrogenases. 1. Liver Alcohol Dehydrogenase with Benzyl Alcohol and Yeast Aldehyde Dehydrogenase with Benzaldehyde. *Biochemistry 23*, 5471–5478.
38. Marcus, R. A. (2006) Summarizing lecture: Factors influencing enzymatic H-transfers, analysis of nuclear tunnelling isotope effects and thermodynamic versus specific effects. *Philos. Trans. R. Soc. London, Ser. B 361*, 1445–1455.
39. Park, H., Girdaukas, G. G., and Northrop, D. B. (2006) Effect of pressure on a heavy-atom isotope effect of yeast alcohol dehydrogenase. *J. Am. Chem. Soc. 128*, 1868–1872.
40. Meyer, M. P., and Klinman, J. P. (2005) Modeling temperature dependent kinetic isotope effects for hydrogen transfer in a series of soybean lipoxygenase mutants: The effect of anharmonicity upon transfer distance. *Chem. Phys. 319*, 283–296.
41. Ulstrup, J., and Jortner, J. (1975) Effect of Intramolecular Quantum Modes on Free-Energy Relationships for Electron-Transfer Reactions. *J. Chem. Phys. 63*, 4358–4368.
42. Meyer, M. P., Tomchick, D. R., and Klinman, J. P. (2008) Enzyme structure and dynamics affect hydrogen tunneling: The impact of a remote side chain (I553) in soybean lipoxygenase-1. *Proc. Natl. Acad. Sci. U.S.A. 105*, 1146–1151.
43. Hatcher, E., Soudackov, A. V., and Hammes-Schiffer, S. (2007) Proton-coupled electron transfer in soybean lipoxygenase: Dynamical behavior and temperature dependence of kinetic isotope effects. *J. Am. Chem. Soc. 129*, 187–196.
44. Hatcher, E., Soudackov, A., and Hammes-Schiffer, S. (2005) Nonadiabatic proton-coupled electron transfer reactions: Impact of donor-acceptor vibrations, reorganization energies, and couplings on dynamics and rates. *J. Phys. Chem. B 109*, 18565–18574.
45. Lucazeau, G. (2003) Effect of pressure and temperature on Raman spectra of solids: anharmonicity. *J. Raman Spectrosc. 34*, 478–496.
46. Benson, A. M., and Drickamer, H. G. (1957) Stretching Vibrations in Condensed Systems:Especially Bonds Containing Hydrogen. *J. Chem. Phys. 27*, 1164–1174.
47. Yenice, K. M., and Lee, S. A. (1992) Raman pectroscopy of Potassium Selenate at High ressure. *J. Raman Spectrosc. 23*, 299–302.
48. Gekko, K. (2002) Compressibility gives new insight into protein dynamics and enzyme function. *Biochim. Biophys. Acta 1595*, 382–386.
49. Cooper, A. (1976) Thermodynamic Fluctuations in Protein Molecules. *Proc. Natl. Acad. Sci. U.S.A. 73*, 2740–2741.

BI8005972