Limits on the Expression of Enzyme-Mediated Solvent Isotope Effects†

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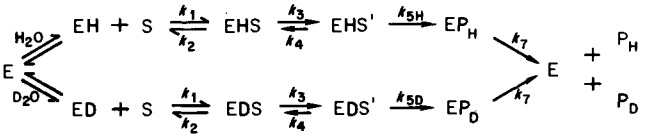
Received February 11, 1980

Abstract: Steady-state analysis of primary solvent isotope effects on enzyme-catalyzed reactions, mediated by solvent-shielded di- or triprotic groups on the enzyme, yields equations describing the upper limit of intramolecular isotopic discrimination. For diprotic groups $[P_H]/[P_D] = (3k_H/k_D + 1)/(k_H/k_D + 3)$, and for triprotic groups $[P_H]/[P_D] = [7(k_H/k_D)^2 + 10k_H/k_D + 1]/[(k_H/k_D)^2 + 10k_H/k_D + 7]$. Given a normal intrinsic isotope effect of $k_H/k_D = 7$, maximal isotopic discrimination in 50:50 H_2O:D_2O is therefore 2.2 and 3.3, respectively, versus 1.0 for a monoprotic group. Intermediate values of isotope discrimination may be interpreted with respect to distinguishing enzyme-mediated catalytic mechanisms from those of direct transfer between solvent and substrate, and to identifying mediating groups, by comparisons of isotopic discrimination at high and low concentrations of substrates and by reference to intrinsic and intermolecular isotope effects.

Primary solvent isotope effects on enzyme-catalyzed reactions may arise either by direct proton transfer from the solvent to the substrate or through the mediation of some catalytic group of the enzyme. Yamada and O'Leary[1] have argued that a distinction between the two can sometimes be made by performing experiments in 50:50 H_2O:D_2O. Specifically, they argue that isotopic discrimination will not be observed if the proton transfer occurs through the mediation of a monoprotic catalytic group which is shielded from exchange with the solvent. The reason for restricting the mediation to monoprotic groups is an expectation of a large discrimination between the mixed population of isotopic hydrogens on groups such as a lysine ammonium group. However, application of steady-state kinetic analysis[2] to these intramolecular isotope effects reveals that their expression as isotopic discrimination cannot be large and may even be absent. Consequently, the method of Yamada and O'Leary fails to provide the desired and important distinction between mechanisms. A new theoretical protocol is presented here for distinguishing not only between mechanisms but also between mono-, di-, and triprotic mediation. The method combines a closer analysis of the kinetic functions governing the expression of the intramolecular component of solvent isotope effects with comparisons to intermolecular isotope effects.

Theory

Upper Limits of Intramolecular Isotope Discrimination. The lack of isotopic discrimination in the monoprotic case can be illustrated by using the general mechanism shown in eq 1. The upper pathway represents exchange of the monoprotic group in H_2O and the lower is in D_2O. Shielding is represented by the absence of exchange from all but free enzyme and the presence of a nearly irreversible step (namely, $k_3 \gg k_4$) which prevents enzyme complexes poised for catalysis from partitioning back to free enzyme and to a second opportunity for exchange with solvent. The isotopically sensitive step is governed by k_5, which may or



(1)

may not be rate limiting. Isotopic discrimination is measured by comparing the ratio of hydrogen to deuterium in the products $[P_H]/P_D]$.

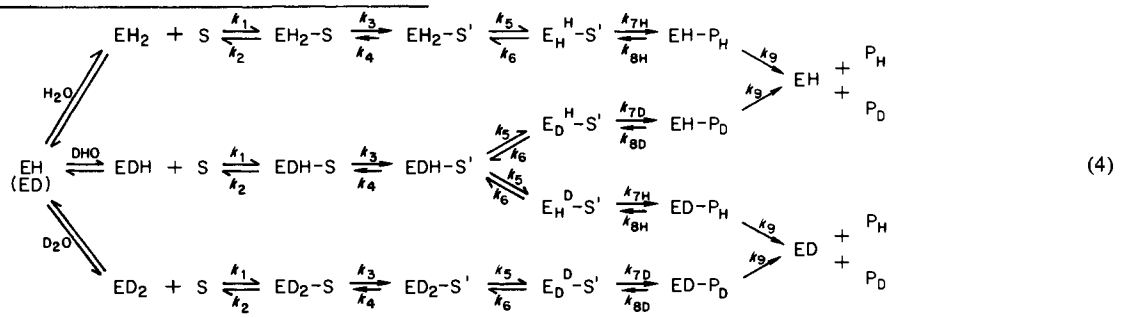
During the first turnover in 50:50 H_2O:D_2O, free enzyme has an equal opportunity for the upper and lower pathways, thus equal amounts of EHS′ and EDS′ will be produced. Because of the isotope effect, however, more enzyme will proceed through the remainder of the upper pathway than the lower to regenerate free enzyme. On the second turnover, the regenerated free enzyme will again have equal opportunity for the two pathways and produce equal amounts of EHS′ and EDS′, but some EDS′ remains from the first turnover (held back by the isotope effect). Hence EDS′ must necessarily exceed EHS′ in an isotopic discrimination experiment. The level of EDS′ continues to increase during subsequent turnovers until the reaction attains steady state, at which time the relative amounts of EDS′ and EHS′ will be defined by the isotope effect:

