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NAD(P)H-NAD(P)+ Models. 74. Entropy-Controlled Kinetics, Stereochemistry, and Tunneling Effect

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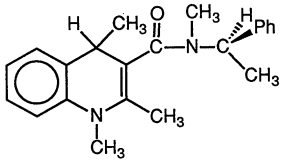
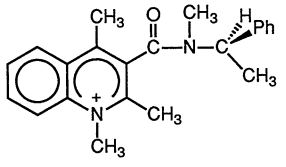
Kinetics have been studied for the reactions of three NAD(P)H analogs and their 4-deuteriated compounds with 2,6-dichloro- and tetrachloro-1,4-benzoquinones. It has been elucidated that the entropy term occupies 50—90% of the Gibbs energy of activation at 298 K. Thus, the reaction is almost entropy-controlled. For certain combinations of the reducing and oxidizing reagents, quantum mechanical tunneling effect plays a large role to determine the reaction rate. The tunneling effect may be another measure of the stereochemistry.

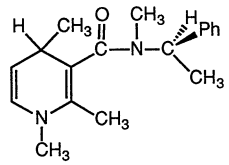
Stereochemistry in the oxidation of 3-(*N*-methyl-*N*-α-methylbenzylcarbamoyl)-1,2,4-trimethyl-1, 4-dihydroquinoline (Me_3MQPH) or 3-(*N*-methyl-*N*-α-methylbenzylcarbamoyl)-1-propyl-2,4-dimethyl-1,4-dihydropyridine (Me_3PNPH) exert free-energy structure–reactivity–stereochemistry relationship; that is, the stereospecificity associated with the transfer of the central chirality in Me_3MQPH or Me_3PNPH into the axial chirality in their corresponding oxidized forms (Me_3MQP^+ or Me_3PNP^+, respectively) depends on the oxidation and reduction potentials of the reducing and oxidizing reagents.[1,2] The reactive pair prefers the anti-transition state while the less reactive pair prefers the syn-transition state.[3] Based on the stereochemical results, it was concluded that the syn/anti preferency in the transition state stems from the earliness or lateness of the transition state.[1,2]



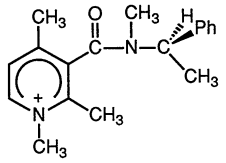
Me_3MQPH



Me_3MQP^+



Me_3PNPH

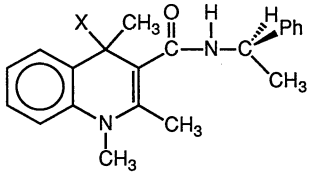


Me_3PNP^+

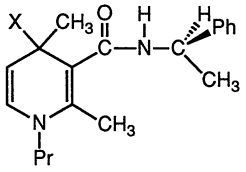
In order to obtain further evidence for the proposed structure of transition state of the reaction, we studied kinetics and elucidated kinetic parameters. Since kinetics become very much complicated in the presence of magnesium ion,[4] the reaction without magnesium ion was studied. 2,6-Dichloro-1,4-benzoquinone (**2Q**) and tetrachloro-1,4-benzoquinone (chloranil, **4Q**) were chosen as the least and most reactive oxidizing reagents, hence, corresponding to the latest and earliest transition states, respectively, under the reaction conditions employed.[5] Although there are many contributions on the kinetics and kinetic isotope effects to discuss on the reaction mechanism of NAD(P)H analogs,[4,6–18] kinetic parameters have scarcely been studied and discussed.[11]

Results

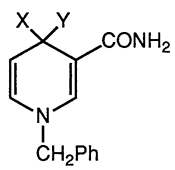
Since the present study does not require to deal with stereochemistry, it is not neccessary to use a compound with a stable axial chirality. Therefore, we employed 3-(*N*-α-methylbenzylcarbamoyl)-1,2,4-trimethyl-1,4-dihydroquinoline (Me_2MQPH) and 3-(*N*-α-methylbenzylcarbamoyl)-1-propyl-2,4-dimethyl-1,4-dihydropyridine (Me_2PNPH) in place of Me_3MQPH and Me_3PNPH.



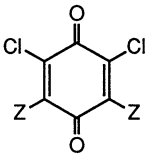
X=H : Me_2MQPH
X=D : Me_2MQPD



X=H : Me_2PNPH
X=D : Me_2PNPD



X=H,Y=H : BNAH
X=H,Y=D : BNAD
X=D,Y=D : $BNAD_2$



Z=H : 2Q
Z=Cl : 4Q

In addition to Me_2MQPH and Me_2PNPH, and their 4-deuteriated derivatives (Me_2MQPD and Me_2PNPD, respectively), *N*-benzyl-1,4-dihydronicotinamide (BNAH) and its 4-deuteriated and 4,4-dideuteriated derivatives (BNAD and $BNAD_2$, respectively) were also studied to convince of the results from the former compounds. BNAH is a compound which has been studied most

extensively.

