Effect of Isotope Scrambling and Tunneling on the Kinetic and Product Isotope Effects for Reduced Nicotinamide Adenine Dinucleotide Model Hydride Transfer Reactions

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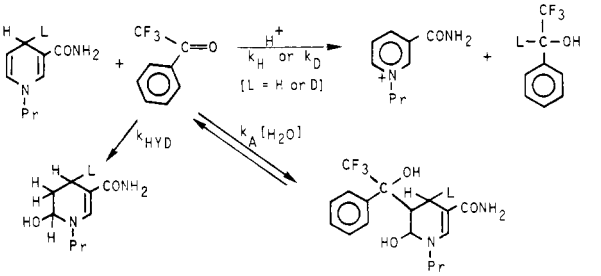
Abstract: The rates and kinetic isotope effects for reaction of several dihydronicotinamides with *N*-methylacridinium ions (MAI) have been measured under conditions that eliminate the possibility of anomalous kinetic (k_H/k_D) and product (Y_H/Y_D) isotope effects brought about by isotope scrambling in the products or by neglect of secondary kinetic isotope effects (k^H/k^D). The large effect of k^H/k^D on the calculated value of k_H/k_D and the effect of isotope scrambling on Y_H/Y_D were demonstrated herein by use of reaction scheme simulation with experimentally observed rate and equilibrium constants. We have critically reexamined several NADH model reactions that were reported to possess different k_H/k_D and Y_H/Y_D values and have found no such discrepancies. This in itself removes much of the evidence for a stepwise e^-, H^+, e^- mechanism for hydride equivalent transfer in these enzyme model systems. The rate and equilibrium data for reaction of MAI and *N*-phenyl-substituted 1,4-dihydronicotinamides (X-PhNADH) support a single-step mechanism for hydride transfer in that the Brønsted plot ($\beta = 0.51 \pm 0.06$) for this reaction is not biphasic. The temperature-dependent (50 to −2 °C) isotope effects ($k_H/k_D = 4–7$) for reaction of MAI with 4,4-diprotio- and 4,4-dideuterio-*p*-CH_3PhNADH indicate that H^- transfer from NADH model compounds proceeds with a moderate amount of tunneling. By use of the Bell equation we have estimated the semiclassical (i.e., in the absence of tunneling) kinetic isotope effects for this reaction and found them to be in the 2–3 range, consistent with rate-determining but nonlinear H^- transfer through a charge-transfer type of complex.

Controversy regarding the detailed mechanism for hydride equivalent transfer from NADH and NADH model compounds (single-step H^- transfer and multistep e^-, H^+, e^- transfer) continues to receive considerable attention. In one of the first studies of NADH model reactions, Abeles et al.[1] proposed that direct hydride transfer was occurring between *N*-benzyl-1,4-dihydronicotinamide (BzNADH) and substituted thiobenzophenones. This conclusion was based on solvent and primary kinetic isotope effects. Other studies supporting a direct hydride transfer mechanism soon followed.[2-6] Some years later it was reported that there was a discrepancy between the kinetic (k_H/k_D) and product (Y_H/Y_D) isotope effect for reaction of *N*-propyl-1,4-dihydronicotinamide (PrNADH) with trifluoroacetophenone (TFA).[2] These data were offered as strong evidence for a multistep mechanism, and it was postulated that formation of a noncovalent intermediate occurred on the reaction pathway and that the rate-determining step preceded hydrogen transfer. Soon thereafter, several others attempted to distinguish between the single-step H^- mechanism and the multistep e^-, H^+, e^- mechanism by what appeared to be an irrefutable experimental method: observed discrepancies between kinetic and product isotope effects (see Table I). Use of these discrepancies to support a multistep mechanism was later shown to be invalid by Chipman et al.[7] when they found that PrNADH reacted with TFA in aqueous solution to give [in addition to the redox (k_H or k_D) products] products of adduct formation (k_A) and hydration (k_{HYD}) (eq 1).



(1)

These kinetically significant side reactions could account for the smaller observed kinetic isotope effects as shown by eq 2, providing

$$k_H/k_D = \frac{2k_H + (k_{HYD} + k_A)}{k_H + k_D + (k_{HYD} + k_A)} \quad (2)$$

the reductant was able to form an adduct (or hydration product) and that the reaction be carried out in a "wet" solvent system in order to provide H_2O (or ROH) for formation of the stable adduct or hydration product. Although this finding resolved some of the discrepancy between k_H/k_D and Y_H/Y_D for reactions in protic solvents, several other nonhydratable NADH model systems studied in aprotic solvents (usually CH_3CN) demonstrated product isotope effects significantly larger than the kinetic ones.[8-10] These disparities in isotope effects were regarded as proof for intermediate formation (inasmuch as the aforementioned kinetic complications should not exist) until we recently reported that isotope scrambling and neglect of secondary isotope effects may also give erroneous results even in the absence of hydration and adduct formation.[11] Support for the existence of such complicating features in the calculation of kinetic and product isotope effects is shown by inspection of the collected studies (Table I) wherein differences between k_H/k_D and Y_H/Y_D measured in CH_3CN are found only when k_H/k_D is determined from the ratio of rate constants when employing 1,4-[4,4-$^1H_2$]- and 1,4-[4-

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Table I. Summary of Kinetic and Product Isotope Effects for NADH Model Reactions

reductant[q]	oxidant[q]	temp, °C	solvent	k_H/k_D (from H,D)[r]	k_H/k_D (from D,D)	y_H/y_D (from H,D)	ref
PrNADH	TFA	50	25% aqueous *i*-PrOH	1.38 ± 0.11		3.8 ± 0.3	*a*
BzNADH	TFA	50	25% aqueous *i*-PrOH	2.77 ± 0.85		3.8 ± 0.3	*a*
PrNADH	MAI	25	phosphate buffer	1.70 ± 0.28	1.46 ± 0.10	5.4 ± 1.0	*b*
N-2′-carboxy-BzNADH	MAI	25	CH_3CN	1.11		3.8	*c*
BzNADH	hexachloroacetone	26.6	CH_3CN		3.96 ± 0.28		
PrNADH	1,10-phenanthroline-2-carboxaldehyde	25	CH_3CN	1.74 ± 0.6		3.7 ± 0.2	*d*
						3.0	*e*
BzNADH	MAI	25	MeOH	1.4	1.38		
BzNADH	MAI	30	CH_3CN	2.76 ± 0.15 (3.70 ± 0.18)		4.0 ± 0.4	*f*
MA	BQ	25	60% CH_3CN/H_2O			4.0 ± 0.2 (3.7 ± 0.2)	*g*
MA	BQ	25	75% CH_3CN/H_2O		8.3 ± 0.6	8.1 ± 1.4	*h*
MA	BQ	25	90% CH_3CN/H_2O		10.6 ± 0.7	9.1 ± 1.9	*h*
MA	CA	25	CH_3CN		11.6 ± 0.8	9.4 ± 2.0	*h*
MA	TCNE	25	CH_3CN		6.7 ± 0.7	6.2 ± 0.7	*h*
MA	DCBQ	25	CH_3CN		4.4 ± 0.5	4.5 ± 1.4	*h*
BzNADH	thiopivalophenc	25	CH_3CN		4.9 ± 0.5	4.7 ± 0.1	*h*
PrNAH	methyl benzoylformate	25	CH_3CN	4.9 ± 0.2 (5.3 ± 0.2)	5.0 ± 0.2 (4.8 ± 0.2)	5.0 ± 0.4 (4.9 ± 0.3)	*i*
PrNADH	TFA	50	CH_3CN	(4.3 ± 0.2)	(4.3 ± 0.2)	(3.7 ± 0.5)	*i*
PrNADH	TFA	50	25% aqueous *i*-PrOH	(3.62 ± 0.15)	(3.65 ± 0.15)	3.65 ± 0.2 (3.44 ± 0.2)	*j*
BzNADH	TFA	50	25% aqueous *i*-PrOH	1.29 ± 0.04	1.93 ± 0.13	6.7 ± 1.8	*k*
BzNADH	2-acetylpyridine	50	CH_3CN	1.55 ± 0.15	2.19 ± 0.11	6.6 ± 1.7	*k*
BzNADH	1-acetylisoquinoline	50	CH_3CN		3.21	3.2	*l*
QNADH	MAI	30	CH_3CN		2.45	3–5	*l*
PrNADH	*p*-ClTFA	50	CH_3CN	2.0 ± 0.2		7.6 ± 0.3	*m*
PrNADH	*p*-BrTFA	50	CH_3CN	2.28 ± 0.41 (3.23 ± 0.29)*		3.6 (3.1)	*n*
PrNADH	*m*-FTFA	50	CH_3CN	2.47 ± 0.49 (2.68 ± 0.35)*		3.0 (3.5)	*n*
PrNADH	*m*-BrTFA	50	CH_3CN	2.80 ± 0.31 (2.91 ± 0.60)*		3.7 (3.4)	*n*
PrNADH	*m*-CF_3TFA	50	CH_3CN	3.01 ± 0.07 (2.84 ± 0.12)*		3.0 (3.0)	*n*
PrNADH	*m*-NO_2TFA	50	CH_3CN	3.21 ± 0.62 (3.46 ± 0.27)*		3.4 (3.5)	*n*
PrNADH	*m*-CF_3/*m*-NO_2TFA	50	CH_3CN	3.17 ± 0.49 (3.23 ± 0.29)*		3.4 (3.2)	*n*
PrNADH	3,5-di-NO_2TFA	50	CH_3CN	2.94 ± 0.94 (2.93 ± 0.58)*		3.4 (3.0)	*n*
p-CH_3OPhNADH	MAI	50	CH_3CN	(3.03 ± 0.93)*		(3.9)	*n*
p-CH_3PhNADH	MAI	50	CH_3CN	4.74 ± 0.31*		5.9	*o*
PhNADH	MAI	50	CH_3CN	5.73 ± 0.40*		5.9	*o*
p-BrNADH	MAI	50	CH_3CN	4.52 ± 0.38*		5.7	*o*
m-BrNADH	MAI	50	CH_3CN	4.31 ± 0.40*		6.1	*o*
m-CF_3NADH	MAI	50	CH_3CN	3.45 ± 0.28		5.9	*o*
BzNADH	MAI	20	CH_3CN	3.30 ± 0.39		6.2	*o*
BzNADH	PhMAI	20	CH_3CN	4.8 ± 1.4	4.6 ± 0.3	4.5 ± 0.3	*p*
BzNADH	PhMAI	20	90% CH_3CN/H_2O	4.4 ± 1.3	4.47 ± 0.3	4.5 ± 0.3	*p*
				3.68 ± 1.0	4.15 ± 0.3	4.7 ± 0.5	*p*