$$[EDS']/[EHS'] = k_{5H}/k_{5D} \quad (2)$$

or

$$k_{5D}[EDS'] = k_{5H}[EHS'] \quad (3)$$

Therefore, the relative flux through the upper and lower pathway will be the same, and no isotopic discrimination will be detected in the product ratio. The governing rule is that intermolecular isotope effects normally expressed in an enzyme-catalyzed reaction may be abolished in an isotopic discrimination experiment by a compensatory shift in the steady-state distribution of enzyme complexes.



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†Supported by Research Career Development Award GM-00245 from the National Institutes of Health.

(1) H. Yamada and M. H. O'Leary, *J. Am. Chem. Soc.*, **99**, 1660 (1977).
(2) D. B. Northrop, *Biochemistry*, **14**, 2644 (1975).

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Extending this rule to mediation of proton transfer by a diprotic group employs the general mechanism shown in eq 4. The reaction sequence is analogous to eq 1 but with one additional step: the positioning of one of the diprotic hydrogens into the transfer site. E_D^H and E_D^D represent diprotic group positioning with deuterium in the transfer site, E_H^D and E_H^H represent the same with hydrogen, and the positioning is in rapid equilibrium (i.e., k_5 and k_6 are much greater than k_7).

Assuming the proton transfer step is essentially irreversible and rate limiting, then at steady-state

$$[E_t] = [E_H^H\text{–}S'] + [E_D^H\text{–}S'] + [E_H^D\text{–}S'] + [E_D^D\text{–}S'] \quad (5)$$

Pathway turnover rates are therefore

$$r_1 = k_{7H}[E_H^H\text{–}S'] \quad (6)$$

$$r_2 = k_{7D}[E_D^H\text{–}S'] \quad (7)$$

$$r_3 = k_{7H}[E_H^D\text{–}S'] \quad (8)$$

$$r_4 = k_{7D}[E_D^D\text{–}S'] \quad (9)$$

The statistical probabilities of initiating the upper, middle, and lower branches of eq 4 are 1:2:1, thus the general rule of compensatory distribution of enzyme complexes requires that

$$r_1 = (r_2 + r_3)/2 = r_4 \quad (10)$$

Substituting eq 6–9 into eq 10 yields the following expressions for compensatory ratios of enzyme complexes at steady state in terms of the intrinsic isotope effect ($k_H/k_D = k_{7H}/k_{7D}$ of eq 4):

$$[E_D^D\text{–}S']/[E_H^H\text{–}S'] = k_H/k_D \quad (11)$$

$$\frac{[E_D^D\text{–}S']}{[E_D^H\text{–}S'] + [E_H^D\text{–}S']} = \frac{1 + k_H/k_D}{4} \quad (12)$$

$$\frac{[E_H^H\text{–}S']}{[E_D^H\text{–}S'] + [E_H^D\text{–}S']} = \frac{1 + k_H/k_D}{4k_H/k_D} \quad (13)$$

The rates of individual reaction pathways can now be expressed in common terms, by combining eq 11–13 and eq 5 in eq 6–9.

$$\frac{r_1}{[E_t]} = \frac{k_H(1 + k_H/k_D)}{(1 + k_H/k_D)^2 + 4k_H/k_D} \quad (14)$$

$$\frac{r_2}{[E_t]} = \frac{2k_H}{(1 + k_H/k_D)^2 + 4k_H/k_D} \quad (15)$$

$$\frac{r_3}{[E_t]} = \frac{2k_H(k_H/k_D)}{(1 + k_H/k_D)^2 + 4k_H/k_D} \quad (16)$$

$$\frac{r_4}{[E_t]} = \frac{k_H(1 + k_H/k_D)}{(1 + k_H/k_D)^2 + 4k_H/k_D} \quad (17)$$

