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The *Saunders-Bell* Analysis of Tunnel Effects in Reactions with Kinetic Isotope Effects

Vladimir Leskovac[1,*], **Svetlana Trivić**[2], **Draginja Peričin**[1], and **Julijan Kandrač**[3]

[1] Faculty of Technology, University of Novi Sad, Yugoslavia
[2] Faculty of Science, University of Novi Sad, Yugoslavia
[3] Faculty of Agriculture, University of Novi Sad, Yugoslavia

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Summary. The primary kinetic isotope effects of deuterium were investigated in 22 hydrogen and deuterium transfer reactions, including enzymatic and nonenzymatic hydride transfer reactions, elimination reactions, and reactions catalyzed by enzymes lipooxygenase, amine dehydrogenase, and methylmalonyl-CoA mutase. In each case, the *Saunders-Bell* analysis was applied to calculate the tunnel effects and the corresponding thermodynamic parameters. The *Saunders-Bell* analysis was effective in 14 cases out of 22. A high degree of correlation was found between the barrier factor, the tunnel factor, and the entropy factor among all reactions studied. From this, a general relationship between the three factors was derived, based on the *Saunders-Bell* analysis of the *Bell* equation; the *Saunders-Bell* analysis is valid within certain limits of the barrier factor. This general relationship is universally valid for all hydrogen/deuterium transfer reactions in nature with moderate tunneling, when the *Saunders-Bell* analysis applies.

Keywords. *Bell* equation; Kinetic isotope effect; Hydrogen transfer reactions.

Introduction

Quantum mechanical tunneling in proton, hydrogen, and hydride transfer reactions occurs in numerous chemical and enzyme-catalyzed reactions in nature [1–10]. It leads to primary kinetic isotope effects of deuterium, larger than the ''semi-classical'' values based on the loss of vibrational zero-point energy in the transition state [11]. For such cases, *Bell* has developed an equation which is a theoretical formulation of the tunnel factor in terms of the energetics and analytical geometry of a presumed reaction barrier (Eq. (1)) [11]. The *Bell* equation assumes that the *Arrhenius* equation adequately described the temperature

* Corresponding author. E-mail: leskovac@neobee.net

dependence of a reaction with tunneling if a temperature-dependent tunnel correction factor, Q_t, is included.

$$k_{\mathrm{OBS}} = A_{\mathrm{OBS}} \cdot e^{-E_a/RT} = k_{\mathrm{SEM}} \cdot Q_t = Q_t \cdot A_{\mathrm{SEM}} \cdot e^{-E/RT} \tag{1}$$

where E_a is the *Arrhenius* activation energy, E is the height of the barrier, A_{OBS} is the observed, and A_{SEM} is the semiclassical preexponential factor that would be found in the absence of tunneling.

According to *Bell* [11], the tunnel correction factor, Q_t, is given by Eq. (2) where u_t and y are defined by Eqs. (3) and (4).

$$Q_t = \left(\frac{\frac{1}{2}u_t}{\sin\frac{1}{2}u_t}\right) - u_t \cdot \exp(E/RT) \times \left(\frac{y}{2\pi - u_t} - \frac{y^2}{4\pi - u_t} + \frac{y^3}{6\pi - u_t}\right) \tag{2}$$

$$u_t = \left(\frac{h}{k_B T}\right)\left(\frac{1}{\pi\sqrt{2m}}\right)\left(\frac{\sqrt{E}}{a}\right) \tag{3}$$

$$y = \exp\left(-\frac{2\pi E}{h\nu_t}\right) \tag{4}$$

In Eq. (3), $\sqrt{E}/a$ is the curvature of the barrier, a is the barrier half-width, and m is the mass of the particle transferred; thus $m_{\mathrm{H}} = 1$ for hydrogen and $m_{\mathrm{D}} = 2$ for deuterium [1, 11].

The experimental evidence for H-tunneling in enzyme-catalyzed reactions is fairly limited. In addition, most experimental data for the temperature-dependence of KIEs with enzymes were treated with the aid of the *Bell* equation, in order to calculate the amount of tunneling. Since the tunneling in enzyme reactions, as it is pointed out below, is a controversial issue, we have analyzed in detail the scope and the application of the *Bell* equation. In doing so, we have applied the *Saunders-Bell* analysis of tunnel effects in reactions with kinetic isotope effects of deuterium, an approach which was not systematically evaluated in the past.

Recently, *Klinman* and coworkers have vigorously advocated the hypothesis that ''the optimization of enzyme catalysis may entail the evolutionary implementation of chemical strategies that increase the probability of tunneling and thereby accelerate the reaction rate'' [12]. In other words, the enzymes may have evolved to enhance the tunneling of hydrogen atoms. Also, recently, this hypothesis was questioned by *Finke* and coworkers [13, 14]. These workers have been able to measure KIEs in reactions catalyzed by methylmalonyl-CoA mutase, in the presence and in the absence of enzyme; the temperature-dependent KIEs are the same in both the enzyme and non-enzyme reactions, indicating that the amount of tunneling is identical in both systems [15]. Thus, the tunneling in enzyme reactions and the extent to which this phenomenon is spread in nature appears to be a controversial issue; this fact underlines the importance of analysis of tunnel effects outlined in this work.

Results

Theory

Relationship between the pre-exponential factor and entropy. From the *Arrhenius* equation it follows that:

$$k = A \cdot e^{-E_a/RT} = A \cdot e^{-[(\Delta H^{\ddagger} + RT)/RT]} \tag{5}$$

The second equality comes from the relationship: $E_a = \Delta H^{\ddagger} + RT$. Thus,

$$\ln k = \ln A - \left(\frac{\Delta H^{\ddagger} + RT}{RT} \right) \tag{6}$$

From Eq. (6) it follows that the ratio of rate constants for hydrogen and deuterium reactants is:

$$\ln k_{\mathrm{D}} - \ln k_{\mathrm{H}} = (\ln A_{\mathrm{D}} - \ln A_{\mathrm{H}}) + \left(\frac{\Delta H^{\ddagger}_{\mathrm{H}} - \Delta H^{\ddagger}_{\mathrm{D}}}{RT} \right) \tag{7}$$