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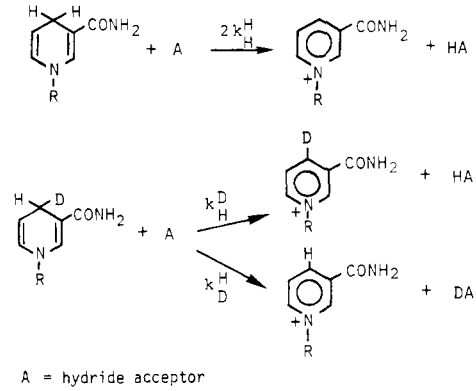
^{1}H,4-^{2}H]dihydropyridines. Determination of the kinetic isotope effect from the H,H and H,D compounds (eq 3 and 4) assumes



$$k_H/k_D = \frac{k_H{}^H}{k_{HD} - k_H{}^H} \quad (5)$$

$$k_{HD} = k_H{}^D + k_D{}^H \quad (6)$$

a secondary isotope effect of unity ($k_H{}^D \simeq k_H{}^H$) for these reactions (eq 5 and 6). We show herein that neglect of secondary kinetic isotope effects may indeed cause isotope effect discrepancies as large as experimentally observed. This problem does not arise when the kinetic isotope ratio is determined by comparison of rate constants for reductions by the diprotio- and dideuterionicotinamides in dry CH_3CN. On transfer from CH_3CN to H_2O solvent there seems to be a difference in k_{HH}/k_{DD} and Y_H/Y_D. For example, reaction of H,H-, H,D- and D,D-PrNADH with *N*-methylacridinium ion (MAI) in phosphate buffer solutions gave $k_{HH}/k_{DD} = 1.46 \pm 0.10$ and $Y_H/Y_D = 5.4 \pm 1.0$.[3] Similarly, reaction of H,H-, H,D- and D,D-BzNADH with TFA in aqueous 2-propanol gave $k_{HH}/k_{DD} = 2.19 \pm 0.11$ and $Y_H/Y_D = 6.6 \pm 1.7$.[7] This is strong evidence in support of Chipman and van Eikeren's proposal that in H_2O solvent hydration and/or adduct formation cause isotope effect discrepancies.

In this paper, we describe our investigation of the kinetic isotope effects for reaction of several NADH model compounds studied previously. Our studies were carried out using the diprotio- and dideuterio-NADH analogues in order to remove any errors caused by neglect of secondary isotope effects. Where also possible, we have taken isotope scrambling into account and by doing so have failed to find *even a single discrepancy* between kinetic and product isotope effects for such NADH model reactions. In addition, we have measured the temperature-dependent isotope effects for an exothermic redox reaction and have found evidence for a moderate amount of quantum-mechanical tunneling. This observation implies that the semiclassical isotope effects (i.e., those due to changes in zero-point energies in reactants and transition state) are much smaller than originally thought. The structure of the transition state for such a hydride equivalent transfer is proposed, based on these reduced isotope effects.

Experimental Section

Materials. Acetonitrile was obtained from Burdick and Jackson Laboratories and was thoroughly deoxygenated before use. Acetic acid and acetic acid-d_4 were distilled under N_2. Doubly distilled deionized water was used for preparation of reaction solutions. *N*-Benzyl-1,4-dihydronicotinamide[12] (BzNADH), *N*-propyl-1,4-dihydronicotinamide (PrNADH), 3-carbamoyl-*N*-benzyl-1,4-dihydroquinoline[13] (QNADH), *N*-phenyl-1,4-dihydronicotinamide[10,14,15] (PhNADH), (*p*-methoxyphenyl)-1,4-dihydronicotinamide (MeOPhNADH), (*p*-methylphenyl)-1,4-dihydronicotinamide (MePhNADH), [*m*-(trifluoromethyl)phenyl]-1,4-dihydronicotinamide (CF_3PhNADH), (*p*-cyanophenyl)-1,4-dihydronicotinamide (CNPhNADH), *N*-methylacridinium iodide[16] (MAI),

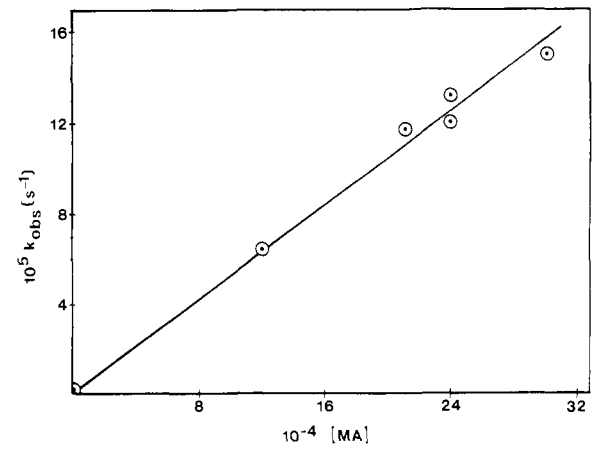


Figure 1. Dependence of k_{obsd} (s^{-1}) on [MA] for reaction of labeled *N*-methylacridinium ion with an excess of *N*-methylacridan in CH_3CN at 30 °C. The least-squares lines has a slope of 0.051 ± 0.002 M^{-1} s^{-1}.

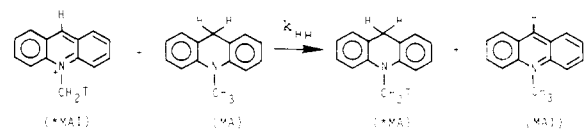
N-methylacridan (MA), and trifluoroacetophenone (TFA) were prepared or purified according to the literature. 4,4-Deuterated analogues of BzNADH, PrNADH, QNADH, MeOPhNADH, MePhNADH, CF_3PhNADH, and CNPhNADH were prepared either by (i) cyanide addition with concomitant isotope exchange in D_2O/dioxane at pD = 11 followed by dithionite reduction in D_2O or by (ii) cyclic (4×) oxidation (I_2 or MAI)–reduction ($Na_2S_2O_4/D_2O$). Tritium labeling of MAI was effected by $NaBH_3T$ reduction or by methylation using ^{3}H-labeled methyl iodide. [4-^{3}H,4-^{1}H]-*N*-propyl-1,4-dihydronicotinamide was prepared by dithionite reduction in tritiated water (~10^{-1} Ci/mL). Deuterium isotope purity was assessed by mass spectral and NMR analysis in which all compounds were >98% isotopically pure. The nicotinamide iodide salts (QNAD, MeOPhNAD, MePhNAD, PhNAD, CF_3PhNAD, and CNPhNAD) used for equilibrium measurements were prepared either by iodine oxidation[13] of the dihydronicotinamides or by anion exchange of the chloride salts using NaI in H_2O.

Kinetic measurements were made at ~2 to 50 °C (temperatures were checked against an NBS-calibrated thermometer) under strict anaerobic conditions in dry (<0.010% H_2O) CH_3CN. Slower runs were carried out in Thunberg cuvettes by using a Cary 118C spectrophotometer. In a typical experiment, 0.5 mL of a solution 2.1×10^{-4} M in MAI was placed in the upper bulb and 3 mL of a solution of dihydronicotinamide [(2–90) $\times 10^{-4}$ M] was placed in the lower bulb. After the cuvettes were temperature equilibrated, the contents were mixed and the disappearance of MAI was observed spectrally with time at λ = 415 nm. For the more rapid reactions, a Durham Gibson Model 13001 stopped-flow spectrophotometer was used. All solutions used in the stopped-flow experiments were prepared under dry N_2 in the same glovebox that housed the stopped-flow instrument.

In all experiments, the concentration of dihydronicotinamide was maintained significantly higher than the concentration of MAI (~3×10^{-5} M). First-order plots for reaction of MAI with the dihydronicotinamides were linear for more than six half-lives and rate constants were calculated from the best fits of these plots. In the case where deuterium-labeled dihydronicotinamide was used, correction for isotope purity was made and was found to affect the kinetic isotope effects by only a few percent.

Initial rates of reaction of H,H- and D,D-PrNADH (1.1×10^{-4} M) with TFA (0–0.05 M) were obtained by following the decrease in [PrNADH] at λ = 350 nm. In separate experiments, $[H,H\text{-PrNADH}]_0 = [D,D\text{-PrNADH}]_0$ such that the ratio of the least-squares slopes of Δ[PrNADH]/Δ time vs. [TFA] for H,H- and D,D-PrNADH is the primary kinetic isotope effect for this reaction.

The rate of reaction of MA with MAI was measured by tritium isotope labeling. In most experiments a small amount ([*MAI] ~ 10^{-12} M) of *MAI was added to a temperature-equilibrated solution of MA ([MA] ≫ [MAI]) in CH_3CN (eq 7). Sample aliquots were removed



at known time intervals and quenched into 8 mL of toluene. The toluene

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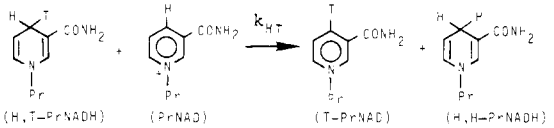
Table II. Rates and Kinetic Isotope Effects for NADH Model Reactions in Acetonitrile

reductant	oxidant	temp, °C	k_{LL}, M^{-1} s^{-1} [a]	k_H/k_D [b]	Y_H/Y_D [b]
H,H-BzNADH	MAI	30	79.9 ± 0.9		
H,D-BzNADH	MAI	30		[2.76 ± 0.15]	[4.0 ± 0.2]
D,D-BzNADH	MAI	30	19.5 ± 0.1	4.11 ± 0.05[c]	
H,H-QNADH	MAI	30	0.762 ± 0.013		
H,D-QNADH	MAI	30	0.447 ± 0.07	4.95	[7.6 ± 0.3]
D,D-QNADH	MAI	30	0.149 ± 0.003	5.09 ± 0.15[c]	
H,H-MeOPhNADH	MAI	50	35.16 ± 1.07		
H,D-MeOPhNADH	MAI	50	21.51 ± 0.54	3.83	[5.9]
D,D-MeOPhNADH	MAI	50	8.84 ± 0.08	3.98 ± 0.13[c]	
H,H-MePhNADH	MAI	50	26.28 ± 0.52		[5.9]
D,D-MePhNADH	MAI	50	6.56 ± 0.64	4.01 ± 0.08[c]	
H,H-CF_3PhNADH	MAI	50	2.903 ± 0.019		[6.2]
D,D-CF_3PhNADH	MAI	50	0.655 ± 0.012	4.43 ± 0.08[c]	
H,H-CNPhNADH	MAI	50	0.727 ± 0.005	4.77 ± 0.06[c]	[6.0]
D,D-CNPhNADH	MAI	50	0.152 ± 0.005		
H,H-MA	MAI	30	0.051 ± 0.002		
H,H-MA	MAI	50	0.103 ± 0.005		
H,T-PrNADH[d]	PrNAD	50	$(5.57 \pm 0.10) \times 10^{-3}$		
H,H-PrNADH	TFA	50	$(4.57 \pm 0.58) \times 10^{-5}$	2.75 ± 0.39[e]	
D,D-PrNADH	TFA	50	$(1.66 \pm 0.11) \times 10^{-5}$		
MA	PhNAD	50	$\sim 1.1 \times 10^{-4}$ [f]		
MA	MeOPhNAD	50	$\sim 4.3 \times 10^{-5}$ [f]		
MA	MePhNAD	50	$\sim 1.2 \times 10^{-4}$ [f]		
MA	CF_3PhNAD	50	$\sim 1.9 \times 10^{-4}$ [f]		
MA	CNPhNAD	50	$\sim 3.0 \times 10^{-4}$ [f]		
MA	QNAD	30	$\sim 3.3 \times 10^{-4}$ [f]		