Finally, the expected net discrimination isotope effect can be expressed in terms of the intrinsic isotope effect:

$$\frac{[P_H]}{[P_D]} = \frac{r_1 + r_3}{r_2 + r_4} = \frac{3k_H/k_D + 1}{k_H/k_D + 3} \quad (18)$$

Following a similar derivation for a triprotic mechanism yields the expression:

$$\frac{[P_H]}{[P_D]} = \frac{7(k_H/k_D)^2 + 10k_H/k_D + 1}{(k_H/k_D)^2 + 10k_H/k_D + 7} \quad (19)$$

Equations 18 and 19 reveal that as the intrinsic isotope effect becomes very large, the observed intramolecular isotopic discrimination will approach maximum limits of $[P_H]/[P_D] = 3$, for proton transfer mediated by a diprotic group, and $[P_H]/[P_D] = 7$, for mediation by a triprotic group. More realistically, given a normal intrinsic isotope effect $k_H/k_D = 7$, the expected isotopic discrimination will have values of 2.2 and 3.3, respectively.[3]

Suppression of Intramolecular Isotope Effects. Equations 18 and 19 were derived by using two simplifying assumptions designed to maximize the expression of intramolecular isotope effects. First, it was assumed that protic positioning in the transfer site was in rapid equilibrium; second, catalysis was assumed to be irreversible. A general equation governing the expression of an intramolecular isotope effect when these assumptions are not true has been derived for an assymetrically labeled substrate.[4] Applied to the intramolecular component of eq 4 it takes the form:

$$\frac{r_3}{r_2} = \frac{k_{7H}/k_{7D} + k_7/k_6 + k_8{}^DK_{eq}/k_9}{1 + k_7/k_6 + k_8/k_9} = \frac{k_H/k_D + C_{if} + C_r{}^DK_{eq}}{1 + C_{if} + C_r} \quad (20)$$

where ${}^DK_{eq}$ is the isotope effect on the equilibrium constant, and C_{if} and C_r are the forward internal and the reverse commitments to catalysis, respectively. If the rapid equilibrium assumption is not true, then the forward internal commitment to catalysis will be significant and will act to suppress the intramolecular effect; if irreversible catalysis is not true, then the reverse commitment to catalysis will similarly act to suppress the intramolecular effect. In either or both cases, $r_3/r_2 < k_H/k_D$ and the lesser value is what enters eq 18 and 19.

Intermolecular vs. Intramolecular Isotope Effects. Equations 18 and 19 were also limited by the presence of an irreversible step, which prevents the dissociation of substrate from the central complex; the substrate shields the central complex from exchange with the solvent, and the lack of exchange prevents the expression of intermolecular isotope effects. The intermolecular component may be kinetically isolated by using the noncompetitive method,[5] in which reaction velocities are measured separately in 100% D_2O and H_2O, instead of the competitive method of isotopic discrimination in 50:50 H_2O:D_2O. Equations governing the expression of intermolecular isotope effects have been described[6,7] and take the form

$$\frac{(V/K)_H}{(V/K)_D} = \frac{k_H/k_D + C_f + C_r{}^DK_{eq}}{1 + C_f + C_r} \quad (21)$$

where V is the maximal velocity, K the varied substrate's Michaelis constant, C_f the forward commitment to catalysis. For the mechanism in eq 4, the equation becomes

$$\frac{(V/K)_H}{(V/K)_D} = \frac{\frac{k_{7H}}{k_{7D}} + \frac{k_7}{k_6}\left[1 + \frac{k_5}{k_4}\left[1 + \frac{k_3}{k_2}\right]\right] + \frac{k_8{}^DK_{eq}}{k_9}}{1 + \frac{k_7}{k_6}\left[1 + \frac{k_5}{k_4}\left[1 + \frac{k_3}{k_2}\right]\right] + \frac{k_8}{k_9}} \quad (22)$$