From the *Eyring* treatment of absolute reactions rates, it follows that [10]:

$$k = \left(\frac{k_B T}{h} \right) e^{-\Delta G^{\ddagger}/RT} = \left(\frac{k_B T}{h} \right) e^{-\Delta H^{\ddagger}/RT} \cdot e^{\Delta S^{\ddagger}/R} \tag{8}$$

The second equality comes from the relationship: $\Delta G^{\ddagger} = \Delta H^{\ddagger} - T\Delta S^{\ddagger}$. Thus,

$$\ln k = \ln \left(\frac{k_B T}{h} \right) + \left(\frac{\Delta S^{\ddagger}}{R} \right) - \left(\frac{\Delta H^{\ddagger}}{RT} \right) \tag{9}$$

From Eq. (9) it follows that the ratio of rate constants for hydrogen and deuterium reactants is:

$$\ln k_{\mathrm{D}} - \ln k_{\mathrm{H}} = \left(\frac{\Delta H^{\ddagger}_{\mathrm{H}} - \Delta H^{\ddagger}_{\mathrm{D}}}{RT} \right) + \left(\frac{\Delta S^{\ddagger}_{\mathrm{D}} - \Delta S^{\ddagger}_{\mathrm{H}}}{R} \right) \tag{10}$$

From Eqs. (7) and (10) it follows that:

$$\ln \left(\frac{A_{\mathrm{H}}}{A_{\mathrm{D}}} \right) = - \left(\frac{\Delta S^{\ddagger}_{\mathrm{D}} - \Delta S^{\ddagger}_{\mathrm{H}}}{R} \right) \tag{11}$$

From Eq. (11) it follows that we can substitute the pre-exponential factor, $\ln(A_{\mathrm{H}}/A_{\mathrm{D}})$, with the entropy factor, $-[(\Delta S^{\ddagger}_{\mathrm{D}} - \Delta S^{\ddagger}_{\mathrm{H}})/R]$, in any thermodynamic relationship.

Saunders computer program [16]. The *Bell* equation (Eq. (2)) is a multiterm equation, but the first term is usually sufficient to describe the moderate tunnel effects. *Bell* [11] and *Kaldor* & *Saunders* [17] have shown that, if one assumes that $A_{\mathrm{H}} = A_{\mathrm{D}}$ in the absence of tunneling, and if we use *only the first term* of the *Bell* equation, the definition of individual thermodynamic expressions for hydrogen and deuterium atoms is following [11, 16, 17]:

$$\left(\frac{E^{\mathrm{D}}_{\mathrm{a}} - E^{\mathrm{H}}_{\mathrm{a}}}{RT} \right) - \left(\frac{E^{\mathrm{D}} - E^{\mathrm{H}}}{RT} \right) = \left(\frac{1}{2} {u_t}^{\mathrm{D}} \cot \frac{1}{2} {u_t}^{\mathrm{D}} \right) - \left(\frac{1}{2} {u_t}^{\mathrm{H}} \cot \frac{1}{2} {u_t}^{\mathrm{H}} \right) \tag{12}$$

$$\ln \left(\frac{Q^{\mathrm{H}}_t}{Q^{\mathrm{D}}_t} \right) = \ln \left(\frac{{u_t}^{\mathrm{H}} \sin \frac{1}{2} {u_t}^{\mathrm{D}}}{{u_t}^{\mathrm{D}} \sin \frac{1}{2} {u_t}^{\mathrm{H}}} \right) \tag{13}$$

$$\ln \left(\frac{A_{\mathrm{H}}}{A_{\mathrm{D}}} \right)_{\mathrm{OBS}} = \ln \left(\frac{{u_t}^{\mathrm{H}} \sin \frac{1}{2} {u_t}^{\mathrm{D}}}{{u_t}^{\mathrm{D}} \sin \frac{1}{2} {u_t}^{\mathrm{H}}} \right) + \left(\frac{1}{2} {u_t}^{\mathrm{H}} \cot \frac{1}{2} {u_t}^{\mathrm{H}} - \frac{1}{2} {u_t}^{\mathrm{D}} \cot \frac{1}{2} {u_t}^{\mathrm{D}} \right) \tag{14}$$

From the Eqs. (12)–(14) it follows that:

$$\ln\left(\frac{A_{\mathrm{H}}}{A_{\mathrm{D}}}\right)_{\mathrm{OBS}} = \ln\left(\frac{Q_t^{\mathrm{H}}}{Q_t^{\mathrm{D}}}\right) - \left[\left(\frac{E_{\mathrm{a}}^{\mathrm{D}} - E_{\mathrm{a}}^{\mathrm{H}}}{RT}\right) - \left(\frac{E^{\mathrm{D}} - E^{\mathrm{H}}}{RT}\right)\right] \tag{15}$$

$$\ln\left(\frac{Q_t^{\mathrm{H}}}{Q_t^{\mathrm{D}}}\right) = \left[\left(\frac{E_{\mathrm{a}}^{\mathrm{D}} - E_{\mathrm{a}}^{\mathrm{H}}}{RT}\right) - \left(\frac{E^{\mathrm{D}} - E^{\mathrm{H}}}{RT}\right)\right] + \ln\left(\frac{A_{\mathrm{H}}}{A_{\mathrm{D}}}\right)_{\mathrm{OBS}} \tag{16}$$

The ratio of pre-exponential factors $\ln(A_{\mathrm{H}}/A_{\mathrm{D}})_{\mathrm{OBS}}$ in Eqs. (15) and (16) includes the steric factors (Eq. (11)) which are usually called the entropy factors [18]. Thus, one can formulate Eqs. (15) and (16) in yet another useful form:

$$\left[\left(\frac{E_{\mathrm{a}}^{\mathrm{D}} - E_{\mathrm{a}}^{\mathrm{H}}}{RT}\right) - \left(\frac{E^{\mathrm{D}} - E^{\mathrm{H}}}{RT}\right)\right] = \left(\frac{\Delta S_{\mathrm{D}}^{\ddagger}\Delta S_{\mathrm{H}}^{\ddagger}}{R}\right) + \ln\left(\frac{Q_t^{\mathrm{H}}}{Q_t^{\mathrm{D}}}\right) \tag{17}$$

The first and the last term in this equation, the barrier factor $\Delta\Delta E/RT$ $\{[(E_{\mathrm{a}}^{\mathrm{D}} - E_{\mathrm{a}}^{\mathrm{H}}) - (E^{\mathrm{D}} - E^{\mathrm{H}})]/RT)\}$, and the tunnel factor $\ln(Q_t^{\mathrm{H}}/Q_t^{\mathrm{D}})$, are temperature-dependent, while the middle term, the entropy factor $[(\Delta S_{\mathrm{D}}^{\ddagger} - \Delta S_{\mathrm{H}}^{\ddagger})/R]$, is temperature-independent.