[a] L = H, D, or T. [b] Values shown in brackets are from the literature. [c] Includes a small secondary kinetic isotope effect. [d] Rate constant for reaction of *N*-propyl-1,4-[4-^{1}H,4-^{3}H]dihydronicotinamide with *N*-propylnicotinamide. [e] Obtained from initial rates. [f] From reaction scheme simulation (see the text).

layer was extracted 3× with distilled water to remove the CH_3CN and MAI before the organic layer was dried (anhydrous Na_2SO_4) and 5 mL counted with 10 mL of PPO/POPOP toluene-base "scintillation cocktail".[17] The sample radioactivity increased [or decreased in the experiments where labeled MA was added to a solution of MAI ([MAI] ≫ [MA])] smoothly over 3–8 half-lives and the observed rates were linearly proportional to the concentration of the catalyst (Figure 1). Studies of the reaction of tritium-labeled PrNADH with PrNAD (eq 8)



(8)

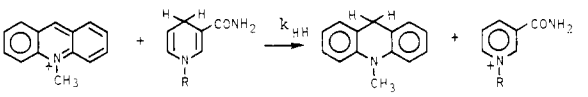
had to be modified since PrNADH was easily washed-out during the aqueous extractions due to its relatively high solubility in H_2O. To circumvent this difficulty, a single 50 mL of NaCl (saturated)/H_2O extraction of toluene was done; this served to remove all traces of PrNAD without extracting significant amounts of PrNADH. The radioactivity of the organic phase decreased smoothly from 10^4 cpm at $t = 0$ to 5×10^1 cpm at $t = \infty$.

^{19}F NMR spectra were obtained on a Nicolet 280-MHz spectrometer. In these instances, reaction of PrNADH and TFA in CD_3CN was followed by recording the ^{19}F NMR spectra of a solution equimolar in PrNADH and TFA (0.02 M) at 50 °C under N_2. Spectra were recorded over the course of several weeks whereby the singlet due to TFA decreased in intensity as other peaks (one doublet, one singlet) formed. Although the CD_3CN contained some water (~0.02%) precautions were taken to minimize further contamination.

Reaction scheme simulations were performed on a department-built, microprocessor-based digital computer. Further calculations were performed on a Hewlett-Packard 9825A desk-top calculator attached to Hewlett-Packard Model 9864A digitizer and Model 9867A plotter. Tunneling calculations were done by using a program employing the truncated Bell equation;[18] a complete description of the program has been given by Saunders et al.[19]

Results and Discussion

Rates of reaction of MAI with dihydronicotinamides were measured spectrophotometrically in anhydrous acetonitrile at 30 °C under strictly anaerobic conditions by monitoring the decrease in [MAI] at A_{415}. Spectral changes monitored from λ = 200 nm to λ = 500 nm were consistent with the formation of MA and nicotinamide (eq 9). The disappearance of MAI in the presence



(9)

of a large excess of dihydronicotinamide followed the first-order rate law to completion of reaction. Plots of the pseudo-first-order rate constants (k_{obsd}) vs. [dihydronicotinamide] were found to be linear and possess zero intercepts on the k_{obsd} axis. The second-order rate constants in Table II were obtained from least-squares analysis of plots of k_{obsd} vs. [dihydronicotinamide]. Charge-transfer-type complex formation between acridinium ion and 1,4-dihydropyridines could cause some error in the reported second-order rate constants if the equilibrium constant favoring complex formation is large, irrespective of whether or not the complex lies on the reaction coordinate.[20] Complex association constants for these and similar systems have, however, been measured and are too small to make a significant change in k.[21] In the case of the extremely endergonic reactions of MA and the substituted nicotinamides, QNAD, MeOPhNAD, MePhNAD, PhNAD, CF_3PhNAD, and CNPhNAD, the approximate reverse rate constants (k_{-1}) were obtained by reaction scheme simulation. For these experiments equal concentrations (2.88×10^{-3} M) of MA and the substituted nicotinamides were allowed to react anaerobically in Thunberg cuvettes; the extent of reaction was followed by monitoring the increase in [MAI] at λ = 415 nm where the absorption due to the other reaction species is negligible. The A_{415} trace was then simulated by using eq 10; the best-fit values for k_{-1} are listed in Table II.

$$\text{MAI} + \text{X-PhNADH} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{MA} + \text{X-PhNAD} \qquad (10)$$

(17) Evans, E. A. "Tritium and its Compounds"; Wiley: New York, 1974.
(18) Bell, R. P. "The Tunnel Effect in Chemistry"; Chapman and Hall: London, 1980.
(19) Kaldor, S. B.; Saunders, Jr., W. H. *J. Am. Chem. Soc.* **1979**, *101*, 7594–7599.
(20) Rappoport, Z.; Horowitz, A. *J. Chem. Soc.* **1964**, 1348–1359.
(21) van Gerresheim, W.; Kruk, C.; Verhoeven, J. W. *Tetrahedron Lett.* **1982**, *23*, 565–568.

$[MA]_0 = [X\text{-}PhNADH]_0 = 2.88 \times 10^{-3}$ M, $[MAI]_0 = [PhNAD]_0 = 0$, and k_1 = appropriate values of k_{HH} from Table II.

The values of k_{HH} reported herein for reaction of MAI with the H,H-dihydronicotinamides agree well with those in the literature except for reaction of MAI and *p*-cyano-1,4-dihydronicotinamide (CNPhNADH) in that our value of 0.727 ± 0.005 M^{-1} s^{-1} is ~25% less than the value reported earlier by Ohno et al.[10] In our hands, we found that CNPhNADH is very photosensitive; for example, irradiation of a solution of CNPhNADH in CH_3CN by a 100-W incandescent light source at 5-cm distance for only 30 s changed the CNPhNADH UV–vis absorption spectrum appreciably. To circumvent this difficulty, reaction solutions were prepared in the dark and reaction rates were measured by using a very narrow slit width in order to minimize any possible photoreactions.

Another discrepancy between our determined values of k_{HH} and those reported in the literature arose in the reaction of QNADH with MAI in CH_3CN wherein our constants are significantly less than those reported by Shinkai et al.[9] We attribute this discrepancy to traces of residual *N*-benzyldihydronicotinamide used in their preparation of QNADH. We have found that H,H-QNADH prepared by reduction of QNAD with *N*-benzyldihydronicotinamide contains traces of reductant, even after repeated recrystallization from ethanol–water mixtures. Support for such contamination in the previous study comes from the length of time required to effect "100%" reaction in the product studies;[9] when their rate constants were used, the reaction should have been completed in approximately 300 s instead of the 2 h they observed. Under the pseudo-first-order conditions of [QNADH] ≫ [MAI] a small contamination of the more reactive dihydronicotinamide in the QNADH will lead to incorrectly large values of k_{obsd}. We have circumvented this problem by preparing the dihydroquinoline via dithionite reduction,[13] which does not afford any unwanted dihydroquinoline isomers or more highly reductive species.

Kinetic isotope effects determined from the ratio of rates of the $[^1H_2]$- and $[^2H_2]$dihydronicotinamides demonstrated little or no discrepancy between k_{HH}/k_{DD} and Y_H/Y_D when the possibility of isotope scrambling in the product studies was accounted for. For example, the highly exothermic reaction of MAI and BzNADH (for which isotope scrambling by possible back reaction is unlikely under the experimental conditions used) gave $k_{HH}/k_{DD} = 4.11 \pm 0.05$, comparable with $Y_H/Y_D = 4.0 \pm 0.2$. Kinetic isotope effects determined from the ratio of rates of the $[^1H_2]$- and $[^2H_2]$dihydronicotinamides (k_{HH}/k_{DD}) have secondary isotope effects ($k_{HH}/k_{DD} = 2k_H{}^H/2k_D{}^D$) incorporated into the reported values. This is expected to modify the "true" value of the primary kinetic isotope effect by less than ~10%. We attribute the differences in k_H/k_D and Y_H/Y_D in earlier studies to the presence of water and/or oxygen in the reaction solutions used or to the extreme sensitivity of k_H/k_D to the value of the secondary kinetic isotope effect when determined by using $[^1H,^2H]$dihydronicotinamide. The dependence of the calculated primary kinetic isotope effect, k_H/k_D, on the secondary isotope effect, k^H/k^D, can be demonstrated by use of eq 5 and 6. For this purpose a primary isotope effect of $k_H{}^H/k_D{}^H = 4$ was chosen and the secondary isotope effect was allowed to vary. The results are shown in Figure 2. If a secondary effect of unity is assumed, then the calculated primary kinetic isotope effect is equal to the "true" primary kinetic isotope effect chosen as 4. However, if the range of the secondary kinetic isotope effect is varied from 0.9 to 1.15 (there is ample evidence in the literature for secondary kinetic isotope effects in this range for systems of this type), then the calculated k_H/k_D ranges from 2.78 to 8.36, far outside acceptable error limits for ~10% uncertainty in secondary kinetic isotope effect. This large error in the calculated (eq 5) k_H/k_D is due to the error in k_D calculated from the expression $k_{HD} \cong k_H{}^D + k_D{}^H$ (eq 6). When k_H/k_D is large, $k_{HD} \cong k_H$ and thus k_D cannot be known with great certainty, especially if $k_H \cong k_H{}^D$.