When k_4 is slow, the forward commitment to catalysis is large, and the intermolecular isotope effect is abolished. When k_4 is fast, eq 22 simplifies to eq 20, which means that isotopic discrimination is fully expressed at low substrate concentrations and equals the V/K isotope effect. At intermediate levels of shielding at low substrate, isotopic discrimination will contain both inter-

(3) Somewhat larger values should be expected if secondary isotope effects are considered as well. These act to decrease the immediate intramolecular effect but increase the compensatory shift of enzyme complexes. The two actions do not cancel but curiously cause the secondary effect to appear in the final equations as a simple multiplying factor of the intrinsic isotope effect. Thus, given normal primary and secondary intrinsic isotope effects of 7 and 1.2, respectively, the value entered into eq 18 and 19 is $7 \times 1.2 = 8.4$, to give limits of 2.3 and 3.58.

(4) G. T. Miwa, W. A. Garland, D. J. Hodshon, A. Y. H. Lu, and D. B. Northrop, *J. Biol. Chem.*, **255**, 6049 (1980).

(5) H. Simon and D. Palm, *Angew. Chem., Int. Ed. Engl.*, **5**, 920 (1966).

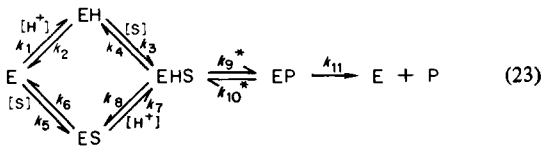
(6) D. B. Northrop in "Isotope Effects on Enzyme-Catalyzed Reactions", W. W. Cleland, M. H. O'Leary, and D. B. Northrop, Eds., University Park Press, 1977, p 122.

(7) D. B. Northrop, *Methods Enzymol.*, in press.

and intramolecular components and have a larger value than the V/K effect.

Intermediate levels of shielding are also possible at high concentrations of substrate if the last restriction, the absence of direct exchange between solvent and enzyme complexes, is relaxed. The kinetic relationships that pertain to a proton transfer mechanism expressing both inter- and intramolecular isotope effects are obviously complex. At present we do not possess adequate theory for containing the simultaneous expression of multiple isotope effects, of this or any other form, within general equations. Nevertheless, additional limits can be established for those situations of direct shielding where only one type of isotope effect is allowed.

Direct shielding may be portrayed as a random addition of substrate and solvent protons:



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The level of shielding is a function of the ease of protonation and deprotonation of the central complex, governed by k_7 and k_8. In the fully shielded case both are zero, only the upper branch of the random mechanism is functional, and only the free enzyme can exchange with solvent, as was the case in the mechanism of eq 4. In a fully unshielded case, solvent exchange with the center complex is in rapid equilibrium, represented by k_8 being much greater than k_9.

The forward commitment to catalysis when substrate is varied in 100% D_2O vs. H_2O is

$$C_f = \frac{k_9}{k_4' + k_8'} = \frac{k_9}{k_4 + k_6k_8/(k_7[H^+] + k_6)} \quad (24)$$

where net rate constants[8] are indicated by primes. At a pH > pK_a (i.e., $[H^+] < K_a = k_8/k_7$), C_f simplifies and eq 21 becomes

$$\frac{(V/K)_H}{(V/K)_D} = \frac{k_{9H}/k_{9D} + k_9/(k_4 + k_8) + k_{10}^{D}K_{eq}/k_{11}}{1 + k_9/(k_4 + k_8) + k_{10}/k_{11}} \quad (25)$$

At a pH < pK_a, C_f further simplifies to yield

$$\frac{(V/K)_H}{(V/K)_D} = \frac{k_{9H}/k_{9D} + k_9/k_4 + k_{10}^{D}K_{eq}/k_{11}}{1 + k_9/k_4 + k_{10}/k_{11}} \quad (26)$$

In the presence of shielding, k_8 equals zero and eq 25 equals eq 26.