Equation (14) was exploited by *Saunders* to develop a computer program which can calculate the tunnel effects [16]. While Eq. (14) cannot be solved explicitly for $u_t{}^{\mathrm{H}}$ and u_t^{D}, it is possible to arrive at values by trial and error by making some assumption concerning the relation between $u_t{}^{\mathrm{H}}$ and $u_t{}^{\mathrm{D}}$. It is convenient to assume that $u_t{}^{\mathrm{H}} = u_t{}^{\mathrm{D}}\sqrt{2}$, which is equivalent to assuming that $m_{\mathrm{H}}{:}m_{\mathrm{D}} = 1{:}2$.

Saunders developed a simple computer program, which varies $u_t{}^{\mathrm{H}}$ until Eq. (14) reproduces $A_{\mathrm{H}}/A_{\mathrm{D}}$ from the experiment, and $E^{\mathrm{D}} - E^{\mathrm{H}}$ value is evaluated from Eq. (12). The barrier curvature, $\sqrt{E}/a$, is obtained from Eq. (3) [16, 17]. This program is simple, efficient, and describes accurately the tunnel effects if only a moderate tunneling is involved. The input values into the *Saunders* program are: the measured values, $E_{\mathrm{a}}^{\mathrm{D}} - E_{\mathrm{a}}^{\mathrm{H}}$ (in kcal/mol) and the ratio of pre-exponential factors $A_{\mathrm{H}}/A_{\mathrm{D}}$; the assumed values are: u_t (min = 1.00), u_t (max = 5.00), $\mathrm{H}_{\mathrm{mass}} = 1.00$, and the standard error in $A_{\mathrm{H}}/A_{\mathrm{D}}$. The output values, calculated by the program at a given temperature, are: $u_t{}^{\mathrm{H}}$, $u_t{}^{\mathrm{D}}$, Q_t^{H}, Q_t^{D}, $Q_t^{\mathrm{H}}/Q_t^{\mathrm{D}}$, $(k_{\mathrm{H}}/k_{\mathrm{D}})_{\mathrm{SEM}}$, $(k_{\mathrm{H}}/k_{\mathrm{D}})_{\mathrm{OBS}}$, $(k_{\mathrm{H}}/k_{\mathrm{D}})_{\mathrm{CALC}}$, $E^{\mathrm{D}} - E^{\mathrm{H}}$ (in kcal/mol), sum of squares of errors (SSQ), and (% Error).

Theoretical analysis of Eq. (17). We have performed a theoretical analysis of Eq. (17) with the aid of the *Saunders* computer program. A number of *arbitrary chosen* input pairs of $(A_{\mathrm{H}}/A_{\mathrm{D}})_{\mathrm{OBS}}$ and of $(E_{\mathrm{a}}^{\mathrm{D}} - E_{\mathrm{a}}^{\mathrm{H}})$ were fed into the *Saunders* program, the $\Delta\Delta E/RT$ values were calculated, and the corresponding plot of $-\ln(A_{\mathrm{H}}/A_{\mathrm{D}})_{\mathrm{OBS}}$ *versus* $\Delta\Delta E/RT$ was constructed in Fig. 1.

The data in Fig. 1 indicate that, theoretically, the entropy factor, $\ln(A_{\mathrm{H}}/A_{\mathrm{D}})$, is related to the barrier factor $\Delta\Delta E/RT$ in the form of a *second order polynomial*. The error analysis shows that (% Error) is small (<1%) when $\Delta\Delta E/RT$ is between 0 and 3, but increases dramatically when $\Delta\Delta E/RT$ becomes larger than 3 or approaches zero. The reason for this are the input limits on $u_t{}^{\mathrm{H}}$ imposed by the *Saunders* program, which are $u_t{}^{\mathrm{H}}(\mathrm{min}) = 1.00$ and $u_t{}^{\mathrm{H}}(\mathrm{max}) = 5.00$; when $u_t{}^{\mathrm{H}} = 1.00$ the value of $\Delta\Delta E/RT$ is zero, and when $u_t{}^{\mathrm{H}} = 5.00$ the value of $\Delta\Delta E/RT$ is 3.00; outside these limits, the value of (% Error) increases dramatically.

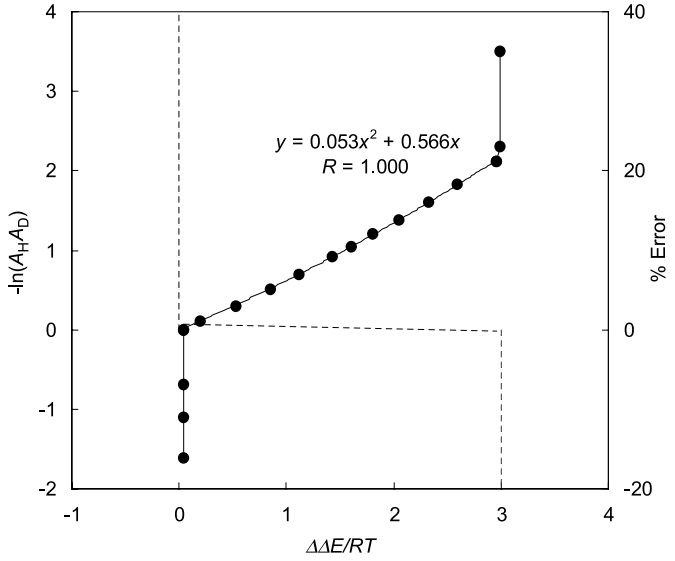

Fig. 1. Theoretical relation between the thermodynamic parameters $-\ln(A_H/A_D)_{OBS}$ and $\Delta\Delta E/RT$ (full line), and the corresponding (% Error) values (dotted line); the lines were calculated by the *Saunders* computer program [16]; the abscissa, $\Delta\Delta E/RT$, represents the function $[(E_a^D - E_a^H) - (E^D - E^H)]/RT$