The secondary kinetic isotope effects for two NADH model redox reactions (MAI + QNADH, MAI + MeOPhNADH) have been determined. We find these to be normal. The following determination of k_H/k_D and k^H/k^D from the rates of reaction of the $[^1H_2]$, $[^1H,^2H]$-, and $[^2H_2]$dihydronicotinamides neglects the breakdown of the rule of the geometric mean;[22] this simplification may introduce a small error in the results. For our analysis, the relationship between the kinetic and secondary isotope effects (SKIE) for reaction of $[^1H_2]$- and $[^2H_2]$dihydronicotinamide $[k_H{}^H/k_D{}^D = k_H{}^H/(k_D{}^H/\text{SKIE})]$ and the expression for reaction of $[^1H,^2H]$dihydronicotinamide $[k_{HD} = k_H{}^D + k_D{}^H]$ were combined to solve for the secondary kinetic isotope effect (eq 11).

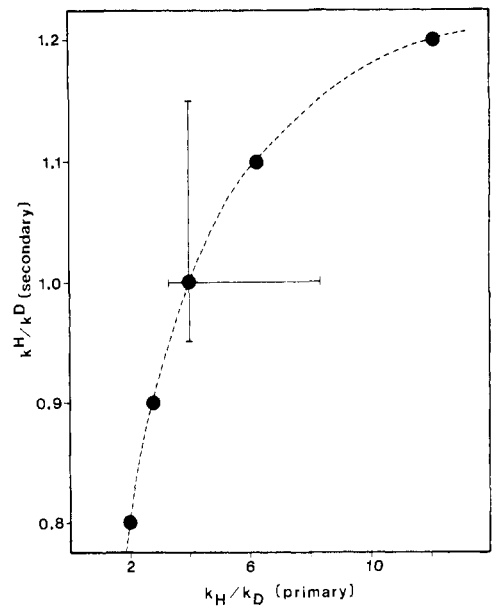


Figure 2. Dependence of the observed primary kinetic isotope effect (k_H/k_D) on the secondary kinetic isotope effect (k^H/k^D) for a true k_H/k_D of 4.0. The horizontal error bar represents a realistic range of k^H/k^D expected for transhydrogenation reaction ($sp_3 \rightarrow sp_2$); the vertical error bar shows the variation in the observed k_H/k_D when a secondary kinetic isotope effect of unity is assumed. The plot shows that unusually large (or small) kinetic isotope effects may be observed when the secondary kinetic isotope effect is neglected.

$$\text{SKIE} = \frac{k_{HD} - [(k_{HD})^2 - k_{HH}k_{DD}]^{1/2}}{k_{DD}} \qquad (11)$$

Reaction of H,H-, H,D-, and D,D-QNADH with MAI gave a secondary kinetic isotope effect of 1.03 ± 0.03; neglect of this secondary isotope effect raises k_H/k_D from 4.95 ± 0.17 to 5.77. MAI reacts with H,H-, H,D-, and D,D-MeOPhNADH to afford a secondary isotope effect of 1.04 ± 0.06 and a k_H/k_D of 3.83 ± 0.18; neglect of the secondary effect gives $k_H/k_D = 4.47$. Thus, even a 3–4% secondary isotope effect changes the calculated primary isotope effect k_H/k_D by ~15–20%. It has also been recently pointed out that an uncertainty in the isotopic composition of the H,D compounds has a profound effect on k_H/k_D and Y_H/Y_D.[23] If $[^1H,^2H]$dihydronicotinamide contains even a few percent of the H,H analogue, the observed product isotope effects are increased and the kinetic isotope effects significantly decreased. This is the type of mismatch in product and kinetic isotope effects reported in the past (Table I).

Isotope scrambling in the product studies for reaction of MAI with QNADH[9] results in a discrepancy between k_H/k_D (2.0 ± 0.2) and Y_H/Y_D (7.6 ± 0.3), even when our value of $k_{HH}/k_{DD} = 5.09 \pm 0.15$ for this reaction is considered. The rate of H^- transfer from MA to MAI was measured by ^3H labeling of MAI and found to be kinetically significant under the reaction conditions employed. This unaccounted-for side reaction will give anomalously large Y_H/Y_D because reaction of H,D-MA product (decreasing Y_D) with remaining MAI reactant gives additional

(22) Bigeleisen, J. *J. Chem. Phys.* **1955**, *23*, 2264–2267. Bigeleisen, J. *Ibid.* **1958**, *28*, 694–699. Bigeleisen, J.; Ishida, T. *Ibid.* **1968**, *48*, 1311–1330.
(23) van Laar, A.; van Ramesdonk, H. J.; Verhoeven, J. W. *Recl. Trav. Chim. Pays-Bas* **1983**, *102*, in press.

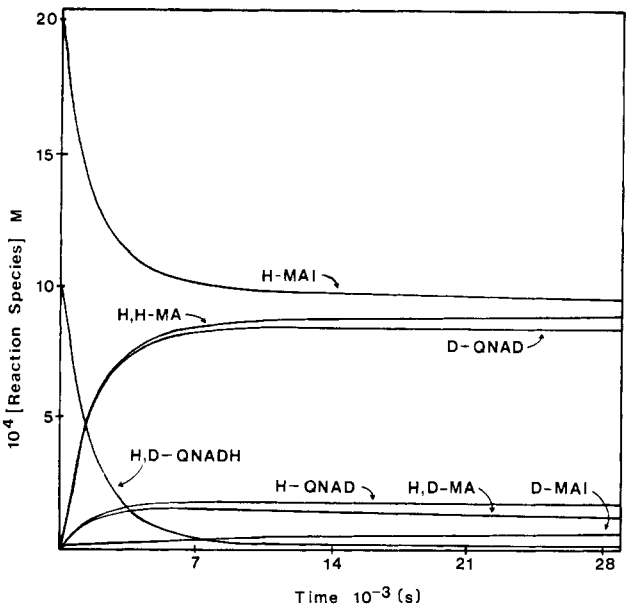


Figure 3. Reaction scheme simulation of the product isotope effect (Y_H/Y_D) for reaction of 3-carbamoyl-*N*-benzyl-1,4-dihydroquinoline (QNADH) and *N*-methylacridinium ion (MAI) in CH_3CN at 30 °C. Y_H/Y_D was determined from the ratio of [H,H-MA]/[H,D-MA] from Scheme I. The plot shows that [H,H-MA]/[H,D-MA] changes during the time course of the reaction due to isotope scrambling.

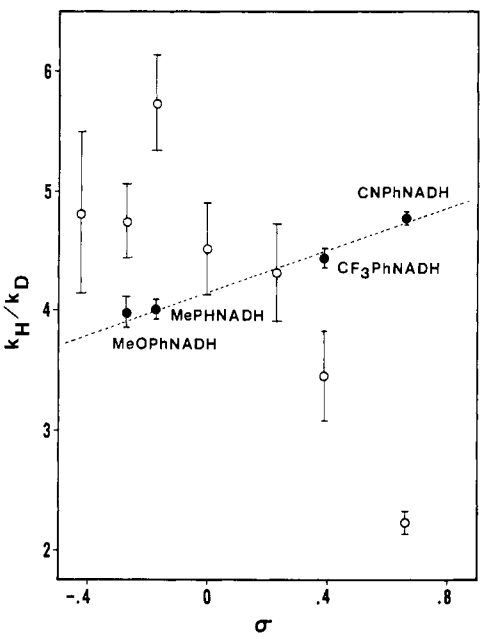


Figure 4. Variation of the kinetic isotope effect determined from the rate ratios of the [$^1H_2$]- and [$^2H_2$]dihydronicotinamides (○) (data from Ohno et al., ref 10) and the [$^1H_2$]- and [$^2H_2$]dihydronicotinamides (●) with the substituent effect (σ) for reaction of MAI + X-PhNADH in CH_3CN at 50 °C. The error bars indicate the standard deviations about each k_H/k_D and were calculated from the square root of the sum of the squares of the errors. The plot shows that the large change in k_H/k_D reported by Ohno et al.[10] is erroneous and that the isotope effects increase with an increase in electronegativity of the substituents.

H,H-MA (increasing Y_H). This is demonstrated by solving Scheme I by computer simulation. By use of the rate constants

Scheme I

$$\text{H,D-QNADH} + \text{H-MAI} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{D-QNAD} + \text{H,H-MA} \quad (12)$$

$$\text{H,D-QNADH} + \text{H-MAI} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \text{H-QNAD} + \text{H,D-MA} \quad (13)$$

$$\text{H,D-MA} + \text{H-MAI} \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} \text{H,H-MA} + \text{D-MAI} \quad (14)$$

$k_1 = 0.37\ M^{-1}\ s^{-1}$, $k_{-1} = k_{-2} = 0$, $k_2 = 0.074\ M^{-1}\ s^{-1}$, $k_3 = 0.025\ M^{-1}\ s^{-1}$, and $k_{-3} = 0.051\ M^{-1}\ s^{-1}$ and initial concentrations $[H,D\text{-QNADH}]_0 = 0.001$ and $[MAI]_0 = 0.002$ M, the concentrations of the reaction species are calculated as shown in Figure 3. The value of k_1 comes from half the rate of reaction of H,H-QNADH + H-MAI by assuming a secondary isotope effect of unity. The other rate constants were derived similarly from the appropriate rate constants given in Table II. Incorporation of other less important isotope scrambling steps or possible back-reactions into the reaction scheme do not change the H,H-MA to H,D-MA ratio appreciably in the time span considered.