Isotopic discrimination in a monoprotic mechanism is also purely intermolecular. For the mechanism in eq 23, the forward commitment to catalysis for solvent protons is

$$C_f = \frac{k_9}{k_4' + k_8'} = \frac{k_9}{k_2k_4/(k_3[S] + k_2) + k_8} \quad (27)$$

At low substrate concentrations, C_f simplifies and isotopic discrimination becomes governed by

$$\left|\frac{[P_H]}{[P_D]}\right|_{S\to 0} = \frac{k_{9H}/k_{9D} + k_9/(k_4 + k_8) + k_{10}^{D}K_{eq}/k_{11}}{1 + k_9/(k_4 + k_8) + k_{10}/k_{11}} \quad (28)$$

Equation 28 equals eq 25, thus isotopic discrimination at low substrate concentrations again equals the V/K isotope effect (compare eq 22, when k_4 is fast, to eq 20) but the present equality is obtained only when the mediating group has an acidic pK_a. At high substrate concentrations, C_f further simplifies and

$$\left|\frac{[P_H]}{[P_D]}\right|_{S\to\infty} = \frac{k_{9H}/k_{9D} + k_9/k_8 + k_{10}^{D}K_{eq}/k_{11}}{1 + k_9/k_8 + k_{10}/k_{11}} \quad (29)$$

In the absence of direct shielding, k_8 greatly exceeds k_9, the forward commitment to catalysis approaches zero, and both eq 28 and 29 reduce to

$$\frac{[P_H]}{[P_D]} = \frac{k_{9H}/k_{9D} + k_{10}^{D}K_{eq}/k_{11}}{1 + k_{10}^{D}K_{eq}/k_{11}} \quad (30)$$

Isotopic discrimination in the absence of direct shielding is therefore independent of substrate concentration and governed only by the reverse commitment to catalysis.

In di- and triprotic mechanisms, eq 30 is also obtained in the absence of direct shielding because a ready access to exchange with solvent precludes a compensatory distribution of enzyme complexes and subsequent distinction between inter- and intramolecular isotope effects, although both effects will be expressed. In the presence of direct shielding at high substrate concentration, isotopic discrimination is purely intramolecular and governed by equations represented by eq 20 as expressed through eq 18 and 19. What remains undefined is the level of expression of isotopic discrimination containing both intermolecular and intramolecular components when shielding is partial, either because of limited exchange directly between the central complex and solvent or by hindered partitioning of the enzyme between the central complex and free enzyme at low substrate concentrations.

Results of Computer Simulations

When multiple isotope effects are present in an enzymatic mechanism, groups of rate constants do not factor into discreet functions represented here by commitments to catalysis. Rather, one must deal with the complete rate equation in terms of individual rate constants. For a mixture of inter- and intramolecular isotope effects, the random binding segment of the mechanism of eq 22 must be superimposed on the multiple pathways of the mechanism in eq 4, which results in very unwiedly rate equations. In order to examine the nature of the expression of mixed isotope effects, various mechanisms were modeled on a computer by assigning arbitrary values to individual rate constants, computing the contribution of individual segments which could be isolated and defined by Cleland's theory of net rate constants,[8] and then introducing the values for these intermediate segments into larger, more general rate equations. The expression of solvent isotope effects was examined as a function of the percentage of shielding of the proton-mediating group, defined as the fraction of protonated enzyme–substrate complex that undergoes catalytic turnover vs. the total disappearance of this complex including the deprotonation step, at high and low concentrations of substrate. For example, for monoprotic group mediation in the mechanism of eq 22 at high substrate concentrations

$$\% \text{ shielding} = \frac{k_9'}{k_4' + k_8' + k_9'} \times 100 = \frac{k_9k_{11}/(k_{10} + k_{11})}{k_9k_{11}/(k_{10} + k_{11}) + k_8} \times 100 \quad (31)$$

Representative results of direct shielding are illustrated in Figure 1, in which the rate constants for protonation and deprotonation of the central complex were varied. The three illustrations depict mono-, di-, and triprotic group mediation, with acidic, neutral, and alkaline pK_a's, respectively, in order to isolate the pH dependence of V/K intermolecular isotope effects. In Figure 1A, catalysis was essentially irreversible to set the reverse commitment to catalysis equal to zero; hence isotopic discriminations at 0% shielding equal the intrinsic isotope effect, which was given a value of seven. In Figures 1B and 1C, the reverse commitment to catalysis is finite and all expressed isotope effects are less than the intrinsic. As shielding increases, isotopic discrimination at low substrate concentrations follows a biphasic curve through values equal to or greater than the V/K effect depending upon the pK_a, to a value at 100% shielding where discrimination equals the V/K value. This equality holds for direct shielding only. Not illustrated in this figure is the effect of hindered partitioning between the central complex and free enzyme (see eq 22).

The major finding of these simulations is the linear conversion of isotopic discrimination at high substrate concentrations from

(8) W. W. Cleland, *Biochemistry*, **14**, 3220 (1975).

