NAD(P)$^+$-NAD(P)H Models. 49. Proximity Effect of a Phenyl Group on the Electron Transfer Process

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1-Propyl-1,4-dihydronicotinamide (PNAH), 1-(3-phenylpropyl)-1,4-dihydronicotinamide (**1a**), 1-(2-phenylthioethyl)-1,4-dihydronicotinamide (**1b**), and 1-(4-phenylthiobutyl)-1,4-dihydronicotinamide (**1c**) and their 4-deuterated analogs are synthesized as NAD(P)H-models and subjected to the reduction of *m*-nitro-α,α,α-trifluoroacetophenone in the presence or absence of magnesium ion in acetonitrile. Kinetics at 323 K have been studied. Kinetic isotope effect is small (2.33) only when **1a** or **1b** is employed for the reduction in the presence of magnesium ion, whereas it appears large (≈3.2) under other conditions. The result has been interpreted in terms of steric interference by a proximate phenyl group on the approach of the substrate to form a binary (in the absence of magnesium ion) or ternary (in the presence of magnesium ion) complexes. Thermodynamic parameters for the formation of complexes between the models and magnesium ion have been studied. It has been elucidated that enthalpy of complexation is small for **1a** than others. However, the small enthalpy of complexation is compensated by small entropy of complexation affording almost constant free-energy of complexation throughout the models studied.

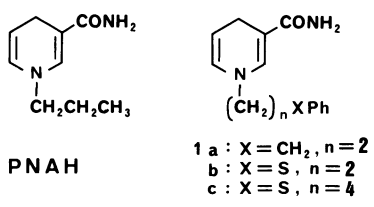
In a series of reports from our laboratory, we have emphasized that a complex of 1,4-dihydropyridine derivative and magnesium ion plays an important role in biomimetic reductions with NAD(P)H-models. The magnesium ion assists polarization of electron cloud on dihydropyridine moiety to migrate an electron onto a substrate.[1,2]

X-Ray crystallography and other evidence have revealed that zinc ion in certain liver alcohol dehydrogenases (LADH) is tetrahedrally coordinated by cysteinyl SH and/or histidinyl nitrogen.[3,4] It is commonly accepted that the zinc ion in the enzyme behaves as a Lewis acid to polarize the carbonyl group of a substrate.[5] However, it is not obvious whether tetrahedrally coordinated zinc ion in an enzyme can accommodate a carbonyl group as an extra ligand.[6] At least in the mimetic reactions, metal ions with an chelating reagent exerted no catalytic activity.[1]

In order to understand the mechanism of enzymic oxidation-reduction with NAD(P$^+$)-NAD(P)H coenzyme, not only interactions between the coenzyme and a substrate but also the effect of environment in the pocket of enzyme should be taken into account. The pocket is entirely surrounded by hydrophobic moieties.[6] Thus, we synthesized NAD(P)H-models with a phenyl or phenylthio substituent. This paper will describe the effect of magnesium ion on the kinetics of reduction with the model of this type in acetonitrile.

Results

Models employed for the study were PNAH and **1**. Although the substrate, *m*-nitro-α,α,α-trifuoroacetophenone (**2**) exerted no evidence for the formation of a complex with magnesium ion in acetonitrile, the models formed finite complexes that were detectable spectrophotometrically. The results are listed in Table 1. Kinetics were studied at 323 K in the presence or absence of magnesium perchlorate. Kinetics with 4-deuterated models were also studied and primary kinetic isotope effects were calculated according to Steffens and Chipman equation.[7] Results are summarized in Table 2.



PNAH

1 a : X = CH_2, n = 2
b : X = S, n = 2
c : X = S, n = 4

It was already reported that water content in the solvent acetonitrile affects the rate of reduction.[8] In order to confirm the constancy of kinetic isotope effect on the variation of water content, we also studied kinetics for the reduction of **2** with PNAH and PNAH-4-*d* in acetonitriles containing 0.1 and 1.0% v/v of water, respectively. The kinetic isotope effect observed in these systems were the same as that listed in Table 2. Adduct-formation was not recognized. Thus, kinetic isotope effects listed in Table 2 can be definitely divided into two classes, small (≈2.3) and large (≈3.2).