Thus, the *Saunders* computer program operates within certain limits of $\Delta\Delta E/RT$. Within these limits, the entropy factor, $\ln(A_H/A_D)$, is strictly related to the barrier factor, $\Delta\Delta E/RT$, in the form of a second order polynomial. This is

Table 1. Thermodynamic parameters for various proton, hydrogen, and hydride transfer reactions

#	Substrate	t/°C	ln $(A_H/A_D)_{OBS}$	ln (Q_t^H/Q_t^D)	(5–4)	$\left(\frac{E_a^D - E_a^H}{RT}\right)$	$\left(\frac{E^D - E^H}{RT}\right)$	(7–8)
1	2	3	4	5	6	7	8	9
Hydride transfer reactions								
1	*NOB*	25	2.1126	0.0211	−2.0915	−0.0686	−0.1126	0.0440
2	*TNBS*	25	1.4748	0.0211	−1.4537	−0.1130	−0.1645	0.0515
3	*DCPIP*	25	0.6313	0.0211	−0.6102	2.0857	2.0428	0.0429
4	Bis*NA*$^+$ intramolec.	25	0.3365	0.0218	−0.3147	0.5648	0.8511	−0.2863
5	Bis*NA*$^+$ intermolec.	25	0.9933	0.0218	−0.9715	0	−0.0434	0.0434
6	*MPAI*	25	−0.3011	0.2386	0.5397	1.7750	1.2322	0.5428
7	*MAI*	25	−1.4586	0.6575	2.1161	3.0980	1.0008	2.0972
8	*NAD*[b]	25	−2.1203	0.8862	3.0065	3.5446	0.5352	3.0094
Lipooxygenase mutants								
9	Wild type	30	2.8904	0.0211	2.8693	1.4940	1.4513	0.0427
10	*Leu*546-*Ala*	30	+1.3863	0.0211	−1.3652	3.1541	3.1114	0.0427
11	*Leu*754-*Ala*	30	+1.0986	0.0211	−1.0775	3.3201	3.2774	0.0427
12	*Ileu*553-*Ala*	30	−2.1203	0.8345	2.9548	6.6402	3.6805	2.9597

(*continued*)

Table 1 (*continued*)

#	Substrate	$t/^{\circ}C$	$\ln(A_H/A_D)_{OBS}$	$\ln(Q_t^H/Q_t^D)$	(5–4)	$\left(\frac{E_a^D-E_a^H}{RT}\right)$	$\left(\frac{E^D-E^H}{RT}\right)$	(7–8)
1	2	3	4	5	6	7	8	9
H-Elimination reactions in:								
13	30% Me_2SO/OH^-	50	−1.3093	0.7275	2.0368	2.8187	0.8065	2.0122
14	40% Me_2SO/OH^-	50	−1.0788	0.5653	1.6441	2.7720	1.1249	1.6471
15	50% Me_2SO/OH^-	50	−1.5606	0.6626	2.2232	2.9122	0.7080	2.2042
16	$EtOH/EtO^-$	50	−0.3425	0.2546	0.5971	1.4327	0.8285	0.6042
H-Elimination reactions in:								
17	30% Me_2SO/OH^-	50	−0.8747	0.4947	1.3694	2.7720	1.4110	1.3610
18	35% Me_2SO/OH^-	50	−1.0385	0.5188	1.5573	3.0212	1.4679	1.5533
19	40% Me_2SO/OH^-	50	−1.5606	0.5933	2.1539	3.4105	1.2782	2.1323
20	50% Me_2SO/OH^-	50	−1.0671	0.4574	1.5245	2.8187	1.2947	1.5240
21	Amine DH[c]	25	−0.5621	0.3816	0.9437	3.3887	2.4323	0.9564
22	MM-CoA mutase[d]	40	−1.9660	0.7999	2.7659	4.9820	2.2154	2.7666

#	Substrate	$t/^{\circ}C$	$\ln(k_H/k_D)_{OBS}$	$\ln(k_H/k_D)_{SEC}$	(10–11)	(% Error)[a]	Ref
1	2	3	10	11	12	13	14
Hydride transfer reactions							
1	*NOB*	25	2.0434	−0.1126	2.1560	88.2	[9]
2	*TNBS*	25	1.3606	−0.1645	1.5251	77.7	[9]
3	*DCPIP*	25	2.7175	2.0428	0.6747	47.9	[9]
4	Bis*NA*$^+$ intramolec.	25	1.2240	0.8515	0.3725	30.0	[7]
5	Bis*NA*$^+$ intermolec.	25	1.1694	−0.0434	1.2128	63.8	[7]
6	*MPAI*	25	1.4712	1.2323	0.2389	<1%	[7]
7	*MAI*	25	1.6582	1.0008	0.6574	<1%	[5]
8	*NAD*[b]	25	1.4244	0.5352	0.8892	<1%	[9]
Lipooxygenase mutants							
9	Wild type	30	4.3844	1.4513	2.9331	94.6	[22]
10	*Leu*546-*Ala*	30	4.5405	3.1114	1.4291	75.0	[22]
11	*Leu*754-*Ala*	30	4.4188	3.2774	1.1414	67.0	[22]
12	*Ileu*553-*Ala*	30	4.5201	3.6805	0.8396	<1%	[22]
H-Elimination reactions in:							
13	30% Me_2SO/OH^-	50	1.5369	0.8065	0.7304	<1%	[17]
14	40% Me_2SO/OH^-	50	1.6901	1.1249	0.5652	<1%	[17]

(*continued*)

Table 1 (*continued*)

#	Substrate	t/°C	$\ln(k_H/k_D)_{OBS}$	$\ln(k_H/k_D)_{SEC}$	(10–11)	(% Error)[a]	Ref
1	2	3	10	11	12	13	14
15	50% Me_2SO/OH^-	50	1.3686	0.7080	0.6606	<1%	[17]
16	$EtOH/EtO^-$	50	1.0852	0.8285	0.2567	<1%	[17]
H-Elimination reactions in:							
17	30% Me_2SO/OH^-	50	1.9036	1.4101	0.4935	<1%	[23]
18	35% Me_2SO/OH^-	50	1.9810	1.4679	0.5131	<1%	[23]
19	40% Me_2SO/OH^-	50	1.8547	1.2781	0.5766	<1%	[23]
20	50% Me_2SO/OH^-	50	1.7440	1.2947	0.4493	<1%	[23]
21	Amine DH[c]	25	2.8137	2.4324	0.3813	<1%	[24]
22	MM-CoA mutase[d]	40	3.0158	2.2155	0.8003	<1%	[13]

[a] (% Error) $= (1-k_{CALC}/k_{OBS}) \times 100$; [b] Alcohol dehydrogenase [9]; [c] Amine dehydrogenase [24]; [d] Methylmalonyl-CoA mutase [13]; all column entries are showing temperature-dependent values, except entry # 4, which shows the temperature-independent one

the *crucial property* of the *Saunders-Bell* analysis of tunnel effects, which states that to any value of $\Delta\Delta E/RT$ corresponds only a single value of $\ln(A_H/A_D)$ and, for that matter, only a single value of $\ln(Q_t^H/Q_t^D)$ (see below).