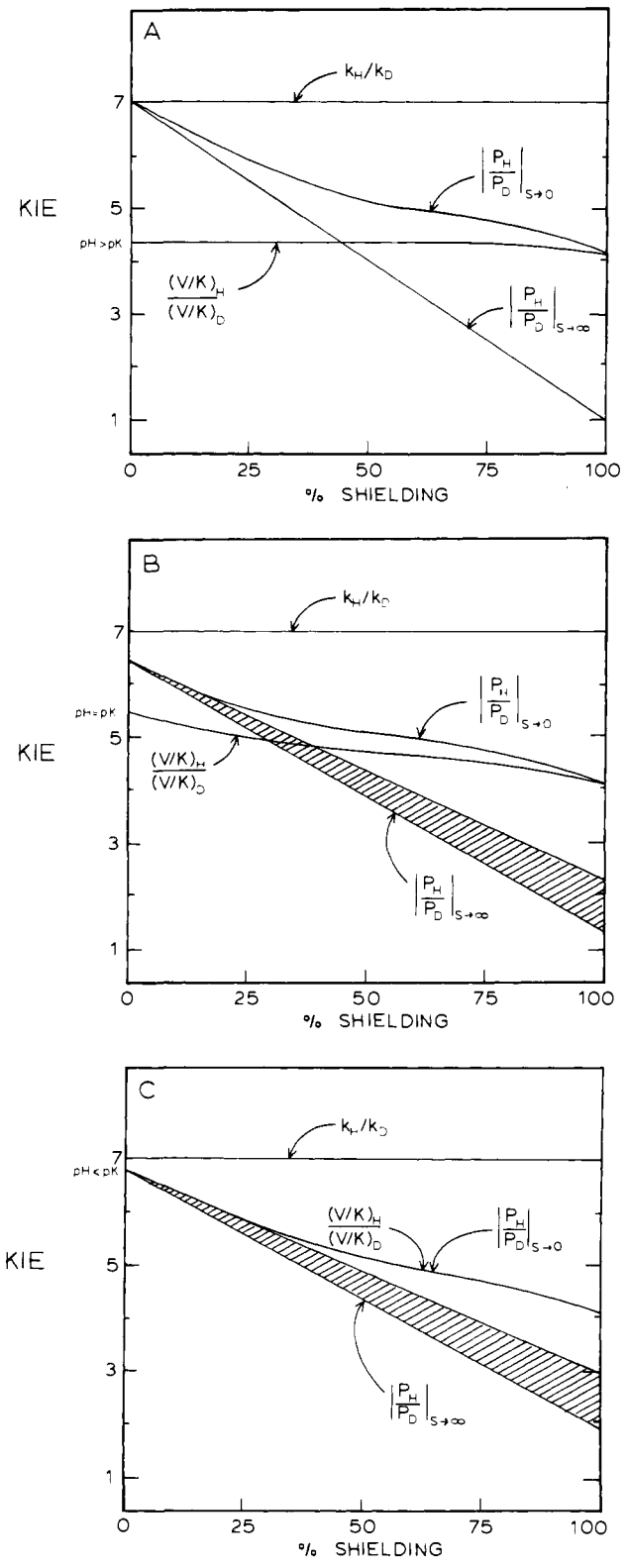

Figure 1. The expression of solvent kinetic isotope effects (KIE) as functions of the percentage of shielding of a proton-mediating group of an enzyme. Values for isotopic discrimination ($[P_H]/[P_D]$) and V/K isotope effects were generated by computer simulations in which a random mechanism was assumed for the binding of substrate and solvent protons. The degree of randomness was progressively varied from one extreme representing 0% shielding (i.e., independent binding at rapid equilibrium) to the other representing 100% shielding (i.e., compulsory ordered protonation of free enzyme followed by addition of substrate) by varying the rate constants for the protonation and deprotonation of the enzyme–substrate complex. (A) An acidic, monoprotic group such as a carboxyl and essentially irreversible catalysis. (B) A neutral diprotic group such as bound water. (C) A basic, triprotic group such as a primary amine.

purely intramolecular to purely intermolecular when portrayed as a function of the percentage of direct shielding. A single line is obtained in the monoprotic case, reaching a final value of one,[9] because it lacks an intramolecular component. A family of lines are obtained in di- and triprotic cases, portrayed by the shaded areas, depending upon the relative values of rate constants contributing to the commitment factors of eq 20. The final values obtained at 100% shielding are equal to or greater than one and represent the initial point of departure of this analysis.