Discussion

It is apparent from Table 2 that the phenyl group on the third atom of the substituent chain exerts small kinetic isotope effect in the coexistence of magnesium ion. The observation is in contrast with that from PNAH, where kinetic isotope effects are large both in the presence and absence of magnesium ion. Energy diagram for the reduction with PNAH has been proposed to be consisted of two energy barriers, one of which that corresponds to the initial electron transfer process is lower than the other that corresponds to the proton (or hydrogen atom) transfer process. Therefore, the rate-determining step of the reduction involves largely the movement of hydrogen nucleus exerting large kinetic isotope effect. Since magnesium ion catalyzes the process of electron transfer, the presence of magnesium ion does not affect the magnitude of kinetic isotope effect, although it accelerates the rate.

TABLE 1. ASSOCIATION CONSTANTS AND THERMODYNAMIC PARAMETERS FOR THE COMPLEX BETWEEN THE MODEL AND MAGNESIUM ION IN ACETONITRILE[a)]

	PNAH	**1a**	**1b**	**1c**
10^{-1} K/mol dm^{-3}				
at 283 K	1901±67	1579±46	—	1729±85
at 293 K	1532±46	1472±44	1310±46	1420±36
at 303 K	1382±64	1368±35	1128±45	1225±32
at 313 K	1132±41	1203±47	892±42	972±43
at 323 K	919±44	948±41	724±24	780±37
$\Delta H°$/kJ mol^{-1}	−13.34±0.04	−6.82±0.02	−15.22±0.04	−14.96±0.05
$\Delta S°$/J mol^{-1} deg^{-1}	34.7±0.12	56.4±0.16	26.8±0.08	28.3±0.11
$\Delta G°$/kJ mol^{-1}	−23.88±0.07	−23.92±0.07	−23.37±0.07	−23.54±0.09

a) Errors are standard deviations.

TABLE 2. RATE CONSTANTS AND KINETIC ISOTOPE EFFECTS FOR THE REDUCTION OF *m*-NITRO-α,α,α-TRIFLUOROACETOPHENONE IN ACETONITRILE[a)]

Model	[Mg^{2+}] = 0 mol dm^{-3}		[Mg^{2+}] = 1.9×10^{-2} mol dm^{-3}	
	10 k[b)]	k^H/k^D	10 k[b)]	k^H/k^D
PNAH[c)]	2.98 ±0.062		6.43±0.127	
PNAH-4-*d*[c)]	1.96 ±0.065	3.17	4.21±0.060	3.23
1a	2.76 ±0.056		5.27±0.052	
1a-4-*d*	1.77 ±0.027	3.61	3.77±0.043	2.33
1b	0.742±0.008[d)]		2.38±0.035	
1b-4-*d*	0.488±0.010[d)]	3.17	1.70±0.024	2.33
1c	2.16 ±0.051		5.51±0.107	
1c-4-*d*	1.38 ±0.021	3.55	3.63±0.044	3.15

a) Errors are standard deviations. Errors in kinetic isotope effects are about 10% for each. [Substrate] = 2.20×10^{-2} mol dm^{-3}. [Model] = 1.00×10^{-3} mol dm^{-3}. b) dm^3 mol^{-1} min^{-1}. c) Data from ref. 2. d) [Substrate] = 4.50×10^{-2} mol dm^{-3}. [Model] = 2.30×10^{-3} mol dm^{-3}.

The present results indicate that **1a** and **1b** behave similarly to PNAH when magnesium ion is absent, whereas, in its presence, contribution of the initial electron transfer process to the rate-determining step becomes quite important in the reductions with **1a** and **1b**. It should be noted, however, that catalytic effect of magnesium ion in the reductions with **1a** and **1b** is as large as that in the reduction with PNAH. When the chain length becomes longer as seen in **1c**, the effect of phenyl group diminishes.

CPK-models for **1** suggest that the phenyl group in **1a** or **1b** locates very closely to the dihydropyridine ring. Thus, there is no doubt that a phenyl group closely situated to the dihydropyridine ring makes, by the assistance of magnesium ion, the movement of hydrogen nucleus easier. There are three possibilities to interpret the present result based on the reaction Scheme 1.

$$\mathrm{MH} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{MH}^{\ddagger}\mathrm{S}^{\bar{\cdot}} \xrightarrow{k_2} \mathrm{M}^{\cdot}\mathrm{HS}^{\cdot} \xrightarrow{k_3} \mathrm{M}^{+} + \mathrm{HS}^{-}, \qquad \mathrm{MH}^{\ddagger}\mathrm{S}^{\bar{\cdot}} \xrightarrow{k'_2} \mathrm{M}^{+} + \mathrm{HS}^{-} \tag{1}$$

where MH and M$^+$ represent reduced and oxidized forms of the models, respectively, and S and SH$^-$ denote the substrate and product, respectively.

