Reduction by a Model of NAD(P)H. XIV. Mechanistic Consideration on the Role of Metal Ion

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(Received November 18, 1976)

The driving force for the catalytic activity of metal ions on the reduction of α-keto esters with an NAD(P)H-model compound has been discussed. The scope of the reaction and spectroscopic investigations as well as molecular orbital consideration have revealed that the transition state of the reaction consists of a ternary complex in analogy with a coenzyme–enzye–substrate complex in an enzymic system. It is concluded that, at the transition state, one electron migrates from a model compound to a substrate through a metal ion, which is followed by the transfer of a proton.

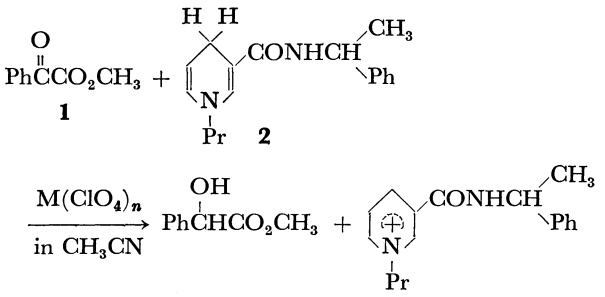
Dihydropyridine nucleotides, NADH and NADPH, are coenzymes which are widely co-operating with dehydrogenases. It has been acepted that the reaction with NAD(P)H proceeds with a one-step hydride transfer from the coenzyme to a substrate.[1,2] On the other hand, recent model reactions revealed that the reduction involves at least one intermediate,[3,4] and that there appear ion-radicals during the reduction.[5,6] ESR signals were also observed with enzymic systems.[7] Yet, the mechanism or the driving force of the reduction with NAD(P)H or its model compounds has not been understood.

In a series of investigations, we found that 1-benzyl-1,4-dihydronicotinamide (BNAH) or its analogs reduces α-keto esters in acetonitrile in the presence of a bivalent metal ion such as magnesium or zinc perchlorate.[8] When the amide-nitrogen is substituted by a chiral group, asymmetric reduction takes place.[8] The ratio of the concentration of metal perchlorate to that of the model compound, but not the absolute concentration of metal perchlorate, influences the optical yield of the product.[9]

The metal ion in the present model reaction may be regarded as a mimetic enzyme in the sense that it catalyzes the reaction as does an enzyme in biological reactions, and the information for the mechanism of the model reaction may provide an insight into the mechanism of enzymic reactions. The purpose of the present paper is focused to elucidate the role of metal-ion catalysts.

Results

Reaction. As was reported, the reaction of methyl benzoylformate (**1**) with *R*-(−)- or *S*-(+)-*N*-(α-methylbenzyl)-1-propyl-1,4-dihydronicotinamide (**2**) afforded *R*-(−)- or *S*-(+)-methyl mandelate, respectively, in quantitative chemical yield with about 15% enantiomeric excess, in the presence of equimolar amount of magnesium perchlorate.[10] When magnesium perchlorate was substituted by lithium perchlorate, the catalytic efficiency decreased remarkably and 6 to 8 molar excess of the lithium salt resulted in the formation



of methyl mandelate in only 60—70% yield. Moreover, it is surprising that the reaction with lithium perchlorate is non-stereospecific. Although less than 1% of enantiomeric excess was observed with the lithium salt, the value was well within the experimental error of ±2%. Tetraethylammonium perchlorate was ineffective to promote the reaction. The reaction was not catalyzed by acetylacetonate magnesium or tris(3-trifluoroacetyl-*d*-camphor)europium, Eu(TFAC)$_3$, effectively; 10 and 12% reductions were observed with these catalysts, respectively.

