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NAD(P)$^+$-NAD(P)H MODEL. 46. KINETIC STUDY ON THE REDUCTION WITH A SULFUR-CONTAINING NAD(P)H MODEL

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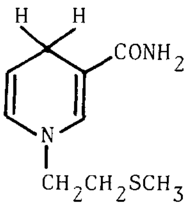
Summary: Kinetics on the reduction with an NAD(P)H model bearing a divalent sulfur atom in the N-alkyl substituent has been studied. The sulfur atom retards the electron-transfer process.

NAD(P)$^+$-dependent dehydrogenases have an indispensable nicotinamide moiety for biochemical transformations. Recently, many organic chemists challenged to create biomimetic systems using simple N-alkyl-1,4-dihydronicotinamide derivatives as NAD(P)H models. Our investigations on NAD(P)H models have revealed a catalytic effect of divalent metal ions such as magnesium and zinc ions in the reductions of carbonyl compounds(*1*). Some natural dehydrogenases, on the other hand, have an essential zinc ion fused by thiol groups from cysteine residues at the active site(*2*). It is important, therefore, to study the effect of sulfur atom on an NAD(P)H model molecule in biomimetic reduction, and we synthesized N-(β-methylthioethyl)-1,4-dihydronicotinamide (1)(*3*). In this letter, we wish to report kinetics for the reduction of substituted



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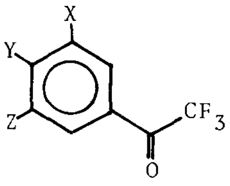

2

a: X = Y = Z = H
b: X = Z = H; Y = Cl
c: X = CF_3; Y = Z = H
d: X = NO_2; Y = Z = H
e: X = NO_2; Y = Cl; Z = H
f: X = CF_3; Y = H; Z = NO_2

and unsubstituted α,α,α-trifluoroacetophenones (2) with 1 in the presence and absence of magnesium perchlorate.

A typical procedure for the kinetics is as follows: A solution (3 ml) of 2a (3.51×10^{-1} M) containing anhydrous $Mg(ClO_4)_2$ (2.71×10^{-2} M) in dry acetonitrile was prepared under an atmosphere of argon and placed in a UV cell sealed by a silicon rubber stopper. The UV cell was kept at 50°C in the cell compartment through the kinetic run. Then, 5μl of acetonitrile solution of 1 was injected into the sample cell (1.58×10^{-4} M) to start the reaction, and decrease in absorption due to 1 at 373 nm was monitored. The obtained first-order rate constant was corrected for the rate constant for the magnesium ion-catalyzed auto-decomposition of 1. A second-order rate constant, k, was calculated by dividing the corrected value by the concentration of 2a. Other kinetic runs were carried out similarly(*4,5*). It was confirmed that all rates were first-order in 1, first-order in 2, and zero-order in $Mg(ClO_4)_2$. The results are summarized in Table 1. Rate constants for reductions with 1-4-*d* were also obtained and kinetic isotope effects, k^H/k^D, were calculated according with Steffens and Chipman's equation(*6,7*). The values of kinetic isotope effects are listed in Table 2.

Figure 1 shows Hammett plots for magnesium ion-catalyzed and uncatalyzed reductions. A good straight line with $\rho = 2.62$ (correlation coefficient $\gamma = 0.998$) is obtained for uncatalyzed reductions, whereas the plot for catalyzed reductions appears as two straight lines with $\rho = 1.50$ ($\gamma = 0.999$) for substrates with relatively less electron-withdrawing substituents and $\rho = 0.768$ ($\gamma = 0.9998$) for those with strongly electron-withdrawing substituents. In Figure 2, the values of k^H/k^D for the reductions are plotted against substituent σ-values. While, for the catalyzed reductions of strongly electron-deficient substrates, the values of k^H/k^D increase with the increase in σ-value, only a small increase in k^H/k^D is observed for the uncatalyzed reductions of the corresponding substrates.

It should be noticed that the present result is a striking contrast to the kinetics for reductions with N-propyl-1,4-dihydronicotinamide (PNAH) which contains no sulfur atom in the side-chain. In the reductions of 2 with PNAH(*1i*), the Hammett plot was rather linear and k^H/k^D remained constant throughout the substituents studied for the catalyzed reductions though two straight lines and initial increase in k^H/k^D were observed for the uncatalyzed reductions. We have proposed a mechanism which involves a successive electron-proton-electron transfer process for the reduction(*1e,1f,1i,4*). Thus, the observation for catalyzed reductions with PNAH was interpreted in terms of less contribution of the initial electron-transfer process for the rate-determining step(*1i*). According to such conception, the present reduction with sulfur-containing NAD(P)H model, 1, should have a higher energy level in the initial electron-transfer step than that with PNAH.