Discussion

The expression of intramolecular solvent isotope effects in measurements of isotopic discrimination cannot be large. Consequently, observations of large isotopic discriminations (such as $P_H/P_D = 6.2$ for enzymatic decarboxylation of α-methylglutamic acid[1]) eliminate mechanisms of proton transfer mediated by di- and triprotic as well as monoprotic catalytic groups which are shielded from exchange with solvent. At the other extreme, observations of small isotopic discriminations (such as $[P_H]/[P_D] = 1_1 \pm 0.1$ for decarboxylation of glutamic acid[1]) do not preclude such participation of di- and triprotic groups. These intramolecular isotope effects are subject to steady-state suppression depending on whether or not protic positioning in the transfer site is in rapid equilibrium and whether or not catalysis is reversible. The first condition is probably true, particularly if the mediating group is small such as a lysine amino group, but is not directly testable. The second is probably not true but is partially testable in that the reverse commitment to catalysis is common to both intra- and intermolecular isotope effects (compare eq 20 and 22 and also isotope effects on the maximum velocity[6,7]): hence, large intermolecular isotope effects argue against suppression by catalytic reversibility alone.

Intermediate values of isotopic discrimination (namely, values between 1.0 and 3.3) may arise from a fully shielded triprotic, a partially shielded diprotic, a moderately shielded monoprotic, or even a solvent-mediated mechanism. Nevertheless, these may in some instances still be distinguished by examining the limits of isotopic discrimination at high and low substrate concentrations, by examining the effect of pH, and by reference to isotope effects on V/K and catalysis. Because isotopic discrimination is normally a V/K effect (when the varied substrate carries the deuterium label), both competitive and noncompetitive methods of measurement normally yield the same experimental value.[5] But when solvent is labeled and substrate is varied, different values for kinetic isotope effects may be obtained by different methods of measurement. Furthermore, as demonstrated in the present analysis, different values may be obtained if the solvent isotope effect is composed of inter- and intramolecular components. These differences are sensitive to both the protonating mechanism and to the nature of group participation. Mechanistic interpretation of solvent isotope effects is obviously best served by determining and comparing all four types of effects: the intrinsic, V/K, and discrimination at high and low concentrations of substrates.

Isotopic discrimination must be equal to or less than the intrinsic isotope effect, depending upon the commitments to catalysis. In the absence of any shielding, the rate of deprotonation is likely to be much greater than catalysis, forcing the forward commitment to catalysis to be low and thereby leaving only the reverse commitment responsible for a lesser value. This situation appears indistinguishable from a mechanism of direct transfer from solvent to substrate, because in direct transfer, the protonating and isotopically sensitive steps are one and the same, eliminating the forward commitment but leaving the reverse commitment factor. A direct transfer may still be distinguished from an enzyme-mediated mechanism, even in the absence of shielding, because of the pH dependence of the V/K isotope effect (see Figure 1 at 0% shielding and eq 24 and 25). A lesser value for the V/K effect is indicative of an enzyme-mediated proton transfer and can aid in the identification of a participating group by indicating a low

(9) A value greater than one will obtain at 100% shielding if the monoprotic group is a sulfhydryl, because of an equilibrium isotope effect on proton exchange with solvent. See R. L. Schowen in ref 6, p 70.

pK_a. However, additional causes for a pH-dependent V/K isotope effect exist, whose complexities lie beyond the scope of the present discussion.

A clear distinction between protonating mechanisms is established if isotopic discrimination is dependent upon the concentration of substrates. What remains is whether one can identify the nature of the participating group. In the presence of shielding, what portion of the commitment factors suppresses the intramolecular component of isotopic discrimination at high concentrations of substrate will be uncertain. Maximal values of shielded isotopic discrimination may be calculated from eq 18 and 19 after first determining the intrinsic isotope effect through a comparison of deuterium and tritium isotope effects on V/K.[2,7] Because the commitment factors governing the V/K isotope effect are at least as great as those governing intramolecular isotopic discrimination (compare eq 22 to eq 20), minimal values may also be calculated, by substituting the V/K effect for the intrinsic in eq 18 and 19. If isotopic discrimination is less than both minima, the group is monoprotic, if less than the triprotic minimum, it is either a shielded diprotic or a partially shielded monoprotic, if less than the triprotic maximum, all three groups are candidates at various levels of shielding, and if greater than the triprotic maximum, then proton transfer is mediated by an unknown group which cannot be fully shielded from exchange with solvent.