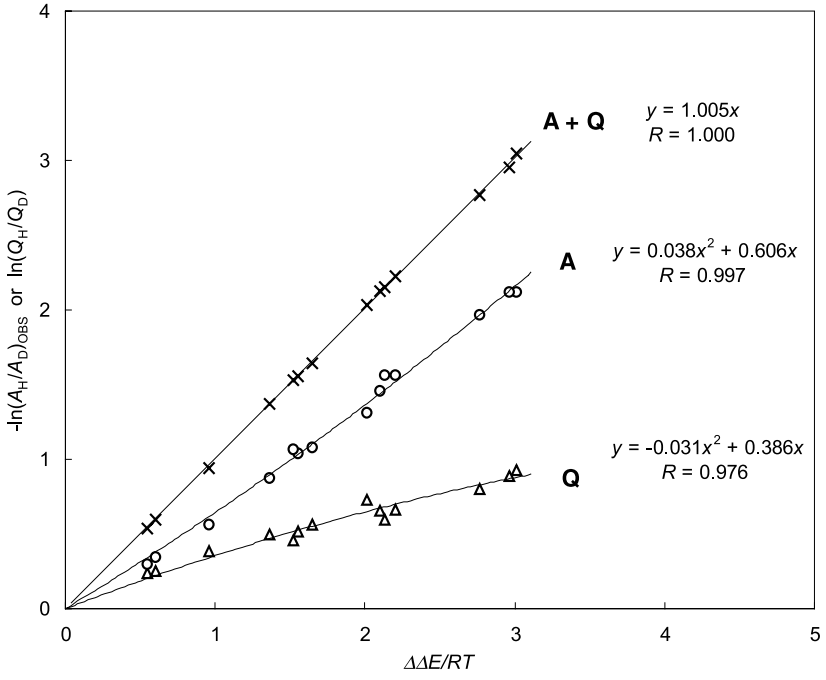

Fig. 2. The nonlinear relationship between the thermodynamic parameters; thermodynamic parameters $\ln(A_H/A_D)_{OBS}$ (**A**) and $\ln(Q_t^H/Q_t^D)$ (**Q**), from Table 2, are plotted according to Eqs. (15) and (16)

Analysis of Experimental Data

In order to support the above conclusion with practical examples, the thermodynamic and rate data for hydride transfer reactions, hydrogen-elimination reactions, and reactions catalyzed by lipooxygenase, amine dehydrogenase, and methylmalonyl-CoA mutase, were collected from the appropriate literature.

Then, the data were systematically analyzed as described in the Experimental section, and the thermodynamic and rate data, $\ln(A_H/A_D)_{OBS}$, $\ln(Q_t^H/Q_t^D)$, $[(E_a^D - E_a^H) - (E^D - E^H)]/RT$, $\ln(k_H/k_D)_{OBS}$, and $\ln(k_H/k_D)_{SEM}$ were extracted and summarized in Table 1. The data from Table 1 are plotted according to Eqs. (15) and (16), and presented in a single plot in Fig. 2.

Figure 2 shows a plot of Eq. (15), $-\ln(A_H/A_D)_{OBS}$ *versus* $\Delta\Delta E/RT$, and a plot of Eq. (16), $\ln(Q_t^H/Q_t^D)$ *versus* $\Delta\Delta E/RT$; a plot of the entropy factor, $\ln(A_H/A_D)$, is concave up, and the plot of the tunnel factor, $\ln(Q_t^H/Q_t^D)$, is convex upwards. Figure 2 shows again that both $\ln(A_H/A_D)$ and $\ln(Q_t^H/Q_t^D)$ are related to the barrier factor $\Delta\Delta E/RT$ in the form of a second order polynomial.

Eqs. (15)–(17) are valid between 10–50°C, the temperature range that was usually examined in this work. In Fig. 2, only the experimental data with $\Delta\Delta E/RT$ values within the limits 0–3 are plotted; thus, only the fourteen data pairs from Table 1 are plotted (entries # 6–8 and # 12–22), but not the entries # 1–5 and # 9–11.

Discussion

An important conclusion, which follows from this work, is that the *Saunders-Bell* analysis of tunnel effects in reactions with primary deuterium kinetic isotope effects was effective in many cases; in 22 enzymatic and nonenzymatic reactions analyzed in this work, the *Saunders-Bell* analysis was effective in 14 cases. The *Saunders-Bell* analysis operates within certain limits of $\Delta\Delta E/RT$ values; these limits are between 0 and 3 $\Delta\Delta E/RT$ (Fig. 1). Within these limits, the entropy factor $\ln(A_H/A_D)$ and the tunnel factor $\ln(Q_H/Q_D)$ are related to the barrier factor $\Delta\Delta E/RT$, in the form of a second order polynomial (Eq. (17)).

The relationship in Eq. (17) is novel and it shows that the enhancement of reaction rates is due to the barrier factor $\Delta\Delta E/RT$; the barrier factor shows the *relative decrease* of the *Arrhenius* activation barrier due to isotope substitution in reactants. The barrier factor is composed of two factors: the entropy factor, $\ln(A_H/A_D)$, which makes approximately three quarters of the barrier factor, and the tunnel factor, $\ln(Q_t^H/Q_t^D)$, which makes the remaining one quarter (Fig. 2).

Thus, the main contribution of this work is a detailed theoretical and practical analysis of tunnel effects with the aid of the *Saunders-Bell* analysis, an analysis that may help people in the field to apply more rationally the *Bell* equation in the future and especially to analyze more critically data from literature that apply the same approach. In this respect, Eq. (17) is crucial for the practical application of the *Bell* equation.