When an acetonitrile solution of Me_2PNPH was mixed with that of **4Q** under argon atmosphere, a yellow color appeared immediately. This strong absorption band at λ_{max}=449 nm (ε=8,000 M^{-1}) was identified to that of the radical anion from **4Q**.[19] **2Q** behaved similarly (λ_{max}=448 nm). The ESR spectrum of each solution exhibited a signal from the radical anion of the quinone.[19-21] The radical anions thus produced were stable in the presence of excess amount of the quinones, but disappeared gradually at room temperature when the NAD(P)H analog existed in excess amount. The kinetics of the reaction of BNAH with quinones have been studied extensively by Fukuzumi and his co-workers,[11] and we employed the reaction scheme proposed by them.

The observed pseudo-first-order rate constants (with more than 10-fold excess of a reagent), calculated by following the increase in absorbances at the absorption maxima of the corresponding radical anions, are plotted against the concentrations of the analog and of the quinone, respectively, indicating that the reaction is first-order in both the analog and the quinone. Temperatures employed for the measurements ranged from 273 to 323 K with 5 to 15 K intervals. The second-order rate constants, primary and secondary kinetic deuterium isotope effects, and kinetic parameters are summarized in Tables 1 and 2. Primary kinetic deuterium isotope effects in the BNAH systems are in good agreement with those reported.[12]

Discussion

It is apparent that the overall reaction is controlled by the entropy; at 298 K, the entropy term amounts about 10 kcal mol^{-1}, which is about 50—90% of the Gibbs energy of activation. This is a brand-new and important observation because there has ever appeared no discussion on the reaction mechanism of the

Table 1. Kinetic Parameters, Isotope Effects, and Stereochemistry of the Oxidation of NAD(P)H Analogs with Chloranil[a]

	Me_2MQPD		Me_2MQPH	$BNAD_2$	BNAD	BNAH	Me_2PNPD		Me_2PNPH
k_2/M^{-1} s^{-1}	27.8		130	233	716	1210	19600		45000
$(k_H/k_D)_{obsd}$		4.68			5.14			2.30	
$(k_H/k_D)_{calcd}$[b]		4.30			8.90			3.22	
$(k_H/k_D)_{second}$[c]					1.01				
$\Delta G^{\neq}$/kcal mol^{-1}	15.5		14.6	14.2	13.6	13.2	11.6		11.1
$\Delta H^{\neq}$/kcal mol^{-1}	5.84		4.98	4.29	3.07	3.00	1.16		0.47
$-\Delta S^{\neq}$/cal mol^{-1} deg^{-1}	32.3		32.7	33.4	35.2	34.3	35.0		35.6
δE_a[d]/kcal mol^{-1}		−0.86			−1.29			−0.69	
A_H/A_D		1.15			0.62			0.72	
Mechanism[e]		Classic			Tunnel			Classic	
R/S Ratio[f]		1.2—1.3/1			—			2.4/1	

a) The values listed in the Table are those at 298 K. Errors in rate constants and kinetic isotope effects are estimated to be ±3 and ±5%, respectively. Those for parameters of activation are less than ±10%. The deuterium content in $BNAD_2$ was 97%, which was corrected to obtain the observed rate constant. b) Calculated from the enthalpy of activation assuming no difference in entropy of activation for protiated and deuteriated compounds. c) Calculated according to the Powell and Bruice's equation; Ref. 28. d) $\delta=X_H-X_D$. e) Ref. 24. f) Ref. 1.

Table 2. Kinetic Parameters, Isotope Effects, and Stereochemistry of the Oxidation of NAD(P)H Analogs with 2,6-Dichloro-1,4-benzoquinone[a]

	Me_2MQPD		Me_2MQPH	$BNAD_2$	BNAD	BNAH	Me_2PNPD		Me_2PNPH
k_2/M^{-1} s^{-1}	6.89		25.9	13.9	62.0	84.4	1990		6030
$(k_H/k_D)_{obsd}$		3.75			6.01			3.03	
$(k_H/k_D)_{calcd}$[b]		5.35			11.48			7.39	
$(k_H/k_D)_{second}$[c]					1.01				
$\Delta G^{\neq}$/kcal mol^{-1}	16.3		15.6	15.9	15.1	14.8	13.0		12.3
$\Delta H^{\neq}$/kcal mol^{-1}	7.98		6.99	6.67	5.50	5.23	2.01		0.829
$-\Delta S^{\neq}$/cal mol^{-1} deg^{-1}	28.0		28.8	30.9	32.2	32.2	36.9		38.4
δE_a[d]/kcal mol^{-1}		−1.00			−1.43			−1.18	
A_H/A_D		0.67			0.54			0.47	
Mechanism[e]		Classic			Tunnel			Tunnel	
R/S Ratio[f]		1.2—1.1/1			—			1/2.3—2.4	

