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Exponential Relationships among Multiple Hydrogen Isotope Effects as Probes of Hydrogen Tunneling[1]

KATHLEEN L. GRANT[2] AND JUDITH P. KLINMAN[3]

Department of Chemistry, University of California, Berkeley, California 94720

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For hydrogen transfer reactions with moderate degrees of tunneling, the semiclassical relationship of deuterium, tritium to protium, tritium isotope effects is predicted to break down, such that $(k_D/k_T)^{3.26} < k_H/k_T$. By contrast, kinetically complex reactions produce $(k_D/k_T)^{3.26} > k_H/k_T$. We now show that these limiting cases may not pertain under the conditions of (i) measured secondary kinetic isotope effects which fall below their equilibrium limits and (ii) extensive hydrogen tunneling. Although condition (i) could lead to a false signature of tunneling, ambiguities of interpretation can be avoided by the simultaneous measurement of exponential relationships for primary and secondary isotope effects. With the exception of reactions where the H-transfer process is known to be fully rate determining, condition (ii) may lead to an underestimate of quantum effects as judged by exponential relationships.

Hydrogen isotope effects are frequently used as probes of quantum mechanical tunneling in simple hydrogen transfer reactions (*1*). Early modeling of the relationship between k_H/k_D and k_H/k_T isotope effects failed to indicate significant anomalies, even under conditions of fairly extensive reaction coordinate tunneling (*2*). More recently, Saunders (*3*) has shown that the semiclassical mass relationship between multiple isotope effects can break down under conditions of moderate degrees of tunneling, such that $(k_D/k_T)^{3.26} < (k_H/k_T)$. In the case of several E-2 elimination reactions (*4*) and an enzyme-catalyzed hydride transfer reaction (*5*), the expected inequity was observed, providing evidence for hydrogen tunneling. However, application of this approach to the bovine serum amine oxidase-catalyzed oxidation of benzylamine indicated $(k_D/k_T)^{3.26} \simeq k_H/k_T$, despite strong evidence from temperature dependencies for hydrogen tunneling (*6*). Additionally, studies of exponential relationships between primary isotope effects in E-2 elimination reactions have failed to detect the expected breakdowns (*7*), contrasting with earlier evidence for $(k_D/k_T)^{3.26} < (k_H/k_T)$ in the measurement of secondary isotope effects (*4*).

In light of the diversity of experimental results to date, we have examined conditions which could lead to a failure to detect exponential breakdowns when tunneling is occurring, as well as situations which could artifactually produce

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[2] Current address: Genetics Institute, Inc., 87 Cambridge Park Drive, Cambridge, MA 02140.

[3] To whom correspondence should be addressed.

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TABLE 1

Exponential Relationship of D/T and H/T Isotope Effects

Case	Primary isotope effects	Secondary isotope effects
I. H-Transfer rate determining		
A. No tunneling[a]	$(D/T)^{3.26} = (k_H/k_T)$	$(D/T)^{3.26} = (H/T)$
B. Moderate tunneling[b]	$(D/T)^{3.26} < (k_H/k_T)$	$(D/T)^{3.26} < (H/T)$
C. More extensive tunneling[c]	Range of values possible	
II. Kinetic complexity		
A. No Tunneling[d]		
$K_{eq,H}/K_{eq,D} \leq k_H/k_D$	$(D/T)^{3.26} > (H/T)$	$(D/T)^{3.26} > (H/T)$
B. No Tunneling[e]		
$K_{eq,H}/K_{eq,D} > k_H/k_D$	N.A.[f]	$(D/T)^{3.26} < (H/T)$
C. Tunneling	Range of values possible	

[a] Swain, C. G., Stivers, E. C., Reuwer, J. F., Jr., and Schaad, L. J. (1958) *J. Am. Chem. Soc.* **80,** 5885–5893.

[b] Ref. (*3*).

[c] Fig. 1 and text.

[d] Ref. (*5*).

[e] Table 2 and text.