Experimental

Source of Experimental Data

In this work, we have analyzed the primary kinetic isotope effect data of deuterium for eight enzymatic and nonenzymatic hydride transfer reactions, described in the works of *Powell & Bruice* [5], *Verhoeven et al.* [6], *van Gerresheim* [7], *Leskovac et al.* [9], *van Gerresheim et al.* [19], *van Gerresheim & Verhoeven* [20], and *van Laar et al.* [21]. Further, we have analyzed the kinetic data for the four reactions catalyzed by lipooxygenase [22], nine hydrogen-elimination reactions [17, 23], and two enzymatic reactions catalyzed by amine dehydrogenase [24], and methylmalonyl-CoA mutase [13].

Hydride transfer reactions (entries # 1–8 in Table 2). Comparative studies of hydride transfer reactions from dihydronicotinamide compounds and from dideuteronicotinamide compounds to various substrates are surprisingly rare in the literature [3, 5–10, 19–21, 25], although they are providing the model chemical reactions for *NAD*(*P*)-dependent dehydrogenases [5–7, 26]. In this communication, we have collected data available from literature that are sufficiently reliable for kinetic analysis; Table 2 summarizes such reactions. In entry # 4, an intra-molecular hydride transfer takes place [7]. In entry # 5, however, a dismutation takes place between the oxidized and the reduced form of Bis*NAD* (Fig. 3).

In Fig. 3 (top), the bent transition state is forced upon the molecule (entry # 4 in Table 2), and the hydride transfer must take place in a sandwich-type transition state [6, 7]. In Fig. 3 (bottom), the hydride transfer reaction is between two molecules (entry # 5 in Table 2), and the transition state may assume the linear orientation.

Hydrogen transfer reactions in lipooxygenase reactions (entries # 9–12 in Table 1) [22]. A soybeen lipooxygenase-1 catalyzes the production of fatty acid hydroperoxides at 1,4-pentadienyl positions, and the product 13-(*S*)-hydroperoxy-9,1-(*Z,E*)-octadecadienoic acid is formed from the physiological substrate linoleic acid. This reaction proceeds by an intital rate-limiting abstraction of the pro-*S* hydrogen from C11 of the linoleic acid by the Fe^{3+}-OH cofactor, forming a substrate-derived radical intermediate and Fe^{2+}-OH_2. Molecular oxygen rapidly reacts with the radical, forming eventually the product, and regenerating the resting enzyme. The primary deuterium KIEs reported for reactions of the wild type and the three mutants of the enzyme [22] were analyzed in this work.

Elimination reactions (entries # 13–16 in Table 1) [17]. The primary deuterium KIEs were reported for the elimination reaction of 2-phenylethyltrimethylammonium and of 2-phenylethyl-*2,2-*d_2-trimethylammonium bromides with hydroxide ion in mixtures of water and dimethyl sulfoxide and with ethoxide ion in ethanol [17], and were analyzed in this work.

Elimination reactions (entries # 17–20) in Table 1) [23]. The primary deuterium KIEs were reported for reactions of [2-[(*p*-trifluoromethyl)phenyl]ethyl]trimethylammonium and [2-[(*p*-trifluoromethyl)phenyl]ethyl-*2,2-*d_2]trimethylammonium iodides with hydroxide ion in mixtures of dimethyl sulfoxide and water [23], and were analyzed in this work.

Tryptophan tryptophylquinone-dependent amine dehydrogenase (entry # 21 in Table 1) [24]. The primary deuterium KIEs were reproted for reactions of this enzyme, and the corresponding thermodynamic parameters were reported [24], and were analyzed in this work.

Methylmalonyl-CoA mutase reaction (entry # 22) in Table 1) [13–15, 27–28]. The enzyme methylmalonyl-CoA mutase catalyzes the conversion of methyl-malonyl-CoA to succinyl-CoA through the use of cofactor adenosyl-cobalamin [13–15, 27–28]. The primary deuterium KIEs for this reaction were compared in the presence and in the absence of enzyme.

Methods

Thermodynamic parameters for hydride transfer reactions, $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ (entries # 1–8 in Table 1), were extracted from the *Eyring* plot of Eq. (18).

$$\ln\left(\frac{k}{T}\right) = \ln\left(\frac{k_B}{h}\right) + \left(\frac{\Delta S^{\ddagger}}{R}\right) - \left(\frac{\Delta H^{\ddagger}}{RT}\right) \tag{18}$$

Table 2. Hydride transfer reactions

#	Substrate	Reductant	Solvent	Source
1	2,4,6-Trinitrobenzene-sulfonic acid (*TNBS*)	N^1-(2,6-Dichlorobenzyl)-1,4-($^1H_2$)dihydronicotinamide (*DBDN*-4H_2) N^1-(2,6-Dichlorobenzyl)-1,4-(^{1}H,^{2}H) monodeuteronicotinamide (*DBDN*-4HD)	0.1 *M* P_i *pH* 7.5	[9]
2	Nitrosobenzene (*NOB*)	N^1-(2,6-Dichlorobenzyl)-1,4-($^1H_2$)dihydronicotinamide (*DBDN*-4H_2) N^1-(2,6-Dichlorobenzyl)-1,4-(^{1}H,^{2}H) monodeuteronicotinamide (*DBDN*-4HD)	dry methanol	[9]
3	Dichloroindophenol (*DCPIP*)	N^1-(2,6-Dichlorobenzyl)-1,4-($^1H_2$)dihydronicotinamide (DBDN-4H_2) N^1-(2,6-Dichlorobenzyl)-1,4-(^{1}H,^{2}H) monodeuteronicotinamide (*DBDN*-4HD)	methanol/ buffer 1:1	[9]
4	Bis-nicotinamide, mixed H_2 Bis*NA*(RedOx) Bis-nicotinamide, mixed D_2 Bis*NA*(RedOx)	Bis-nicotinamide, mixed H_2 Bis*NA*(RedOx) (*intramolecular* reaction) Bis-nicotinamide, mixed D_2 Bis*NA*(RedOx) (*intramolecular* reaction)	dimethyl-sufoxide-$^2H_6$	[7]
5	Bis-nicotinamide, Bis*NA*(Ox)	Bis-nicotinamide, reduced-dihydro-Bis*NA*(4-$^1H_2$) (*intermolecular* reaction) Bis-nicotinamide, reduced-dideutero-Bis*NA*(4-$^2H_2$) (*intermolecular* reaction)	borate *pH* 8.22	[7]
6	10-Methyl-9-phenyl-acridinium ion (*MPAI*)	N^1-(Benzyl)-1,4-($^1H_2$) dihydro nicotinamide (*BDN*H_2) N^1-(Benzyl)-1,4-($^2H_2$) dideutero nicotinamide (*BDN*H_2)	acetonitrile	[7]
7	10-Methylacridinium ion (*MAI*)	N^1-(Benzyl)-1,4-($^1H_2$) dihydronicotinamide (*BDN*H_2) N^1-(Benzyl)-1,4-($^2H_2$) dideuteronicotinamide (*BDN*H_2)	acetonitrile	[5]
8	NAD^+	2-Propanol[a] 2-Propanol-d_8[a]	0.1 *M* P_i *pH* 7.0	[9]