a) The values listed in the Table are those at 298 K. Errors in rate constants and kinetic isotope effects are estimated to be ±3 and ±5%, respectively. Those for parameters of activation are less than ±10%. The deuterium content in $BNAD_2$ was 97%, which was corrected to obtain the observed rate constant. b) Calculated from the enthalpy of activation assuming no difference in entropy of activation for protiated and deuteriated compounds. c) Calculated according to the Powell and Bruice's equation; Ref. 28. d) $\delta=X_H-X_D$. e) Ref. 24. f) Ref. 1.

NAD(P)H analog from the viewpoint of entropy.

The entropy of activation changes most evidently on changing Me_2PNPH to Me_2PNPD in the reaction with **2Q**, where the syn-transition state is preferred: That is, the primary kinetic deuterium isotope effect calculated on the basis of enthalpy of activation (7.39) is more than twice as large as that observed (3.03). The result strongly suggests that the quantum mechanical tunneling effect plays a major role in differentiating the reactivities of protiated and deuteriated compounds.[22,23] The importance of the tunneling effect can also be recognized by Kwart's diagnosis.[24] Although a part of Kwart's proposal has been claimed,[25,26] the reaction with a large difference in E_a (ca. 1.5—6 kcal mol^{-1}) and very small A_H/A_D value (<0.6) can safely be attributed to a tunneling transition state. With this criterion, the reaction of Me_2PNPH with **2Q** as well as the reactions of BNAH with both **2Q** and **4Q** is believed to pass through the tunneling transition state.[14–18] This is not true for the reaction with Me_2MQPH which passes through the anti-transition state preferentially.

Namely, although the changes in Gibbs energy of activation at 298 K on deuteriation are the same for the Me_2MQPH and the Me_2PNPH systems in the reaction with **2Q** ($\delta\Delta G^{\neq}=-0.7$ kcal mol^{-1}), the changes in enthalpy and entropy of activation for the Me_2MQPH system ($\delta\Delta H^{\neq}=-0.99$ kcal mol^{-1}; $\delta\Delta S^{\neq}=-0.8$ cal mol^{-1} deg^{-1}) are largely different from those for the Me_2PNPH system ($\delta\Delta H^{\neq}=-1.18$ kcal mol^{-1}; $\delta\Delta S^{\neq}=-1.5$ cal mol^{-1}deg^{-1}) in accord with the proposal of classical transition state for Me_2MQPH and quantum mechanical tunneling effect for Me_2PNPH.

In the reactions with **4Q**, the tunneling effect is smaller (or even negligible) than that in the reaction with **2Q**, which reveals that the proton (or hydrogen atom)-transfer process in a reaction with the former quinone requires larger movement of the nucleus than that in a reaction with the latter quinone. Since the reactivity of **4Q** for the electron-transfer process is much larger than that of **2Q**, the electron-transfer takes place at the early stage of the ring-deformation, or the C_4–H bond in the NADH analog is less elongated, and the C_4-proton (or hydrogen atom) of the NADH analog in the reaction with **4Q** has to migrate longer distance than that in the reaction with **2Q** in the succeding proton (or hydrogen atom)-transfer process.[1]

The present result again supports the multi-step reaction mechanism for the transfer of a (net) hydride by proving that when the electron-transfer process is associated with a low activation energy and proceeds fast, the succeeding proton (or hydrogen atom)-transfer process becomes slow due to a high activation energy.[7,8]

The oxidation potentials of Me_2MQPH, Me_2PNPH, and BNAH measured by the scanning rate method[6,13] are +0.83, +0.62, and +0.59 (lit,[12] 0.57) V (vs. SCE), respectively, which means that the rate of the initial electron-transfer increases in this order. Taking into consideration the discussion mentioned above for the proton (or hydrogen atom)-transfer process, the energy diagrams along the total reaction coordinate may be those that are represented in Fig. 1.[1]