Communications to the Editor

First Ionization Band of 1,1-Dimethylsilaethylene by Transient Photoelectron Spectroscopy

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Received September 29, 1980

The properties of alkenes and arenes containing trigonal silicon atoms have attracted the recent attention of both experimentalists[1] and theoreticians.[2] This interest derives, in part, from the fact that such compounds are not isolable in the ordinary sense. 1,1-Dimethylsilaethylene (**1**) is such a compound, and we presently wish to report our observation of the first band of its photoelectron spectrum.

The instrument used here was designed[3] to allow chemical studies on the same samples that give rise to the observed spectra and to permit variable distance between generating reaction and photoionization [He I, 584 Å]. Figure 1 summarizes our transient photoelectron (TPE) spectral results on the pyrolysis of 1,1-dimethylsilacyclobutane (**2**, Scheme I).[4]

The lower trace shows the PE spectrum of the precursor **2**. The middle trace shows the spectrum observed at a pyrolysis temperature of 650 °C and a flight distance from furnace tip to photoionization chamber of 30 cm. This spectrum shows the presence of ethylene, indicating the cracking reaction has proceeded. The main product, under these conditions, is a polymeric oil[4] which coats the 40-cm zone between furnace and cold trap (10 cm downstream from photoionization chamber). The upper trace of Figure 1 shows the spectrum obtained with the furnace (720 °C) tip located 5 mm from the center of the photoionization chamber. The new band at 8.3 eV can be assigned to the first ionization state of **1**.[5]

The mass spectrum of the volatile fraction of material collected on the cold (liquid N_2) trap, while the middle spectrum was recorded, showed the presence of the symmetrical dimer (**3**, m/z 144, 129). When water was injected into the photoionization chamber, immediately downstream from the furnace, the 8.3-eV photoelectron band disappeared. The mass spectrum of the volatile fraction of material collected on the cold trap when water was present showed new peaks, indicating the formation of ether (**4**) (m/z 147; **4** - CH_3).[4] The result strongly supports the assignment of the 8.3-eV band as the first ionization of **1**.

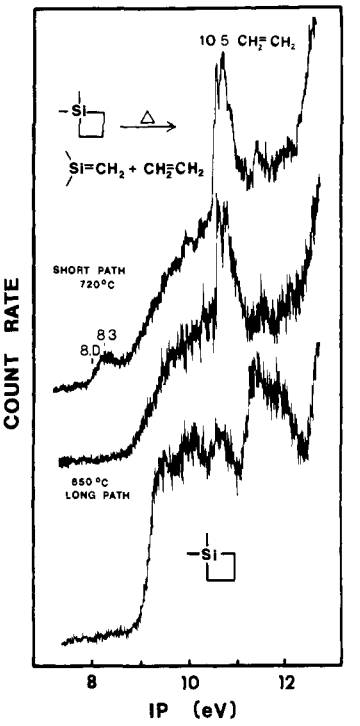
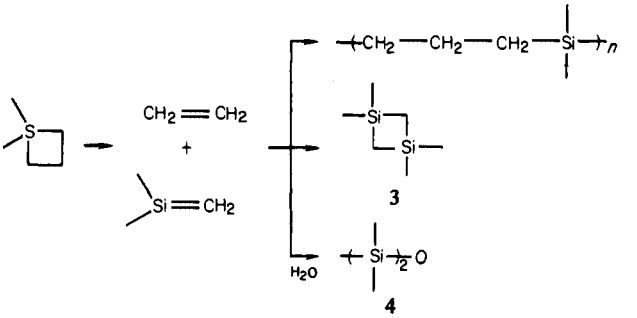


Figure 1.

Scheme I



The presently observed lowest vertical ionization potential is not in very good agreement with the empirically corrected STO-3G Koopmans theorem[7] value (7.53 eV). It is in better agreement

(1) Mahaffy, P. G.; Gutowsky, R.; Montgomery, L. K. *J. Am. Chem. Soc.* **1980**, *102*, 2855 and references therein.
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(5) The middle trace of Figure 1 is a good approximation to the background which should be subtracted from the upper trace to leave the spectrum of **1**. The high background relative to **1** in the upper trace is an indication of the high reactivity of **1** at the pressures needed for the TPE technique.
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