[a] Catalyzed by yeast alcohol dehydrogenase

It was possible because all the rate data for hydrogen and deuterium substrates were available from the literature. The *Arrhenius* activation energy, E_a, was calculated at a constant temperature from the *Arrhenius* equation and/or from the relationship: $E_a = \Delta H^{\ddagger} + RT$ [10].

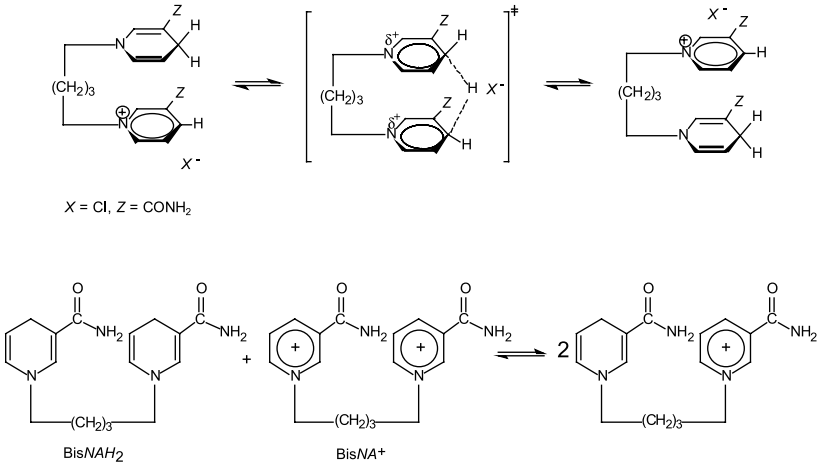

Fig. 3. Intramolecular and intermolecular hydride transfer reactions of bis-dihydronicotinamides

If tunneling is present, both the *Arrhenius* and the *Eyring* plot are by definition slightly curved; however, both plots provide a relatively accurate estimate of $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ [10]. For hydride transfer reactions, $(E_a^D - E_a^H)$ and A_H/A_D values were estimated from the experimental data (Eq. (5)), and all thermodynamic values were calculated with aid of the *Saunders* computer program.

For entries # 13–20 in Table 1, all thermodynamic values were lifted directly from the corresponding literature [17, 23]; the literature values were checked with the *Saunders* program and a close fit was obtained.

In all other cases, entries # 9–12 and # 21–22 in Table 1, $(E_a^D - E_a^H)$ and A_H/A_D values were lifted directly from the corresponding literature [13, 22, 24] and all other thermodynamic values were calculated with aid of the *Saunders* program. The data in Fig. 1 were calculated with aid of the *Saunders* program, assuming that the standard error in A_H/A_D is 50%.