1) The k_2 (or k'_2)-step is accelerated by magnesium ion remaining the k_1-step unchanged.

2) Although both k_1- and k_2 (or k'_2)-steps are accelerated, the latter is more affected than the former.

3) The k_1-step is decelerated remaining others unchanged.

Schematic energy diagrams for these three situations are illustrated in Fig. 1.

Although none of the possibilities has definite evidence to be proved or to be discarded, there arises no reason to rationalize catalytic effect of magnesium ion on the k_2 (or k'_2)-step. The effect must be specific only for the reductions with **1a** and **1b**, not with PNAH. Thus, the possibility 3) or its analogy seems to be most plausible.

In the reduction with PNAH in the presence of magnesium ion, complex between the magnesium ion and PNAH ligates a substrate forming a ternary complex (probably of charge transfer type), in which an electron is transferred from the dihydropyridine moiety to the substrate. In this way, the initial one-electron transfer process is catalyzed by magnesium ion.[2)] When PNAH is substituted by **1a** or **1b**, the phenyl group closely situated to the dihydropyridine moiety also ligates to the magnesium ion. Since the formation of complex was confirmed by the change in absorption at around

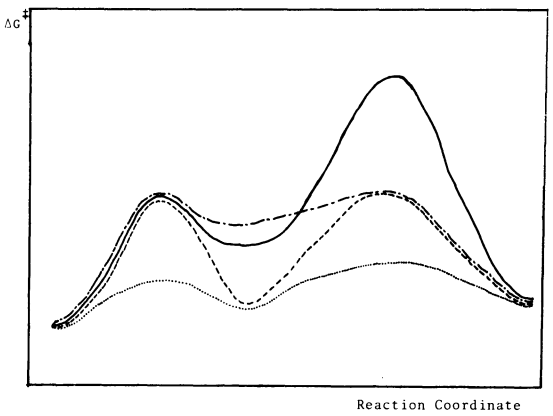
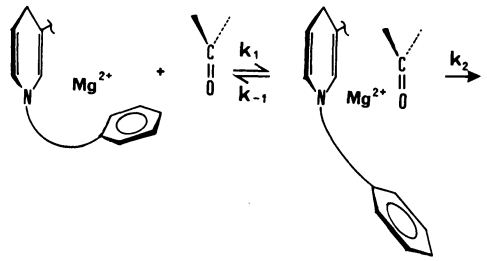


Fig. 1. Schematic energy diagram for the Reduction. The full line corresponds to the reduction without magnesium ion. Broken, dotted, and dashed lines correspond categories **1**, **2**, and **3** described in the text, respectively.



Scheme 1.

350 nm, there is no doubt that the magnesium ion primarily interacts with the dihydropyridine moiety. Nevertheless, the phenyl group in the model interrupts the approach of a substrate to form a composite ternary complex. The free-energy of transition state for the formation of initial intermediate, therefore, becomes higher than that with PNAH.

Once the intermediate is formed, on the other hand, competition takes place between the substrate and the phenyl group to ligate on the magnesium ion, and the free-energy level of the intermediate may be the same as that with PNAH (*cf.* Scheme 1). In other words, partition of the intermediate toward the reactants and products becomes less favored in the direction to the former. The energy level of the transition state for the k_2 (or k_2')-step, or migration of a hydrogen nucleus, is lowered and kinetic isotope effect becomes smaller.

Thermodynamic parameters listed in Table 1 indicate an interesting classification: although all models studied have equal free-energy of complexation, the enthalpy for **1a** is much smaller than those for others and the entropy compensates its minority. We do not have an appropriate explanation for this phenomenon. However, it seems probable that proximate phenyl group again interferes the approach of magnesium ion on complexation. The situation is improved in **1b** by the affinity of lone-pair electrons on sulfur.