The kinetic isotope effects, k_{HH}/k_{DD}, for reaction of MAI with *N*-phenyl-substituted 1,4-dihydronicotinamides are in the 4–5 range in contrast to the varied (2–6 range) values reported earlier[10] obtained from the ratio of rates of the [$^1H_2$]- and [$^1H,^2H$]dihydronicotinamides. Plotted in Figure 4 are values of k_H/k_D vs. the σ value of the substituent X in XPhNADH. The points marked as open circles are from ref 10 with error bars calculated from provided deviations. The points marked with closed circles are from the data reported herein and have substantially smaller errors. Ohno proposed[10] that the dramatic increase and decrease of k_H/k_D (open circles) with a change in the substituent X was proof for a multistep mechanism in that X = *p*-CH_3 (k_H/k_D = 5.72) should result in stabilization of the nicotinamide radical cation[24,25] formed upon one-electron transfer, resulting in a fully rate-determining H-transfer step. Substituents (i.e., CN) that destabilize the radical cation were proposed to raise the ΔG^* for initial one-electron transfer such that H transfer would no longer be fully rate-determining, resulting in decreased k_H/k_D's. Isotope effects determined herein from the [$^1H_2$]- and [$^2H_2$]dihydronicotinamides clearly show the reported maximum in k_H/k_D is erroneous. We attribute this earlier error in the determination of k_H/k_D to the aforementioned sensitivity of the secondary isotope effect and to the previously unmentioned photoreactivity of CNPhNADH which causes kinetic complications. The increase in primary kinetic isotope effect with a decrease in reaction rate is predicted by the Hammond postulate inasmuch as the less exothermic reaction should have the more symmetrically located transition state and, hence, show larger isotope effects based upon maximal loss of zero-point energy from the C–H stretching vibration. The large variation in k_H/k_D accompanying the change of substituents on the Ph moiety claimed earlier[10] is unexpected, especially when one considers (a) only a single substituent is being varied some eight atoms from the reaction center and (b) the large intrinsic barriers associated with H transfer from carbon should manifest themselves in only subtle changes in k_H/k_D.[26] Furthermore, the lack of variation in Y_H/Y_D for reaction of *N*-phenyl-substituted dihydronicotinamides with MAI cannot be explained when the reversible nature of these redox reactions is accounted for. The kinetically significant back-reactions cause isotope scrambling on the time scale employed in the product studies,[10] which reduces the observed Y_H/Y_D. We have demonstrated this by use of Scheme II and $k_1 = k_5 = 1.45\ M^{-1}\ s^{-1}$, $k_2 = k_8 = 0.327\ M^{-1}\ s^{-1}$, $k_3 = k_4 = 2.90\ M^{-1}\ s^{-1}$, $k_6 = k_7 = 0.655\ M^{-1}\ s^{-1}$, $k_{-1} = k_{-4} = 1.90 \times 10^{-4}\ M^{-1}\ s^{-1}$, $k_{-2} = k_{-6} = 2.14 \times 10^{-5}\ M^{-1}\ s^{-1}$, $k_{-3} = k_{-5} = 9.50 \times 10^{-5}\ M^{-1}\ s^{-1}$, $k_{-7} = k_{-8} = 4.29 \times 10^{-5}\ M^{-1}\ s^{-1}$, $[H\text{-MAI}]_0 = [H,D\text{-CF}_3\text{PhNADH}]_0 = 0.2$ M (these were the initial concentrations used in the product studies of ref 10), and all other initial concentrations were set to zero. The rate constants k_4, k_{-4}, and k_6 are those reported in Table II for these reactions; all others were obtained from a statistical correction of the measured rates with the assumption that all secondary kinetic and equilibrium isotope effects are unity. For example,

(24) Nelson, R. F.; Adams, R. N. *J. Am. Chem. Soc.* **1968**, *90*, 3925–3930.
(25) Nelsen, S. F.; Landis, R. T.; Kiehle, L. H.; Leung, T. H. *J. Am. Chem. Soc.* **1972**, *94*, 1610–1614.
(26) Marcus, R. A. *J. Phys. Chem.* **1968**, *72*, 891–899; *Symp. Faraday Soc.* **1975**, *10*, 60–68.

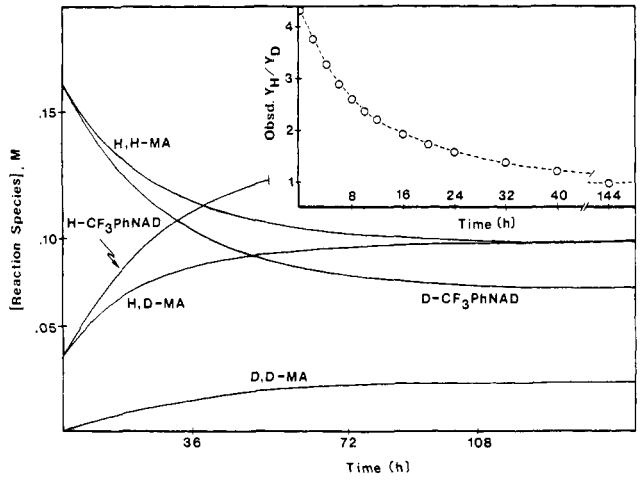


Figure 5. Reaction scheme simulation of the product isotope effect (Y_H/Y_D) for reaction of [*m*-(trifluoromethyl)phenyl]-1,4-[^{1}H,^{2}H]dihydronicotinamide (H,D-CF_3PhNADH) + MAI in CH_3CN at 50 °C. The observed Y_H/Y_D (=[H,H-MA]/[H,D-MA] decreases from its initial value of 4.43 (the value of the kinetic isotope effect) to its equilibrium value of 1 (see the inset) over the time of the product studies[10] when the reverse rates (k_{-1}–k_{-8}) are included.

calculation of the rate constant for H transfer from H,D-CF_3PhNADH to D-MAI (k_5 in Scheme II) neglects the secondary isotope effects caused by epi D substitution at C(4) in H,D-CF_3PhNADH and C(9) D substitution in MAI so that k_5 is taken as $k_4/2$. This scheme does not account for reaction of either MAI with MA or CF_3PhNADH with CF_3PhNAD, both of which only serve to further scramble the D isotope.

Scheme II

$$\text{H,D-CF}_3\text{PhNADH} + \text{H-MAI} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{D-CF}_3\text{PhNAD} + \text{H,H-MA} \quad (15)$$

$$\text{H,D-CF}_3\text{PhNADH} + \text{H-MAI} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \text{H-CF}_3\text{PhNAD} + \text{H,D-MA} \quad (16)$$

$$\text{H,H-CF}_3\text{PhNADH} + \text{D-MAI} \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} \text{H-CF}_3\text{PhNAD} + \text{H,D-MA} \quad (17)$$

$$\text{H,H-CF}_3\text{PhNADH} + \text{H-MAI} \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} \text{H-CF}_3\text{PhNAD} + \text{H,H-MA} \quad (18)$$

$$\text{H,D-CF}_3\text{PhNADH} + \text{D-MAI} \underset{k_{-5}}{\overset{k_5}{\rightleftharpoons}} \text{D-CF}_3\text{PhNAD} + \text{H,D-MA} \quad (19)$$

$$\text{D,D-CF}_3\text{PhNADH} + \text{H-MAI} \underset{k_{-6}}{\overset{k_6}{\rightleftharpoons}} \text{D-CF}_3\text{PhNAD} + \text{H,H-MA} \quad (20)$$

$$\text{D,D-CF}_3\text{PhNADH} + \text{D-MAI} \underset{k_{-7}}{\overset{k_7}{\rightleftharpoons}} \text{D-CF}_3\text{PhNAD} + \text{D,D-MA} \quad (21)$$

$$\text{H,D-CF}_3\text{PhNADH} + \text{D-MAI} \underset{k_{-8}}{\overset{k_8}{\rightleftharpoons}} \text{H-CF}_3\text{PhNAD} + \text{D,D-MA} \quad (22)$$

Reaction scheme simulation of Scheme II (Figure 5) shows that the ratio of concentrations of H,H-MA to H,D-MA varies with time and that after 24 h the value of [H,H-MA]/[H,D-MA] decreased from 4.43 (the value of the kinetic isotope effect) to 1.60. Values of Y_H/Y_D (i.e., ~6) larger than k_H/k_D are inconsistent with such a scheme unless the "true" values of Y_H/Y_D are unusually large, i.e., >30. From this scheme we are able to show that after several days under the reaction conditions employed, the deuterium has effectively scrambled throughout the products such that $[H,H\text{-MA}]_\infty = [H,D\text{-MA}]_\infty = 0.088$ M, $[D,D\text{-MA}]_\infty = 0.022$ M, and $[H\text{-CF}_3\text{PhNADH}]_\infty = 2[D\text{-CF}_3\text{PhNADH}]_\infty = 0.132$ M. Similar results were obtained by reaction scheme simulation of the other phenyl-substituted dihydronicotinamides with MAI. Possible explanations for the previously observed large product isotope effects may be due to either 1,2-dihydronicotinamide formation followed by reoxidation and isotope exchange at the 2-position with traces of water in the acetonitrile solvent or difficulty in obtaining accurate results by electron impact mass spectroscopy as was observed earlier.[23]

Adduct formation between PrNADH and TFA in relatively dry acetonitrile was thought[27] not to occur (as it does in wholly aqueous media[3,7]) in support of the small kinetic isotope effects observed for this redox reaction. Ohno et al. proposed[27] that the presence of electron-withdrawing substituents on the phenyl moiety of TFA should destabilize radical intermediates and make H^- transfer fully rate determining whereas e^- transfer to TFA should be partly rate limiting, resulting in small observed isotope effects. We have studied the reaction between PrNADH and TFA by ^{19}F NMR and have found the PrNADH–TFA adduct (eq 1) is readily formed when even traces of H_2O are present. Over the course of several weeks the NMR signal due to TFA (reference, 0 ppm/CD_3CN) decreased in intensity and a doublet at −6.51 ppm (J = 7 Hz) and a singlet at −12.38 ppm formed. We attribute these peaks to the fluorinated redox product and adduct, respectively (eq 1). After 2.5 h the singlet was approximately 10× the intensity of the doublet, indicating the rate of adduct formation was substantially faster than the redox reaction under the conditions employed. After 19 h the product doublet and singlet were approximately the same intensity, presumably due to the "mopping up" of free H_2O by the adduct. The intensity of the product singlet continued to increase (albeit much more slowly than for the doublet) for several weeks.

These ^{19}F NMR experiments serve to show that under the experimental conditions of the kinetic studies ($[H_2O] \gg$ [PrNADH]), adduct formation will successfully compete with the redox reaction and thus give an observed isotopic rate ratio less than the true kinetic isotope effect. Our measurements of reaction of H,H- and D,D-PrNADH with TFA (20 experiments) in CH_3CN give a rate ratio of 2.74 ± 0.40 (plot not shown), somewhat larger than that predicted by Ohno et al.[27] for reaction of H,H- and H,D-PrNADH with TFA. This value may be somewhat smaller than the true kinetic isotope effect due to adduct formation and is identical (within experimental error) with the isotope effects reported for reaction of the more electronegative trifluoroacetophenones.[10] An isotope effect of this magnitude precludes the possibility of rate-determining electron transfer from this NADH model compound to an activated carbonyl and indicates that the other kinetic isotope effects reported for reaction of substituted trifluoroacetophenones with PrNADH may be low.