TABLE 1. ELECTRONIC SPECTRA OF *N*-(α-METHYLBENZYL)-1-PROPYL-1,4-DIHYDRONICOTINAMIDE (**2**) IN ACETONITRILE IN THE ABSENCE OR PRESENCE OF MAGNESIUM PERCHLORATE

$([Mg^{2+}]/[\mathbf{2}])$	λ_{max}, nm	ε_{max}
0	351	7765
0.25	352	7647
0.50	352	7718
1.0	353	7605
2.0	354	7824
3.0	354	8047
4.0	355	7906
6.0	356	8194
8.0	357	8194
400	367	—

Spectroscopy. The absorption maximum of **2** at 351 nm shifted toward the region of longer wave-length with the addition of magnesium perchlorate as listed in Table 1. With lithium perchlorate, no shift was observed up to the ratio of 10. However, 18 nm of the shift was recorded at the point of $[Li^+]/[\mathbf{2}]=400$. Tetraethylammonium perchlorate did not change the

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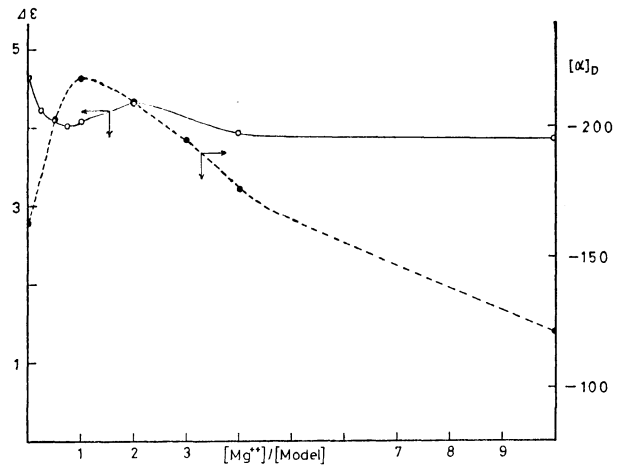

Fig. 1. Variations of $[\alpha]_D$ (----) and maximum intensity in CD spectrum (——) of *N*-(α-methylbenzyl)-1-propyl-1,4-dihydronicotinamide (**2**) as a function of $[Mg^{2+}]/[2]$ (in CH_3CN).

spectrum even at its saturated concentration. The dependencies of the intensity of the CD spectrum, observed at around 350 nm, and $[\alpha]_D$ of **2** on the molar ratio are illustrated in Fig. 1. Chemical shifts of protons in NMR spectrum of **2** were not altered significantly by the addition of magnesium perchlorate.[11] Remarkable shift was observed, however, with the addition of chiral shift reagents, $Eu(TFAC)_3$ and its ytterbium analog. Nevertheless, the protons at the chiral and prochiral centers in *R*- and *S*-**2** behaved similarly (Fig. 2). No appreciable difference was detected from IR spectra of **2** with and without magnesium perchlorate.

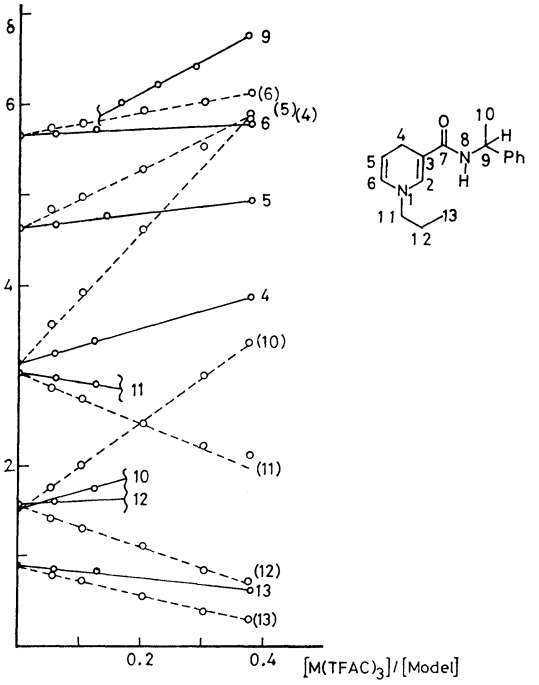

Fig. 2. Variations of chemical shift of protons in *N*-(α-methylbenzyl)-1-propyl-1,4-dihydronicotinamide (**2**) as functions of $[Eu(TFAC)_3]/[2]$ (——) and $[Yb(TFAC)_3]/[2]$ (----) (in $CDCl_3$).