Table 1. Rate Constants for the Reductions of 2 with 1.[a)]

Sub-strate	k, $M^{-1}sec^{-1}$ with Mg^{2+}	without Mg^{2+}
2a	0.0270	——[b)]
2b	0.0660	——[b)]
2c	0.122	0.0222
2d	0.321	0.0995
2e	0.474	——[c)]
2f	0.676	1.56

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a) At 50°C in acetonitrile.

b) Too slow to be observed.

c) Side reaction occured at benzene-ring of the substrate(*1i*).

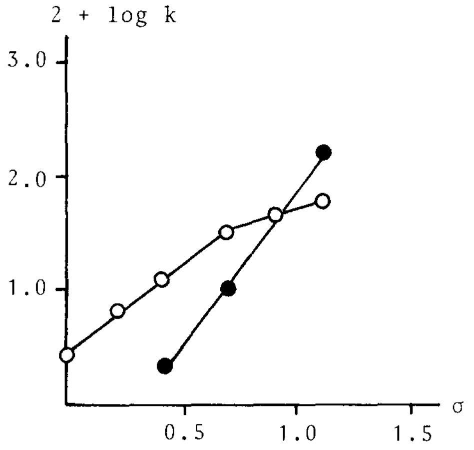

Figure 1. Hammett plots for the reduction of 2 with 1 in the presence (o) and absence (●) of Mg^{2+}

Table 2. Isotope Effects for the Reductions of 2 with 1.[a)]

Sub-strate	k^H/k^D with Mg^{2+}	without Mg^{2+}
2a	3.11	
2c		2.43
2d	3.13	2.41
2e	4.31	
2f	5.51	3.29

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a) At 50°C in acetonitrile

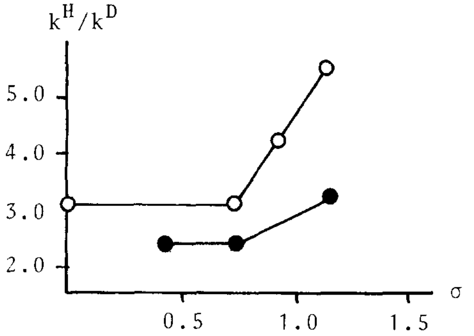

Figure 2. Plots of isotope effects against σ-values in the presence (o) and absence (●) of Mg^{2+}

Table 3. Kinetic Parameters for the Reductions with 1[a)]

Substrate	$\Delta H^{\ddagger}$ kJ/mol	$\Delta S^{\ddagger}$ J/deg.mol	$\Delta G^{\ddagger}$ kJ/mol
2a	56.0	-151.2	104.9
2d	48.5	-155.5	98.6
2f	40.4	-173.5	96.6

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a) Values at 50°C.

In order to evaluate this argument, kinetic parameters were estimated from rate constants at four different temperatures (30 , 40 , 50 , and 60 °C) for the reductions of 2a, 2d, and 2f in the presence of magnesium perchlorate. Whereas the free energy of activation remains almost constant for these three substrates, a less dependence on entropy is observed for the reductions of 2a and 2d as compared with that of 2f. If the reduction proceeded through a one-step hydride transfer mechanism, the transition-state from the more electron-deficient substrate should resemble to the reactant more than that from less electron-deficient substrate. The result indicates that a charge-transfer-type interaction between two reactants plays an important role, and the interaction becomes more important as the substrate becomes more electron-deficient.

Although diminished reactivity of 1 compared with that of PNAH was unfortunately observed in the reduction, a noticeable effect of sulfur atom appeared in the substituent effect for reactivity and kinetic isotope effect. This result may imply an enzymic regulation in a living system. Further studies are in progress to establish the steric and electronic effects of sulfur atom in NAD(P)H models.

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3. Elemental analyses and spectral data gave satisfactory results. The corresponding pyridinium salt was prepared by Shiffrin's method; S. Shiffrin, *Biochemistry*, 3, 829 (1964).
4. All runs gave excellent first-order plots over three-half lives with correlation coefficients better than 0.9999.
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