Brønsted correlation of the equilibrium and rate constants for reaction of MAI and phenyl-substituted dihydronicotinamides (X-PhNADH) (eq 23) was made from the forward (k_1) and

$$\text{MAI} + \text{X-PhNADH} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{MA} + \text{X-PhNAD} \quad (23)$$

reverse (k_{-1}) rate constants given in Table II and ref 10. A plot of ln k_1 vs. ln K ($K = k_1/k_{-1}$) for the reductants MeOPhNADH, MePhNADH, PhNADH, CF_3PhNADH, and CNPhNADH gives a slope of 0.70 ± 0.06 and an intercept of −5.71 ± 0.66 whereas a plot including the points for the degenerate reactions between MAI + MA and PrNADH + PrNAD gives a slope of 0.51 ± 0.06 and an intercept of −3.57 ± 0.57 (Figure 6). The rate of reaction of PrNADH + PrNAD probably overestimates the free energy of activation at $\Delta G = 0$ (i.e., $\Delta G^{\ddagger}_0{}'$) for reaction of MAI + X-PhNADH whereas the faster rate of reaction of MAI + MA probably underestimates it somewhat. ($\Delta G^{\ddagger}_0{}'$ can be broken into two parts; the work term, w_r, which represents the work required to form the reaction complex from the separated reactants, and

(27) Ohno, A.; Yamamoto, H.; Oka, S. *J. Am. Chem. Soc.* **1981**, *103*, 2041–2045.

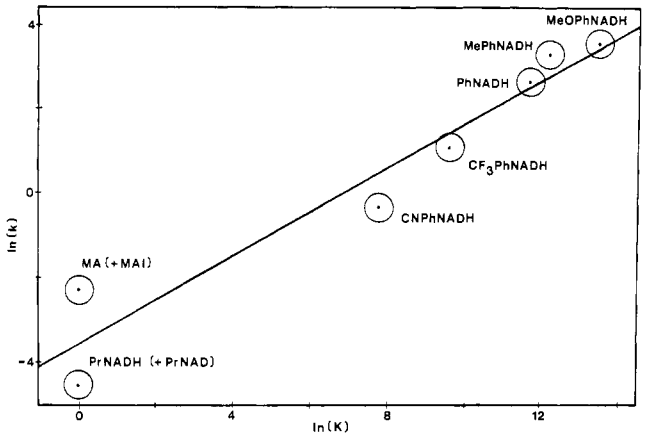
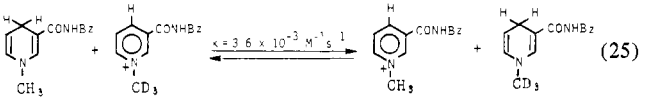


Figure 6. Brønsted plot for hydride transfer between X-PhBzNADH + MAI, PrNADH + PrNAD, and MA + MAI. The least-squares line has a slope of 0.51 ± 0.06 in agreement with a centrally located transition state on the reaction coordinate.

the intrinsic barrier, $\Delta G^{\ddagger}_0$, for the hydride transfer step. The latter is identified with the height of the reaction barrier for an isoendergonic reaction such that $\Delta G^{\ddagger}_0' = w_r + \Delta G^{\ddagger}_0$.) This is shown in Figure 6 where the point for reaction of MAI + MA lies above the least-squares line and that for reaction of PrNADH + PrNAD lies below it. This deviation in rate constants for similar degenerate reactions has been reported previously;[28] for example, reaction of MAI + MA is somewhat faster than for a substituted nicotinamide degenerate reaction measured in aqueous 2-propanol (eq 24 and 25).[28] Thus, $\Delta G^{\ddagger}_0'$ for reaction of MAI + X-PhNADH

$$CD_3\text{-MAI} + CH_3\text{-MA} \underset{}{\overset{k = 4.3 \times 10^{-2}\ M^{-1}\ s^{-1}}{\rightleftharpoons}} CD_3\text{-MA} + CH_3\text{-MAI} \quad (24)$$



(25)

is probably somewhat between the free energies of activation for reaction of MA + MAI and PrNADH + PrNAD, and so the β of 0.51 is reasonably accurate. This value of $\beta \sim 0.5$ is in agreement with theory when $\ln K = 1$; larger values of $\ln K$ should reduce β slightly depending on $\Delta G^{\ddagger}_0$.

The Brønsted β value has often been used to assess the degree of H transfer in the transition state. Our value of 0.51 is in accord with a symmetrical transition state in which H is centrally located between donor and acceptor. This is supported by the size of the isotope effects and their increase with electron withdrawal (as ΔG_0 decreases), which may be taken to indicate that the transition state is becoming evermore symmetric (Figure 7). Unfortunately, one cannot distinguish between the single and multistep mechanisms for hydride equivalent transfer from our β values or kinetic isotope effect in that both mechanisms may give $\beta \simeq 0.5$ and substantial kinetic isotope effects. It has been argued,[28] however, that a change in mechanism from rate-determining electron transfer to proton transfer in the multistep mechanism (as was proposed earlier[10]) will result in a biphasic Brønsted plot, contrary to experimental observation (see Figure 6).

We have correlated k_{HH}/k_{DD} and $\Delta G°$ for reaction of MAI + X-PhNADH by use of eq 26 derived[29] from the Melander–Westheimer principle[30,31] and Marcus theory.[26] The difference $\Delta G^{\ddagger}_H - \Delta G^{\ddagger}_D$ is proportional to $\ln (k_{HH}/k_{DD})$ whereas $\delta(ZPE)^{\ddagger}_{min}$

$$\Delta G^{\ddagger}_H - \Delta G^{\ddagger}_D = [\delta(ZPE)^{\ddagger}_{min} - \delta(ZPE)_{IS}][1 - (\Delta G°/4\Delta G^{\ddagger}_0)^2] \quad (26)$$

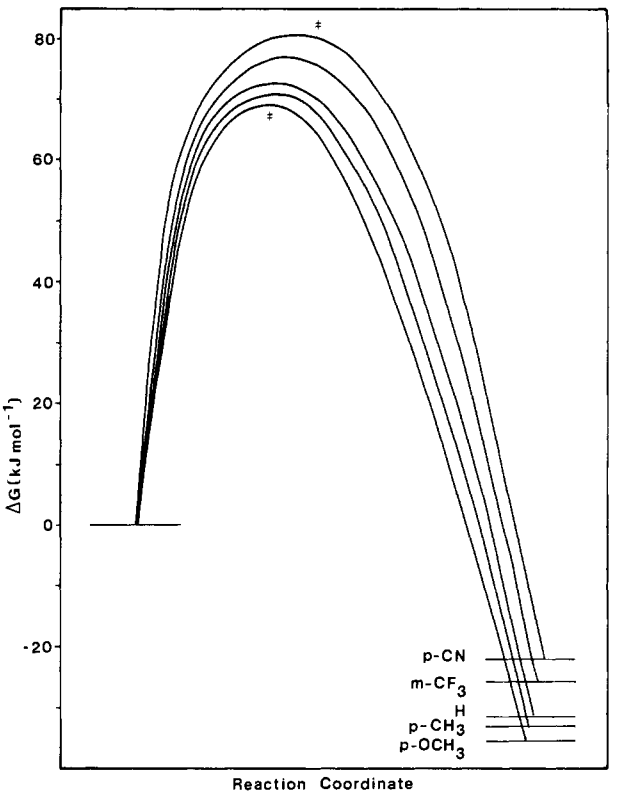


Figure 7. Dependence of $\Delta G^{\ddagger}$ on $\Delta G°$ for reaction of X-PhNADH + MAI in CH_3CN at 50 °C. A transition state that moves to the right (more productlike) as the overall reaction is made more exothermic (Hammond postulate) is in accord with the slightly larger kinetic isotope effects observed for the slower reaction.

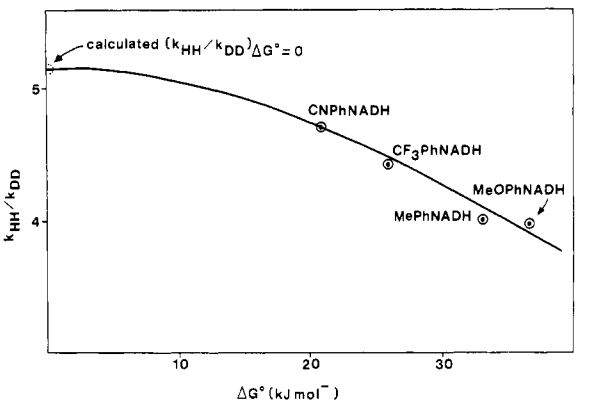


Figure 8. Marcus theory correlation of $\Delta G°$ and k_{HH}/k_{DD} for reaction of MAI and X-PhNADH in CH_3CN at 50 °C. The best fit curve to eq 26 is shown; $\Delta G^{\ddagger}_0 = 22.2$ kJ mol^{-1} and $\ln (k_{HH}/k_{DD})_{\Delta G°=0} = 1.64$.

$- \delta(ZPE)_{IS}$ represents the double difference of H − D for both the zero-point energy in the initial state and in the transition state and is proportional to $\ln (k_{HH}/k_{DD})$ at $\Delta G° = 0$. Other nonlinear rate equilibria correlations such as the BEBO expression[32] and the hyperbolic equation offered by Lewis, Shen, and More O'Ferrall[33] give slightly larger values of $\Delta G^{\ddagger}_0$ in that Marcus' simpler equation[26] provides a lower limit for $\Delta G^{\ddagger}_0$, resulting in the maximum curvature for a rate-equilibrium relationship.[34] A nonlinear least-squares fit of our data [$\Delta G°$ vs. $RT \ln (k_{HH}/k_{DD})$ for reaction of MAI + X-PhNADH] to eq 26 gives $\delta(ZPE)^{\ddagger}_{min} - \delta(ZPE)_{IS} = 4.40 \pm 0.08$ [$(k_{HH}/k_{DD})_{\Delta G° = 0} = 5.16 \pm 0.17$] and $\Delta G^{\ddagger}_0 = 22.2 \pm 1.7$ kJ mol^{-1} (see Figure 8).[35] This calculated

(28) Roberts, R. M. G.; Ostovic, D.; Kreevoy, M. M. *Faraday Discuss. Chem. Soc.* **1983**, in press.
(29) Kresge, A. J. *J. Am. Chem. Soc.* **1980**, *102*, 7797–7798.
(30) Melander, L. "Isotope Effects on Reaction Rates"; Ronald Press: New York, 1960; pp 24–32.
(31) Westheimer, F. H. *Chem. Rev.* **1961**, *61*, 265–273.
(32) Agmon, N.; Levine, R. D. *Chem. Phys. Lett.* **1977**, *52*, 197–201.
(33) Lewis, E. S.; Shen, C. C.; More O'Ferrall, R. A. *J. Chem. Soc., Perkin Trans. 2* **1981**, 1084–1088.
(34) For example, see: More O'Ferrall, R. A. *Symp. Faraday Soc.* **1975**, *10*, 92–93. Saunder, W. H., Jr. *J. Phys. Chem.* **1982**, *86*, 3321–3323.