All spectra were recorded with acetonitrile solutions and were not affected by the addition of **1**. The electronic and vibrational spectra of **1** remained unchanged in the presence of magnesium ion.

Discussion

The absorption at around 350 nm is attributed to the π,π^* transition of the dihydropyridine moiety.[12,13] It should be noted that only closed (ring) enamine-structure can account for the absorption at such a long wavelength as 350 nm.[12] All spectral data are in accord with the concept that the metal ion coordinates onto the dihydropyridine moiety instead of the amide-carbonyl; the absorption at 350 nm was affected by the addition of magnesium ion; but not by the addition of tetraethylammonium ion; only slight difference was observed in the CD spectrum; the chiral NMR shift reagent did not discriminate the chiral protons; no shift was observed for the stretching frequencies of the amide–C–O, C=N, and N–H groups; the fact that the band at 350 nm is the longest-wavelength absorption indicates that the π-orbital of the dihydropyridine ring is the highest-occupied molecular orbital (HOMO) of **2**. Coordination of metal ions onto the pyridine ring of nicotinamide has also been witnessed.[14,15] The meaning of minimum and maxima in curves shown in Fig. 1 is equivocal because the absorption spectra did not show an isosbestic point to calculate the dissociation constant of the complex. A complex containing four moelcules of BNAH coordinated onto a magnesium ion has been isolated from an acetonitrile solution.[11] It is also reported that the rate *vs.* molar ratio, $[Mg^{2+}]/[BNAH]$, profile for the reaction of BNAH with 2-benzoylpyridine has a maximum at the ratio of 0.4.[11]

Since the prochiral C_4-protons were shifted in the same extent by the chiral NMR shift reagent, it is apparent that the coordination does not introduce the chirality at the C_4-position, or the C_4-protons still remain to be prochiral in the coordination complex.

The red-shift of the absorption maximum at 350 nm indicates that the decrease of the energy level of the lowest-unoccupied molecular orbital (LUMO) of **2**, by the influence of magnesium ion, is larger than that of the HOMO.[17] Consequently, the hyperconjugative participation of the C_4–H σ-bond to the π-orbital of the dihydropyridine moiety (or, more precisely, the contribution of the C_4–H σ-bond to the molecular orbital which is mainly constituted by π-orbitals of dihydropyridine moiety) becomes larger when magnesium ion coordinates onto the ring. The importance of the C_4-moiety was emphasized at the beginning of this discussion. Under such a circumstance, it is highly unlikely to expect that a hydride ion dissociates from the C_4-position, whereas a proton may easily dissociate because of the developing positive charge on the dihydropyridine ring in the complex. The localization of large positive-charge density on the C_4-protons is also proved by the large down-field shift of the corresponding NMR signal on complexation of **2** with $Yb(TFAC)_3$.[16]

On the other hand, molecular orbital theories have proposed that a positive charge operates to decrease