[f] Not applicable.

exponential deviations diagnostic of tunneling when none is occurring. Reactions have been divided into two limiting cases, involving either a rate limiting H-transfer (Case I) or multiple rate limiting steps (Case II), Table 1. As discussed previously for a kinetically isolated H-transfer step, moderate tunneling is predicted to produce experimental k_H/k_T values which are in excess of $(k_D/k_T)^{3.26}$. The original study (*3*) employed the force field approach of Buddenbaum and Shiner for the calculation of isotope effects (*8*). The observed rate constant (k_{obs}) is formulated as the product of a semiclassical term (k_{sc}) and a tunnel correction (Q):

$$k_{obs} = (k_{sc})(Q). \qquad [1]$$

From Eq. [1], a comparison of experimental isotope effects will reflect the interrelationship of $(k_L/k_T)_{sc}$ and (Q_L/Q_T) terms where L is either H or D:

$$(k_D/k_T)^{3.26}_{sc}(Q_D/Q_T)^x = (k_H/k_T)_{sc}(Q_H/Q_T). \qquad [2]$$

Since the semiclassical component of Eq. [2] has an exponent which is fixed at 3.26 to 3.34 (*3*), deviations from these values arise from the exponent (x) relating (Q_D/Q_T) to (Q_H/Q_T).

In earlier calculations (*3*), data were modeled using a truncated model for the Bell correction, which corresponds to low reaction coordinate frequencies. We now extend this work to include greater tunnel corrections, using the full expression for Q, Eq. [3], where $\alpha = 1/kT$, $\beta = 2\pi/h\nu\ddagger$, and E is the reaction barrier height:

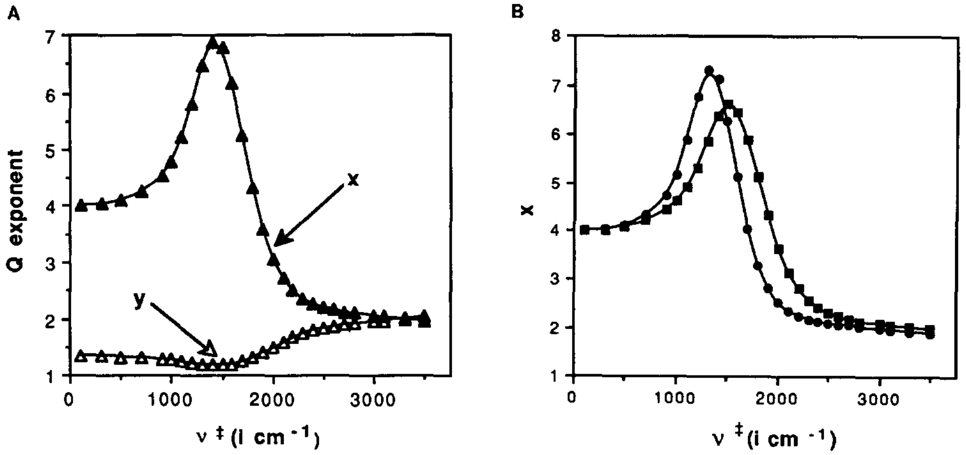

FIG. 1. (A) The dependence of the exponent relating Q_D/Q_T to Q_H/Q_T (x, ▲) and Q_H/Q_D to Q_H/Q_T (y, △) on reaction coordinate frequency. Q exponents are obtained from individual values for Q_L at 25°C, Eq. [3], using $E_T = 70.9$ kJ/mol, $E_D = 68.5$ kJ/mol, and $E_H = 62.8$ kJ/mol. (B) The frequency dependence of x at 0°C (●) and 45°C (■).

$$Q(\mathrm{L}) = (\pi\alpha/\beta)/\sin(\pi\alpha/\beta) - \sum_{n=0}^{n=\infty} \{(-1)^n \alpha \exp[\alpha - (n+1)\beta]E/(n+1)\beta - \alpha\}. \quad [3]$$

A potential limitation in applying the expanded Bell correction is the need to evaluate the reaction barrier height for the reaction under examination. We have used our studies on bovine serum amine oxidase as a frame of reference for model calculations, since measured temperature dependencies offer an estimate of the energies of activation for an enzyme-catalyzed C–H, C–D, and C–T cleavage.[4] In calculations of Q_L as a function of reaction coordinate frequency, the first eight terms of the summation of Eq. [3] have been found to be sufficient to reach a limiting value for Q.