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Annex

Saunders computer program [16]

```
C     TUNNEL.F. TUNNEL EFFECT ON THE ISOTOPE EFFECT FROM ARRHENIUS
C     PARAMETERS OF THE ISOTOPE EFFECT. SEE BELL, THE PROTON IN
C     CHEMISTRY, 2ND EDITION, PAGE 279.
      REAL KHKDSC, KHKDOB, KHKDC
      DIMENSION TI (20), KHKDC (20), KHKDSC (20), KHKDOB (20)
      DIMENSION QHQD (20), UH (20), UD (20), QH (20), QD (20), ERR (20)
      DIMENSION DADU (20), DQH (20), DQHQD (20), DU (20), SQH (20), SQHQD (20)
      COSEC (DUM) = 1./SIN(DUM)
      COTAN (DUM) = COS (DUM)/SIN (DUM)
      PRINT *, 'PLEASE INPUT NUMBER OF TEMPERATURES'
      READ *, NTEMP
```

```
      DO 104 J=1, NTEMP
      PRINT *, 'PLEASE INPUT TEMPERATURE NO.', J, 'IN DEGREES C'
      READ *, TI(J)
  104 CONTINUE
      PRINT *, 'PLEASE INPUT AHAD'
      PRINT *, 'AHAD IS THE RATIO OF ARRHENIUS PREEXPONENTIAL PARAMETERS'
      READ *, AHAD
      PRINT *, 'PLEASE INPUT EHED'
      PRINT *, 'EHED IS ED-EH, THE DIFFERENCE IN ARRHENIUS ACTIVATION
     1ENERGIES'
      READ *, EHED
      PRINT *, 'PLEASE INPUT MIDPOINT TEMPERATURE'
      READ *, T
      PRINT *, 'PLEASE INPUT UHMIN'
      READ *, UHMIN
      PRINT *, 'PLEASE INPUT UHMAX'
      READ *, UHMAX
      PRINT *, 'PLEASE INPUT HMASS'
      PRINT *, 'HMASS IS THE EFFECTIVE MASS ALONG THE REACTION
     1COORDINATE'
      READ *, HMASS
      PRINT *, 'PLEASE INPUT STD DEV OF AHAD'
      READ *, DA
      WRITE(*,*)'TUNNEL CORRECTION FROM FIT TO BELL EQUATION'
      OPEN(3, FILE='UPIS')
      WRITE (3, 1100) AHAD, EHED, T
 1100 FORMAT (' INPUT AHAD=', F5.3, 'EHED=', F5.3, 'T=', F5.1)
      WRITE (3, 1101) UHMIN, UHMAX, HMASS
 1101 FORMAT ('UHMIN=', F4.2, 'UHMAX=', F4.2, 'HMASS=', F4.2)
      TAV=T+273.16
C     FOR TRITIUM ISOTOPE EFFECTS USE FRAT=1./(2.+1./ HMASS)
      FRAT=1./(1.+1./HMASS)
C     DETERMINE THE VALUE OF UH THAT GIVES THE BEST FIT TO THE
C     EXPERIMENTAL TEMPERATURE DEPENDENCE. SEE KALDOR, S. B.;
C     SAUNDERS, W. H., JR. J. AM. CHEM. SOC. 1979, 101, 7594-7599.
      SMRAT=SQRT (FRAT)
      UDMIN=UHMIN*SMRAT
      AHADS=EXP(ALOG((UHMIN*SIN(0.5*UDMIN))/(UDMIN*SIN(0.5*UHMIN)))-0.
     15*UDMIN*COTAN(0.5*UDMIN)+0.5*UHMIN*COTAN(0.5*UHMIN))
      DELTA1=ABS (AHADS-AHAD)
      UHTEST=UHMIN
      UDTEST=UDMIN
      UHBEST=UHMIN
      IMAX=(UHMAX-UHMIN)*100.
      DO 200 I=1, IMAX
      UHTEST=UHTEST+0.01
      UDTEST=UHTEST*SMRAT
      AHADTR=EXP(ALOG((UHTEST*SIN(0.5*UDTEST))/(UDTEST*SIN(0.5*UHTEST)
     1))-0.5*UDTEST*COTAN(0.5*UDTEST)+0.5*UHTEST*COTAN(0.5*UHTEST))
      DELTA2=ABS (AHADTR-AHAD)
```

```
      IF (DELTA1-DELTA2) 601, 600, 600
  600 UHBEST = UHTEST
      DELTA1 = DELTA2
  601 CONTINUE
  200 CONTINUE
      UDBEST = UHBEST*SMRAT
      FREQH = UHBEST*TAV/1.4387
      FREQD = UDBEST*TAV/1.4387
      C = 0.0046053*FREQH*SQRT (2.0*HMASS)
      EHEDSC = EHED-0.001987*TAV*(0.5*UDBEST*COTAN(0.5*UDBEST)-0.5*UHBES
     1T*COTAN (0.5*UHBEST))
      WRITE (3, 1200) EHEDSC, C
 1200 FORMAT ('EHEDSC =', F9.5, 'SQRT(E)/A =', F8.3)
      WRITE (3, 1201) FREQH, FREQD
 1201 FORMAT ('VLH =', F9.3, 'CM1-1, VLD =', F9.3, 'CM-1',/)
      SUM = 0.0
      DO 400 K = 1, NTEMP
      TI (K) = TI(K) + 273.16
      KHKDOB (K) = AHAD*EXP (EHED/(0.001987*TI(K)))
C     KHKDOB IS AN ''OBSERVED'' VALUE CALCULATED FROM THE ARRHENIUS
C     PARAMETERS TO AVOID THE EFFECT OF RANDOM ERRORS IN THE
C     ACTUAL OBSERVED VALUES
      KHKDSC (K) = EXP (EHEDSC/(0.001987*TI(K)))
C     KHKDSC IS THE SEMICLASSICAL ISOTOPE EFFECT
      UH (K) = 1.4387*FREQH/TI (K)
      UD (K) = 1.4387*FREQD/TI (K)
      QH (K) = (0.5*UH (K))/SIN (0.5*UH (K))
      QD (K) = (0.5*UD (K))/SIN(0.5*UD (K))
      QHQD (K) = QH(K)/QD (K)
      KHKDC (K) = QHQD (K)*KHKDSC (K)
C     KHKDC IS THE CALCULATED ISOTOPE EFFECT INCLUDING TUNNELING
      ERR (K) = (KHKDOB (K)-KHKDC (K))*100./KHKDOB (K)
C     DIFFERENCE BETWEEN CALCULATED AND OBSERVED ISOTOPE EFFECTS.
      SUM = SUM + (KHKDOB(K)-KHKDC (K))**2
      DQH (K) = 0.5* (COSEC (0.5*UH (K))-QH (K)*COTAN(0.5*UH (K)))
      DQHQD (K) = 0.5*QHQD (K)* (SMRAT*COTAN (0.5*UD (K))-COTAN (0.5*UH (K)))
      DADU (K) = EXP (0.5*UH (K)* (COTAN (0.5*UH (K))-SMRAT*COTAN (0.5*UD (K))))
     1* (QHQD (K)* (0.25*UH (K)* (FRAT* (COSEC (0.5*UD(K)))**2-(COSEC (0.5*UH (K)
     2))**2) + 0.5* (COTAN (0.5*UH (K))-SMRAT*COTAN (0.5*UD(K)))) + DQHQD (K))
      DU (K) = (1./DADU (K))*DA
      SQH (K) = SQRT ((DQH (K)**2)*(DU (K) **2))
      SQHQD (K) = SQRT ((DQHQD(K)**2) * (DU (K)**2))
  400 TI(K) = TI (K)-273.16
      WRITE (3, 1300)
 1300 FORMAT (1X,'TEMP', 5X,'UH', 7X,'UD' , 7X,'QH', 7X,'QD' , 6X,'QH/QD', 4X,'K
     1HKD (S)' , 1X, 'KHKD(O)' , 2X, 'KHKD (C) ' )
      WRITE(3, 1400)(TI (K), UH(K), UD(K), QH(K), QD(K), QHQD(K), KHKDSC(K), KHKD
     1OB (K), KHKDC (K), K = 1, NTEMP)
 1400 FORMAT (F6.2, 8F9.6)
      WRITE (3, 1450)
```

```
1450 FORMAT (' ')
     WRITE (3, 1500) SUM
1500 FORMAT (4X, 'SUM OF SQUARES OF ERRORS =' , E10.4, /)
     WRITE (3, 1710) DA
1710 FORMAT (4X, 'STD DEV OF AHAD =' , F8.5,/)
     WRITE (3, 1720)
1720 FORMAT ('TEMP', 4X, '%ERR', 5X,'DEL(U)', 3X, 'S(QH)', 4X, 'S(QH/QD)' )
     WRITE (3, 1730) (TI(K), ERR (K), DU (K), SQH (K), SQHQD (K), K = 1, NTEMP)
1730 FORMAT (F6.2, 4F9.3)
     END
```

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