the energy level of the LUMO of a carbonyl group so that the carbonyl group is activated to accept an electron.[17-19] Since magnesium ion is a bivalent cation, a one-to-one complex of **2** and magnesium ion remains one more positive charge on the magnesium ion formally, which, in the present reaction, may play to activate the substrate, **1**.[20] In other words, an electron migrates from **2** to **1** in the transition-state ternary-complex, **2**-Mg^{2+}-**1**, which is followed by the migration of a proton from the cation-radical of **2** to the anion-radical of **1**.[21,22] The transfer of an electron may or may not precede the migration of a proton. What is the most important here is that an electron and proton move separately and the movement of an electron triggers the migration of a proton. Catalytic activity of alkali and alkaline earth metal ions in an electron-transfer process has been reported and discussed in relation to the polarizabilities of metal ions and ligands.[23] The same kinetic deuterium isotope effect for an electron-transfer process[25] and for the reduction of an organic substrate ($\approx$1.7)[4] as well as large and similar isotope partitioning ratios in products from various reductions ($\approx$4)[1,3,4] also suggest the existence of two distinguishable processes for electron- and proton-migrations.[25] The migration of a second electron seems to take place almost spontaneously, because the pyridinyl radical can gain large stabilization energy by converting into the pyridinium ion.[26] Thus, the chirality in **2** is recognized by **1** in a complex, or with intramolecular fashion. When magnesium ion is substituted by univalent lithium ion, on the other hand, the corresponding binary complex, **2**-Li^+, remains no positive charge on the metal ion and another lithium ion (or ions) has to be used to activate the substrate. In this case, therefore, the reaction takes place bimolecularly and the transition state is so loose that the chirarity in **2** cannot be recognized by **1**. The idea of a positive-charge-promoted reaction discussed above is supported by the inability of tetraethylammonium and chelated metal ions to catalyze the reaction.[20] The dependency of the optical yield on the molar ratio of $[Mg^{2+}]/[\mathbf{2}]$[9] can also be interpreted with the present proposal: under the condition of $[Mg^{2+}]/[\mathbf{2}]<1$, is favored the formation of complexes composed of a magnesium ion and more than two molecules of **2**. The positive charge in such a complex is so diffused that it has no facility to activate the substrate and the situation becomes similar to that with lithium perchlorate.

The scheme for the stereospecific reaction may be represented as follows:

$$MgX_2 \rightleftharpoons MgX^+ + X^-,$$

$$NH + MgX^+ \rightleftharpoons (NH\cdot MgX)^+,$$

$$(NH\cdot MgX)^+ + S \longrightarrow [(NH\cdot Mg\cdot S)^{2+}]^{2+} + X^-$$

$$\downarrow$$

$$N^+ + HSMg^+$$

$$2HSMg^+ + 2X^- \rightleftharpoons Mg(SH)_2 + MgX_2$$

where X, NH, N^+, S, and SH are perchlorate ion, reduced form of a coenzyme-model, oxidized form of a coenzyme-model, α-keto ester, and the anion of α-hydroxy ester, respectively. The double dagger stands for the transition state, which may be depicted as shown in Fig. 3. It seems better, as a minor effect, to take into account the interaction between the amide-carbonyl group and magnesium ion, because the stereospecificity of the reaction depends on the basisity of the carbonyl-oxygen.[27]

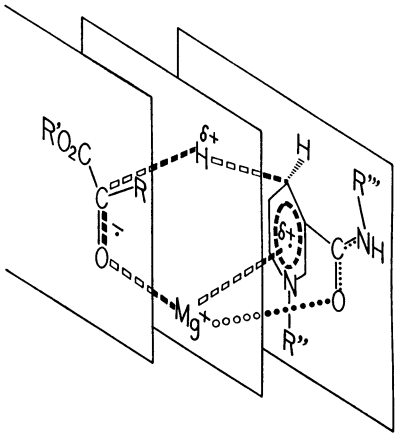

Fig. 3. Schematic representation of the transition state of the reaction.

Mechanistic studies so far reported have had no facility to discriminate the one-step hydride transfer and three-step electron-proton-electron transfer mechanisms and the present paper is the first one to deal with the detailed interaction of molecules at the transition state of the reaction in favor of the latter process. Kinetic studies will provide further support for the discussed concept and the research in our laboratories is in progress toward this end.

Experimental

Preparation and purification of materials and general procedure of the reduction were described previously.[10] Tris(3-trifluoroacetyl-*d*-camphor)-europium and -ytterbium were prepared according to the literature.[28]

UV, IR, NMR, and CD spectra were recorded on Union Giken SM-401, Hitachi EPI-S2, Varian T-60, and Union Giken CD-1000 spectrometers, respectively. Optical rotations were observed with a JASCO DIP-180 automatic polarimeter.

Support for a part of this research by the Ministry of Education, Japanese Government, with a Scientific Research Grant is acknowledged. The authors also wish to thank Dr. S. Inagaki of Gifu University for fruitful discussions on molecular orbital theory.

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