Table III. Rate Constants and Activation Parameters for Reaction of MAI with (*p*-Methylphenyl)-1,4-[$^1H_2$]- and (*p*-Methylphenyl)-1,4-[$^2H_2$]dihydronicotinamide

H,H-MePhNADH		D,D-MePhNADH	
temp, °C	k_H, M^{-1} s^{-1}	temp, °C	k_D, M^{-1} s^{-1}
50.0	26.28	50.0	6.56
41.4	18.56	43.0	4.72
33.3	13.37	34.5	3.07
27.4	10.36	25.3	1.80
18.7	7.02	16.8	1.17
10.0	4.33	7.0	0.576
−2.2	2.19	−2.2	0.341

$E_A^H = 34.26 \pm 0.46$ kJ mol^{-1} $E_A^D = 41.95 \pm 0.57$ kJ mol^{-1}
$\ln A^H = 16.06 \pm 0.18$ $\ln A^D = 17.52 \pm 0.23$
$\Delta H^{\ddagger} = 31.91$ kJ mol^{-1} $\Delta H^{\ddagger} = 39.49$ kJ mol^{-1}
$\Delta S^{\ddagger} = -119.56$ J K^{-1} mol^{-1} $\Delta S^{\ddagger} = -105.97$ J K^{-1} mol^{-1}

$E_A^D - E_A^D = 7.68 \pm 1.03$ kJ mol^{-1}
$A^D/A^H = 4.30 \pm 1.30$

intrinsic barrier is significantly smaller than that observed for reaction of MAI + MA or PrNADH + PrNAD, even when a reasonable estimate for the work term (w_r = 8 − 25 kJ mol^{-1}) is accounted for. Assuming $\Delta G^{\ddagger}_0{}' = 88.5$ kJ mol^{-1} (the mean for reactions MAI + MA and PrNADH + PrNAD) and $\Delta G^{\ddagger}_0 = 22.2$ kJ mol^{-1}, w_r is calculated to have an unusually large value of 66.3 kJ mol^{-1}. This value is too high to account for phenomena associated with w_r such as diffusion, desolvation of reactants, and correct orientation for hydride transfer. A smaller w_r of 20 kJ mol^{-1} would give $\Delta G^{\ddagger}_0 = 68$ kJ mol^{-1}; this would give rise to a ~2% change in k_{HH}/k_{DD} over the experimentally measured range of $\Delta G°$ contrary to the ~20% change we observed. This "large" increase in k_{HH}/k_{DD} with greater transition-state symmetry (for MeOPhNADH → CNPhNADH) must be due to something other than just the factors that determine isotopic zero-point energy differences during bond breaking. It could possibly be due to a dependence of the secondary kinetic isotope effect on $\Delta G°$; this, however, is not expected to account for such a large change in k_{HH}/k_{DD} based on known (vide infra) k^H/k^Ds for reactions of this type. The increase noted in k_{HH}/k_{DD} could also be due to quantum-mechanical tunneling since tunneling exhibits a somewhat parabolic dependence on the free energy of reaction with a maximum at $\Delta G° = 0$[18], and thus, could increase k_{HH}/k_{DD} faster than expected from Marcus theory as the transition state becomes more symmetrical.

The extent of tunneling during hydride transfer in NADH model systems was investigated by measuring the temperature dependence of the isotope effects (Figure 9) for reaction of MAI with (*p*-methylphenyl)-1,4-[$^1H_2$]- and (*p*-methylphenyl)-1,4-[$^2H_2$]dihydronicotinamide. Rate measurements were made over a wide temperature range (−2 to 50 °C) by conventional UV–vis spectrophotometry; the rate constants and activation parameters are given in Table III.

To assess the magnitude of the tunnel effects, we employed a computer program of Kaldor and Saunders.[19] This program assumes that the Arrhenius equation adaquately describes the temperature dependence of a reaction with tunneling if a tunnel correction factor, Q, is included (eq 27).

$$k_H = Q_t^H A_s^H \exp[-E_a^H/(RT)] \quad (27)$$

When the additional assumption of isotopically insensitive semiclassical preexponential factors ($A_S^H = A_S^D$) is made, the temperature dependence for the isotope effects is given by eq 28

$$k_H/k_D = Q_t^H/Q_t^D \exp[(E_a^D - E_a^H)/(RT)] \quad (28)$$

(35) Correlation of $\Delta G^{\ddagger}_H - \Delta G^{\ddagger}_D$ and $\Delta G°$ for reaction of MAI + X-PhNADH by use of the expression $\Delta G^{\ddagger}_H - \Delta G^{\ddagger}_D = [(\Delta G°^2 + 4\Delta G^{\ddagger}_{O,H}{}^2)^{1/2} - (\Delta G°^2 + 4\Delta G^{\ddagger}_{O,D}{}^2)^{1/2}]/2$ derived from the hyperbolic equation[33] for rate equilibria relationships gives $\Delta G^{\ddagger}_{O,H} - \Delta G^{\ddagger}_{O,D} = 4.56$ [this corresponds to $(k_{HH}/k_{DD})_{\Delta G°=0} = 5.47$] and $(\Delta G^{\ddagger}_{O,H}\Delta G^{\ddagger}_{O,D})^{1/2} = 25.4$ kJ mol^{-1}. The latter quantity approximates ΔG_0 and is only slightly larger than $\Delta G^{\ddagger}_0 = 22.2$ kJ mol^{-1} calculated from eq 26.

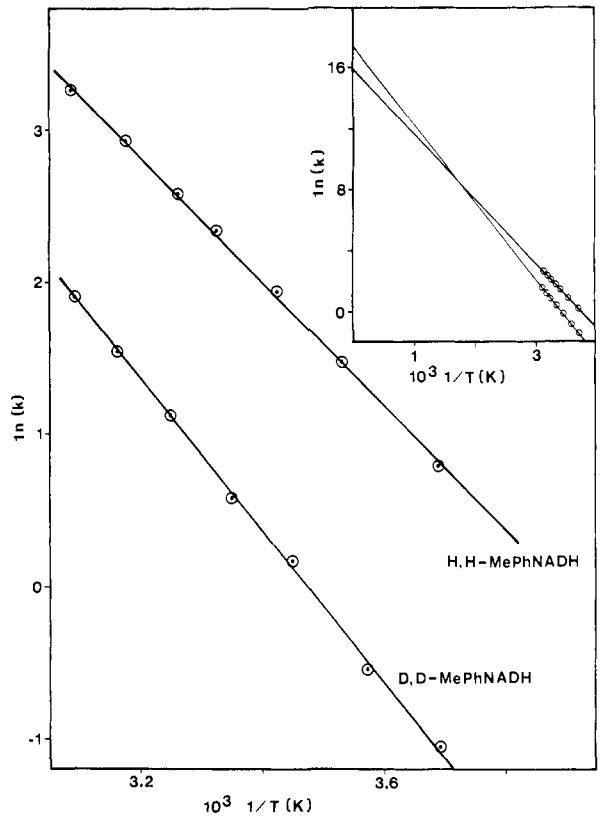


Figure 9. Rates of reaction for H,H-CH_3PhNADH and D,D-CH_3PhNADH with MAI in CH_3CN. The inset shows the inversion of the kinetic isotope effects at high temperatures to give $A_D/A_H = 4.30 \pm 1.30$.

where Q_t is calculated by using the first term of the Bell equation[18] (eq 29)

$$Q_t = (1/2)u_t/\sin[(1/2)u_t] \quad (29)$$

with $u_t = [h/(kT)][E^{1/2}/[\pi a(2m)^{1/2}]]$. The quantity u_t relates the barrier height, E, and the barrier half-width, a, to the mass, m, of the particle being transferred and the temperature, T. If it is assumed that $u_t^D = u_t^H m_D^{-1/2}$ where $m_D = 2amu$, the quantity u_t^H can be varied to reproduce experimental values of A^D/A^H and $E_a^D - E_a^H$. After calculation of Q_t^H and Q_t^D, the semiclassical isotope effect $(k_H/k_D)_s$, i.e., that isotope effect that would be found in the absence of tunneling, was calculated from eq 30. The

$$k_H/k_D = (Q_t^H/Q_t^D)(k_H/k_D)_s \quad (30)$$

semiclassical isotope effect is that part of the isotope effect due to zero-point energy effects and is the quantity one should consider in discussing the effect of the extent of hydrogen transfer in the transition state in terms of the Melander–Westheimer three-center model.[30,31] Table IV lists the quantities derived from the fits of our data.