The interrelationship of isotope effects on Q is obtained from the ratio of Q terms, Eq. [4].

$$Q_H/Q_T = (Q_D/Q_T)^x = (Q_H/Q_D)^y, \quad [4]$$

where x reflects the exponential relationship for the H,T/D,T pair and y the H,T/H,D pair. Values for x and y, determined from individual calculations of Q_L at 25°C, are shown in Fig. 1A. In regions of low amounts of tunneling (small $\nu\ddagger$ values), Q exponents are seen to lie near 4 for x and 1.3 for y. Semiclassical rates dominate in this region, leading to observed exponents[5] in the semiclassical range

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[4] The measured value of E_T = 70.9 kJ/mol for C–T cleavage has been assigned to the classical reaction barrier height, on the assumption that the tritium is tunneling very little or not at all; values for E_H and E_D are then available, following subtraction of maximal semiclassical differences in energies of activation.

[5] Observed exponents have been calculated by setting values for k_{sc1}/k_{sc2} at 9.8(H/D), 2.7(D/T), and 27(H/T), with values for Q_1/Q_2 obtained from Eq. [3]. The value of the exponent relating two isotope effects is then calculated using semiclassical exponents for k_{sc} terms (frequency independent) and calculated exponents for Q terms (frequency dependent, Fig. 1A).

(*10*). As the reaction coordinate frequency increases and tunneling becomes more significant, the exponents relating Q isotope effects begin to change, rising above 4 for the D,T/H,T pair and falling below 1.3 for the H,D/H,T pair. While the model used here is a simple one, the trends shown in Fig. 1A at low values for $\nu\ddagger$ are as predicted by Saunders: specifically, (i) $(k_D/k_T)^{3.26} < (k_H/k_T)$ when tunneling occurs and (ii) deviations from semiclassical exponents are greater when comparing k_D/k_T to k_H/k_T than k_H/k_D to k_H/k_T.[6]

Although the data in Fig. 1 support the earlier predictions (*3*), *this is only true for moderate degrees of tunneling.* As the contribution of hydrogen tunneling to the reaction rate increases, the magnitude of x, for example, is seen to peak, followed by a decline to *below* its semiclassical value ($x \simeq 1.9$ and $y \simeq 2.1$ in the limit of high $\nu\ddagger$, Fig. 1A). An independent estimate for x and y at high $\nu\ddagger$ values can be obtained from the WKB approximation for H, D, and T tunneling through a rectangular barrier (*10*). This approach yields values for x and y of 2.3 and 1.7, in reasonable accord with the limiting values shown in Fig. 1A. These considerations indicate that for a large degree of tunneling, $(k_D/k_T)^{3.26}$ may exceed k_H/k_T.

In conjunction with studies of the variation of x and y with reaction coordinate frequency, we have examined the temperature dependence of exponents relating Q isotope effects. As shown in Fig. 1B, a decrease in temperature leads to a shift in the x curve to lower frequencies. This shift can produce either an increase or a decrease in the magnitude of x with temperature (depending on the extent of tunneling) and is in marked contrast to semiclassical isotope effects which predict temperature-independent exponential relationships. The trends in Fig. 1B may be relevant to the proton transfer catalyzed by bovine serum amine oxidase which, on the basis of anomalously large isotope effects and their temperature dependencies, has been determined to occur via extensive tunneling (*6*). Despite the strong evidence for tunneling, exponents relating measured k_D/k_T and k_H/k_T isotope effects were found to decline from a near semiclassical value at 45°C to a value below 3.26 at 0°C (*6*).[7] This behavior could indicate an increasing contribution of nonisotopically sensitive kinetic steps with decreasing temperature (*cf* Case II Table 1), but comparative studies of stopped flow and steady-state isotope effects (*6*) have provided strong support for a single rate limiting proton transfer step.