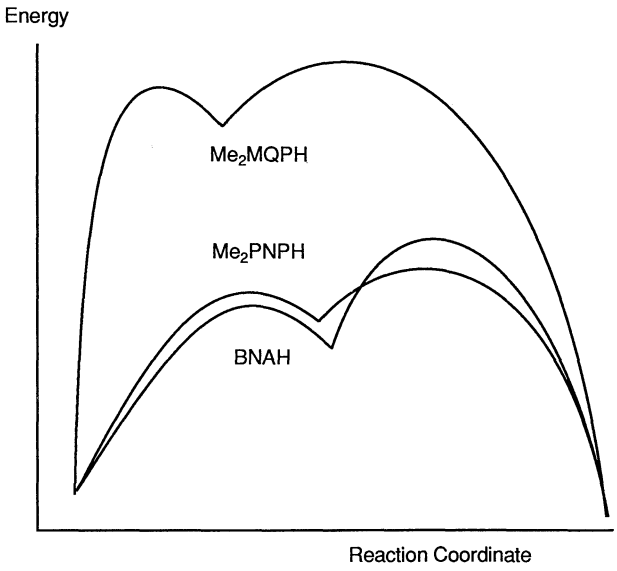


Fig. 1. Energy diagram along the reaction coordinate in the reactions of Me_2MQPH, Me_2PNPH, and BNAH.

The present conclusion is in good agreement with the idea proposed previously that the syn-configuration is favored enthalpically in a late transition state but the anti-configuration is favored entropically in an early transition state.[1,2] That is, the NAD(P)H analog in a reaction which has to pass through a hindered transition state as a result of strong interaction between two reacting reagents prefers to assume entropically favorable anti-conformation to make the transition state less hindered and entropically favorable, whereas that in another reaction which is accompanied by less small entropy of activation can assume enthalpically favored syn-conformation at the transition state.

It is thus interesting to point out that, although the syn/anti selection is controlled by the electronic effect from the reagent(s), or there holds a linear free-energy reactivity-stereochemistry relationship,[1,2] the essential of the effect stems from the entropic (or steric) effect instead of the enthalpic effect. That is, the electronic effect controls molecular assembly at the intermediate and/or transition state, and the molecular assembly is the subject of entropy-control, at the same time. Consequently, the electronic effect is related to the entropic effect, then to stereospecificity. In this sense, the electronic effect discussed in this paper is entirely

different from the stereoelectronic effect proposed and developed by Delongchamps and other researchers.[27]

A careful inspection of Tables 1 and 2 suggests that the classical (which corresponds to the early or anti-configurational) or quantum mechanical tunneling (which corresponds to the late or syn-configurational) transition state may be another measure of the stereochemistry of the reaction. We need more data before concluding the stereochemistry–transition-state structure relationship.

Experimental

Instruments. ^{1}H NMR spectra were recorded at 200 and 400 MHz on a Varian VXR 200 FT-NMR and a JEOL JNM-GX400 FT-NMR spectrometers, respectively. ^{2}H NMR spectra were recorded at 31 MHz on a Varian VXR 200 FT-NMR spectrometer. UV spectra were obtained on a Hitachi U-3210 spectrometer with a Hitachi SDR-30 temperature controller. ESR spectra were recorded on a JEOL JES-RE 2XG spectrometer. Kinetic measurements were performed with a Union Giken RA-401 Rapid Reaction Analyzer equipped with a Union Giken K2R temperature controller and a Union Giken System 77 or a NEC PC9801 microcomputer system. Elemental analyses were performed with a Yanako MT-3 elemental analyzer.

Cyclic voltammetry and controlled potential macro-electrolysis were performed with a Hokuto Denko HAB-151 potentiostat/galvanostat. Cyclic voltammograms were recorded on a Riken Denshi F-35 X-Y recorder. A platinum electrode sealed in heat-shrinkable Teflon was employed as the working electrode. A platinum wire and an Ag/AgCl electrodes were used as the counter and reference electrodes, respectively.

Materials. Detailed procedures for the preparation and purification of reagents employed for the present study were described in the previous report.[1]

Kinetics. Kinetics measurements were carried out by using a stopped-flow apparatus from 273 to 323 K with 5 to 15 K intervals under argon atmosphere. Reaction rate was followed by observing the increase in absorbance at the absorption maxima of the anion radical of quinones in acetonitrile under pseudo-first-order conditions by using more than 10-fold excess quinone. Pseudo-first-order rate constants and kinetic parameters were calculated by the least-squares curve-fit, using a personal computer.

Oxidation Potential. The oxidation peak potentials of Me_2MQPH, Me_2PNPH, and BNAH were measured with cyclic voltammetry at 298 K in 10 ml acetonitrile containing 0.1 M (M=mol dm^{-3}) tetraethylammonium perchlorate and 2 mM NADH analog by changing the scan rate in the range 20—2,000 mV/s. The error in observation was estimated to be ±0.005 mV. The detailed procedure has been described in a literacture.[12] The observed potential against Ag/AgCl electrode was corrected for the potential against SCE by subtracting a value of 0.042 V.

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3) The syn-transition state is the one in which the C_4–H and C=O bonds in Me_3MQPH or Me_3PNPH point the same face of the molecular plane, whereas these bonds point the opposite face in the anti-transition state.

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