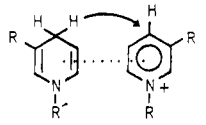
The tunnel correction factors for Q_t^H vary from approximately 2.5 to 4.5; this is indicative of a moderate amount of tunneling but not enough to give rise to any qualitatively striking phenomena. The first term of the Bell equation is deemed adequate to estimate the extent of tunneling for values of Q_t^H less than ~5. The ratios of Q_t^H/Q_t^D reflect the extent to which tunneling is affecting k_H/k_D. At 50 °C, less than half of the observed isotope effect arises from the tunneling contribution, but at lower temperatures slightly more than half of k_H/k_D is due to tunneling. From the ratio of tunneling correction factors, the semiclassical isotope effect was calculated and, although increasing with decreasing temperature, was found to be significantly below the predicted isotope effect maximum for loss of zero-point energy in the C–H stretching vibrations. Several factors could account for small semiclassical isotope effects. An unsymmetrically located H in the transition state would reduce k_H/k_D somewhat; however, it would also qualitatively be expected

Table IV. Tunnel Corrections for Reaction of MAI with (*p*-Methylphenyl)-1,4-[$^1H_2$]- and (*p*-Methylphenyl)-1,4-[$^2H_2$]dihydronicotinamide

temp, °C[a]	Q_t^H	Q_t^H/Q_t^D	$(k_H/k_D)_s$	k_H/k_D	$(k_H/k_D)_{calcd}$[b]
5.0	4.00 ± 0.68	2.25 ± 0.26	2.92	6.43	6.57
14.0	3.54 ± 0.49	2.07 ± 0.19	2.83	5.79	5.85
23.0	3.19 ± 0.39	1.93 ± 0.15	2.74	5.25	5.29
32.0	2.91 ± 0.32	1.83 ± 0.13	2.66	4.79	4.85
41.0	2.69 ± 0.11	1.74 ± 0.11	2.58	4.39	4.49
50.0	2.51 ± 0.10	1.66 ± 0.10	2.52	4.05	4.19

[a] Temperatures used which span observed temperature range. [b] Kinetic isotope effect (including tunneling contribution) calculated from the first term of the Bell equation.[18]

to reduce the effect of tunneling since both depend on the symmetry of the transition state. Such an unsymmetrical transition state is unlikely based on the similar structures of reactants and products, the large $\Delta G^{\ddagger}$ as compared with $\Delta G°$ (Figure 6), and the Bronsted β of ~0.5 for this reaction. Nonlinear H transfer in the transition state would also have the effect of reducing k_H/k_D. A bent transition state could easily be envisaged for a hydride equivalent transfer from dihydronicotinamide to nicotinamide if the reactants form a face to face charge-transfer type of complex.



Such interactions have been reported previously for related compounds.[36] Model calculations[37] for variation of the transition-state geometry show that semiclassical isotope effects for a bent transition state (~90–120°) lie in the 2–3 range at 25 °C, similar to the semiclassical isotope effects calculated herein when the tunneling contribution to k_H/k_D is removed. In the bent transition state, the kinetic isotope effect is composed of zero-point energy differences arising from an out-of-plane bend and a composite stretching and bending motion of high frequency that offsets the combined zero-point energy changes in the stretching and bending modes of the reactants. This gives rise to isotope effects much smaller than for the linear transition state. Although the accuracy of these calculations rests on various assumptions of transition-state geometry and force constants, they do serve to show that the reduced semiclassical isotope effects are in accord with a transition state favoring a charge-transfer interaction. It has been argued, however, that H^- transfer in our systems can be made nearly linear by partial tilting of the nicotinamide planes, which would result in only a slight loss of charge-transfer stabilization. Since two carbons and two nitrogens are changing their valence angles during the H^- transfer, it is reasonable to expect that such nonhydrogenic motion to the reaction coordinate may reduce the observed kinetic isotope effects even though heavy atom motion usually makes its largest contribution when the transition state is unsymmetric. On the basis of their vibrational analysis calculations, Huskey and Schowen[38] have proposed that a realistic explanation for the isotope effect observations in these hydride transfer systems is due to the combination of α-hydrogen motion in the reaction coordinate and concomitant tunneling. Further study of isotope effects in geometrically constrained systems[21] where nonlinear H^- transfer is enforced should elaborate upon the importance of tunneling and nonlinear H^- transfer in these reactions.

Conclusions

(1) The previously reported (see Table I) discrepancies between kinetic and product isotope effects for reaction of NADH model compounds (the dihydronicotinamides) with various hydride acceptors (such as acridinium ion) were shown to be anomalous when isotope scrambling and/or secondary kinetic isotope effects were considered. The dependence of the deuterium kinetic isotope effect (k_{HH}/k_{DD} = 4–4.8) upon the substituent (X = MeO, Me, CF_3, or CN) for reaction of MAI + X-PhNADH and the temperature-dependent (~0–50 °C) isotope effects (k_{HH}/k_{DD} = 7–4) for reaction of MAI + *p*-MePhNADH suggest tunneling makes a significant contribution to the overall rate.

(2) The moderate kinetic isotope effects (k_H/k_D = 3–6) observed in NADH model reactions do not necessitate linear H^- transfer in the transition state if tunneling is occurring. Recent modeling studies of alcohol dehydrogenase structure and X-ray crystallographic studies of enzyme–substrate complexes hint that H^- transfer in these systems may be nonlinear. For example, Dutler and Branden[39] found that the rates of reduction of alkylated cyclohexanones by horse liver alcohol dehydrogenase (HLAD) could be correlated with the size and structure of the active site; they were able to show that the kinetic data were consistent with a model involving nonlinear [providing the nicotinamide and C(1) are held fixed] H^- transfer from C(1) of the cyclo alcohol to C(4) of the nicotinamide ring. Similarly Eklund et al.[40] showed that substrate molecules of *p*-bromobenzyl alcohol in HLAD could easily undergo simple rotation (about the axis formed with the Br and O atoms) to bring it into position for direct (but nonlinear) hydride transfer. The moderate kinetic isotope effects for reaction of substituted benzyl alcohols with HLAD[41] suggest some tunneling must be occurring if the H transfer is nonlinear. The structure of the active ternary complex of pig heart lactate dehydrogenase with (3*S*)-5-(3-carboxy-3-hydroxypropyl)-NAD also indicates a possible nonlinear H transfer.[42] Distortion of the nicotinamide ring prior to hydride transfer so that N(1) becomes pyramidal and C(4) develops increased carbonium ion[43] structure is allowed on steric grounds for these active site models but is not expected to significantly change the nonlinearity of the H transfer. X-ray crystallographic studies of glutathione reductase[44] also show that direct hydride transfer from C(4) of dihydronicotinamide to N(5) of flavin must be nonlinear inasmuch as the flavin and dihydronicotinamide form a tight "sandwich" type of complex approximately 0.35 Å apart. Evidence for "face-toface" complex formation between flavins and dihydronicotinamides has been given by Bruice et al.[45] and Blankenhorn;[46] other more indirect evidence for such complexes may be found in the slow rates of reduction of flavins by borohydride,[47] which precludes such complex formation.

(3) The findings reported herein (k_H/k_Ds vs. Y_H/Y_Ds and a linear Brønsted plot for reaction of MAI + X-PhNADH) support single-step hydride transfer from dihydronicotinamides to acridinium ion, although they do not unequivocally rule out a stepwise mechanism with e^-, H^+, e^- transfer in other special cases. Indeed, there exists evidence in the literature for electron transfer from NADH model compounds *to strong oxidants*. For example,

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replacement of NO_2 by H in activated compounds by NADH occurs via an electron-transfer chain mechanism as demonstrated by the influence of free radical inhibitors and scavengers on the reaction.[48] Evidence for a stepwise path of hydride equivalent transfer in the oxidation of MA by 2,3-dicyano-1,4-benzoquinone was provided by Lai and Colter[49] when they found the *N*-methylacridinyl radical was trapped by 2-methyl-2-nitrosopropane. Even PrNADH has been shown to react (although the kinetics are somewhat complex) with ferricyanide, presumably through a one-electron transfer mechanism.[50] In view of our earlier findings[51,52] that flavin radicals (whose reaction with NADH model compounds is exothermic) do not undergo detectable one-electron transfer with dihydronicotinamides, the aforementioned observations of one-electron transfer may be explained as follows: if the redox potential of the oxidant is high enough to form the putative nicotinamide radical intermediate, it will presumably do so; otherwise hydride transfer from dihydronicotinamides to less powerful one-electron or two-electron oxidants will follow a two-electron pathway—that of direct H^- transfer. A correlation of kinetic and secondary isotope effects and reaction rates with the redox potential[53] for various NADH model reactions would elucidate the factors governing such a dual mechanistic pathway for hydride equivalent transfer.

Acknowledgment. This work was supported by grants from the National Institutes of Health and the National Science Foundation. M.F.P. expresses appreciation to the Natural Sciences and Engineering Research Council of Canada for a postdoctoral fellowship. We thank James Wu for his experimental assistance.

Registry No. NADH, 18940-08-4; MAI, 948-43-6; PrNAD·I, 5463-59-2; TFA, 434-45-7; PhNAD-I, 87412-97-3; MeOPhNAD·I, 87412-98-4; MePhNAD·I, 87412-99-5; CF_3PhNAD·I, 87413-00-1; CNPhNAD·I, 87413-01-2; QNAD·I, 17260-82-1; BzNADH, 952-92-1; H,D-BzNADH, 17750-30-0; D,D-BzNADH, 60172-94-3; QNADH, 17260-79-6; H,D-QNADH, 79798-57-5; D,D-QNADH, 83077-37-6; MeOPhNADH, 87413-02-3; H,D-MeOPhNADH, 87413-03-4; D,D-MeOPhNADH, 87413-04-5; MePhNADH, 87413-05-6; D,D-MePhNADH, 87432-52-8; CF_3PhNADH, 83077-40-1; D,D-CF_3PhNADH, 83077-41-2; CNPhNADH, 87413-06-7; D,D-CNPhNADH, 87413-07-8; MA, 4217-54-3; H,T-PrNADH, 87413-08-9; PrNADH, 17750-24-2; D,D-PrNADH, 60764-07-0.

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(53) For example, see: Kellogg, R. M.; Piepers, O. *J. Chem. Soc., Chem. Commun.* **1982**, 402–404.

Effects of Hydration on the Dynamics of Deoxyribonucleic Acid

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Abstract: The effect of hydration on motions in the sugar California 94305 backbone of double-stranded DNA fragments 200 ± 50 base pairs in length has been examined by using ^{31}P NMR. It is shown that very little motion occurs in lyophilized DNA, but motions increase in amplitude with increasing hydration. Changes in the relaxation behavior with increasing hydration suggest that several different types of motion are present at different hydration levels, with the changes between them occurring at hydration levels that correlate with the successive hydration at various molecular sites previously described. In the hydration range of B-form DNA, the changes in the spectrum induced by increasing hydration are found to be similar to the changes induced by increasing temperature.

In the past several years, a number of experiments have shown that double-stranded B-forms DNA, both in solution and in ordered phases, undergoes some relatively fast motions in addition to the normal reorientation of a rigid cylinder.[1-9] Hogan and Jardetzky[1-3] and Bolton and James[5] reported correlation times of the backbone motions to be in the range of 1×10^{-9} s. Early, Feigon, and Kearns[4] showed that base pairs also show the effects of such motions. Opella, DiVerdi, and Wise[6,7] showed that ordered B-form DNA similarly showed motions of the sugar phosphate backbone. Optical measurements have additionally demonstrated internal flexibility for long DNA helicides in solution.[16,17] Keepers

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