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[6] Northrop (*8*) has recently shown that this advantage of D,T/H,T isotopes for the detection of tunneling is partially offset by a greater propagation of error in the D,T/H,T pair than in H,D/H,T measurements.

[7] Using Fig. 1A, the value of Q_H is 70.8 at the peak value for x (1400 i cm^{-1}) with $Q_H/Q_D = 20$, in excess of the observed $k_H/k_D = 12$ for bovine serum amine oxidase. It should be emphasized that Fig. 1 was constructed as a qualitative model of possible trends in exponential values relating Q_L/Q_T terms and was not intended as a quantitative fit to the experimental data for bovine serum amine oxidase. Although models with more realistic barrier shapes than the Bell correction could be employed, these require additional assumptions (e.g., barrier width as well as height). We find that the Bell correction alone shows a dramatic dependence between the energy assigned to the barrier height and the value of Q_H/Q_D. For example, using E_H = 40, 50, 70, and 90 kJ/mol, Q_H/Q_D = 5.8, 13, 50, and 197 at the turnaround point for the x exponent. The value of $\nu\ddagger$ corresponding to the maximum value for x varies very little, by contrast ($\nu\ddagger$ = 1300, 1400, 1450, and 1500 i cm^{-1}, respectively). This suggests that the barrier height for the bovine serum amine oxidase reaction may be less than 63 kJ/mol, which was initially assigned in the assumption of no tritium tunneling.

The above considerations demonstrate how extensive tunneling can produce exponential relationships which deviate from those predicted for moderate amounts of tunneling (Case IC, Table 1). We now turn to a discussion of the possible appearance of exponential relationships diagnostic of tunneling when none is occurring. A detailed analysis of the consequence of kinetically complex reactions on exponential relationships has already been presented (*5*), showing that multiple, rate limiting steps lead to inequities between k_D/k_T and k_H/k_T which are opposite to those seen with tunneling (Case IIA, Table 1). Implicit in these derivations is the assumption that the equilibrium isotope effect on the H transfer step is smaller than the kinetic isotope effect. This is a valid assumption for primary isotope effects, where $K_{eq,H}/K_{eq,D}$ generally ranges from 1.0 to 1.2 and $k_H/k_D = 5$ to 10, as well as for secondary isotope effects in the case of tunneling plus coupled motion (since this situation leads to inflated values for k_H/k_D). Neglecting tunneling, however, secondary isotope effects can be expected to range from unity to $K_{eq,H}/K_{eq,D}$, with their magnitude indicating the nature of the transition state (*11, 12*).

We now examine the consequences of $k_H/k_D < K_{eq,H}/K_{eq,D}$ on exponential relationships. A simple, two-step reaction has been modeled, which allows for reversibility of the H-transfer step:

$$\mathrm{RH} + \mathrm{B} \underset{2}{\overset{1}{\rightleftharpoons}} \mathrm{RH}\cdot\mathrm{B} \underset{4}{\overset{3}{\rightleftharpoons}} \mathrm{R}\cdot\mathrm{BH} \xrightarrow{5} \mathrm{R} + \mathrm{BH}, \qquad [5]$$

where RH · B and R · BH reflect either encounter complexes for a reaction in solution or bound complexes in the case of enzyme reactions.[8] Using the expression which relates an observed secondary isotope effect, $(k_L/k_T)_{obs}$, to the intrinsic value on the H-transfer step (*13*), $(k_L/k_T)_3$, we obtain:

$$(k_L/k_T)_{obs} = \frac{(k_L/k_T)_3 + C_{f,L} + (K_{eq,L}/K_{eq,T})C_{r,L}}{1 + C_{f,L} + C_{r,L}}, \qquad [6]$$

where $C_f = k_3/k_2$, $C_r = k_4/k_5$, $K_{eq,L}/K_{eq,T}$ is the equilibrium isotope effect for conversion of RH · B to R · BH, and L is either H or D. By way of example, the magnitude of $(k_L/k_T)_{obs}$ has been modeled using $k_H/k_T = 1.1$, $K_{eq,H}/K_{eq,T} = 1.35$, $C_f = 0$, and $C_{r,H} = 1.0, 0.1$, and 0.05 ($C_{r,D} = 0.25, 0.025$, and 0.0125, corresponding to a primary deuterium isotope effect of 4).[9]