Table 2 also shows that substitution of a methylene group in **1a** by a sulfur atom decreases the reactivity regardless the presence or absence of magnesium ion in the reaction system. The same trend was reported and the inhibitory effect of sulfur atom was attributed to the interference on the initial one-electron transfer process.[9] Characteristic absorption at around 350 nm from dihydropyridine moiety appears at shorter wavelength in **1b** than in other models (see Experimental), which suggests that **1b** is suffered from electronic distortion in its cyclic dienamine structure. Probably this is the reason of less ability to donate an electron to an acceptor. It is apparent, from Tables 1 and 2, that the sulfur substituent in the side-chain of model compound exerts different effects both in thermodynamics and kinetics from the corresponding methylene group. However, the proximity effect of the phenyl group is so large that present result alone is not sufficient to discuss on the effect of sulfur. Further and detailed discussion on the effect of sulfur substituent will be reserved for a forthcoming report from our laboratory.

Experimental

Melting and boiling points were not corrected.

Instruments. UV, NMR, and mass spectra were obtained on Union Giken SM-401, JEOL JNMFX-100, and Hewlett Packard 5992B GC/mass spectrometers, respectively. A Yanaco G-1800F was used for GLC analyses.

Materials. Acetonitrile was distilled over phosphorus pentaoxide five times and kept over potassium carbonate under argon atmosphere. The acetonitrile was refluxed over calcium hydride several hours, then distilled from the same pot under argon atmosphere prior to the use. Magnesium perchlorate was dried over phosphorus pentaoxide for a couple of days under reduced pressure at 400 K. The water content in a solution which was subjected to the kinetic or thermodynamic measurement was determined on GLC (30% bis(2-cyanoethyl) ether, 1 m, 313 K) prior and after the experiment and found to be less than 0.075% v/v.

1-Propyl-1,4-dihydronicotinamide (PNAH) and PNAH-4-*d* were prepared as described previously.[10] 1-(3-phenylpropyl)-1,4-dihydronicotinamide (**1a**), 1-(2-phenylthioethyl)-1,4-dihydronicotinamide (**1b**), and 1-(4-phenylthiobutyl)-1,4-dihydronicotinamide (**1c**) and their 4-deuterated analogs were prepared similarly. Deuterium contents were measured on GC/MS and NMR to be more than 99.5% for all deuterated compounds.

1a: λ_{max}=232 nm (ε=5.13×10³) and 352 nm (ε=5.36×10³) in acetonitrile at 303 K. Found: C, 74.05; H, 7.59; N, 11.42%. Calcd for $C_{15}H_{18}N_2O$: C, 74.35; H, 7.49; N, 11.56%.

1b: λ_{max}=254 nm (ε=8.48×10³) and 347 nm (ε=5.41×10³) in acetonitrile at 303 K. Found: C, 64.51; H, 6.26; N, 10.71%. Calcd for $C_{14}H_{16}N_2OS$: C, 64.59; H, 6.19; N, 10.76%.

1c: λ_{max}=256 nm (ε=1.01×10⁴) and 351 nm (ε=5.45×10³) in acetonitrile at 303 K. Found: C, 66.35; H, 7.00; N, 9.88%. Calcd for $C_{16}H_{20}N_2OS$: C, 66.63; H, 6.99; N, 9.71%.

m-Nitro-α,α,α-trifluoroacetophenone (**2**) was prepared according to a literature procedure.[11]

Kinetic Procedure. Solutions for kinetic studies were prepared under an atmosphere of argon and placed in a UV cell (0.1 dm) equipped with a silicon rubber stopper. The cell compartment of the spectrometer was also filled with dry argon and kept at 323±0.05 K. As a standard procedure, both sample and reference celles were filled with solutions of a substrate (and magnesium perchlorate, when necessary) in acetonitrile to obtain a difference spectrum. Then an acetonitrile solution of a model compound was injected into the sample cell by using a syringe to start the reaction.

The kinetics was followed by observing the decrease in the

intensity at 380 nm from a model. The spectrometer was attached by an NEC PC-8001 computor and absorptions at appropriate time intervals were monitored over 3 half-lives through the computer. One observed absorption was the avarage of 80 consecutive input. More than 50 points were monitored for a run. The data were automatically subjected to the least-squares treatment to give a pseudo-first order rate constant. Standard deviation and correlation coefficient for each run were better than ±1% (better than ±0.75% in most runs) and 0.9999, respectively. More than 5 runs were averaged to obtain rate constants listed in Table 2.

Thermodynamic Parameters. Equilibrium constants at appropriate temperatures were obtained by similar procedure as described above and according to the equation proposed by Creighton.[12]

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