Results of these simulations are given in Table 2, revealing an inflation of the exponent relating k_D/k_T to k_H/k_T from 3.26 to 4.78 when $C_{r,H} = 1.0$. A value of unity for C_r is often seen in enzyme reactions, producing reduced but sizeable primary isotope effects. Even when the size of C_r is as small as 0.05, the magnitude of the exponent relating isotope effects continues to be slightly above 3.26. These consider-

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[8] The transfer of hydrogen from RH to B in Eq. [5] refers to the primary hydrogen. Although not shown explicitly in Eq. [5], we are concerned with the effect of a hydrogen left behind on RH in its conversion to R, i.e., the secondary isotope effect.

[9] The assumption of $C_f = 0$ introduces the maximum impact for the $(K_{eq,L}/K_{eq,T})C_r$ term; for kinetic situations in which the $C_{f,L}$ term dominates Eq. [6], the exponential relationship is that seen when $k_H/k_D > K_{eq,H}/K_{eq,D}$ (Case IIA, Table 1).

TABLE 2

Modeled Exponents for Secondary Isotope Effects, when Equilibrium Isotope Effects Exceed Kinetic Isotope Effects and Tunneling Is Absent[a]

$C_{r,H}$	$(k_H/k_T)_{obs}$	$(k_D/k_T)_{obs}$	Exp[b]
1.0	1.23	1.04	4.78
0.10	1.12	1.03	3.65
0.05	1.11	1.03	3.54

[a] Observed isotope effects calculated from Eq. [6] in text, where $C_f = 0$, $k_H/k_T = 1.1$ ($k_D/k_T = 1.03$), $K_{eq,H}/K_{eq,T} = 1.35$ ($K_{eq,D}/K_{eq,T} = 1.096$).

[b] $(k_D/k_T)^{exp} = k_H/k_T$.

ations indicate that an examination of exponential relationships relating secondary isotope effects could produce a false signature of tunneling (Case IIB, Table 1). Ambiguities of interpretation can be avoided, however, by the simultaneous measurement of exponential relationships for primary and secondary isotope effects.

The trends in Table 2 are also relevant to the application of the breakdown in the rule of the geometric mean as a probe of tunneling. As discussed previously (*14, 5, 15*), tunneling with coupled motion inflates the magnitude of the secondary isotope effect; replacement of H by D in the primary position leads to a decrease in tunneling and, hence, a reduction in the secondary isotope effect. Deviation from the rule of the geometric mean has been used to implicate tunneling in both enzymatic and solution reactions (*4, 16, 17*). While this approach is perfectly valid when the magnitude of the secondary isotope effect approximates or exceeds the limit set by the equilibrium isotope effect for the reaction in question (Case IIA, Table 1), care must be exercised when the initially measured secondary isotope effect falls below its equilibrium limit. In the latter case, a measured secondary isotope could be reduced upon replacement of hydrogen by deuterium in the primary position, due to a change in rate limiting step.[10]

In summary, we have examined the range of possible exponents relating measured D,T and H,T isotope effects (Table 1). As illustrated (Fig. 1), failure to observe the expected inequity between k_D/k_T and k_H/k_T for moderate tunneling at a single temperature may be diagnostic of more extensive tunneling, and should be further explored to distinguish this possibility from the presence of multiple rate limiting steps. We also show that measurement of secondary isotope effects alone may produce a false signature of tunneling (Table 2). Primary isotope effects should therefore be measured in conjunction with secondary effects, unless the exponential breakdown from 3.26 is very large or it can be demonstrated that the kinetic secondary effect approximates or exceeds its equilibrium limit.

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[10] By reference to Eq. [5] in text, a measured secondary isotope effect would reflect $K_{eq,L}/K_{eq,T}$, when k_5 is rate limiting. Cleavage of C–D rather than C–H at the primary position would increase the extent to which k_3 (and k_4) are rate limiting, relative to k_5, leading to a decrease in the measured isotope effect.

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