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Coenzyme Models. 18. Metal Catalysis of NADH Model Reduction in Aqueous Systems

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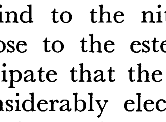
Metal ions (Ni^{2+} and Zn^{2+}) affected the NADH model reduction of 2-pyridinecarbaldehyde and Schiff bases in aqueous systems. In particular, the rate of the dihydroquinoline reduction of *N*-(2-pyridylmethylene)-2-pyridylmethylamine was augmented by more than 200-fold, compared with that in the absence of metal ions. On the other hand, the reduction of benzil, ethyl benzoylformate, and trifluoroacetophenone was not subject to the metal catalysis. The enhanced reactivity is accounted for by the affinity of Schiff bases toward metal ions. The efficiency of the metal catalysis was compared with that of the proton catalysis. This is the first example of the systematic examination of the metal catalysis in aqueous systems.

It is known that some alcohol dehydrogenases contain NAD^+ and Zn^{2+} ion at the active sites and catalyze the interconversion of aldehydes and alcohols coupled to the oxidation-reduction of NAD^+ coenzyme.[1] The role of Zn^{2+} ion, as well as that of NAD^+, has long been a subject of investigation and debate. Recent model studies have established that metal ion not only activates substrates and/or NADH analogues but also helps retaining stereoselective hydrogen transfer from NADH analogues to substrates.[2–6] These successful demonstration in the model system may provide the information relevant to biological NADH dependent oxidation-reduction, but the metal catalysis has been confined strictly to aprotic solvents (*e.g.*, acetonitrile). In fact, it has often been mentioned that metal ion does not act as catalyst in aqueous NADH model reduction.[5,6]

It is proposed that the transition state of NADH reduction is analogous to that pictured for S_N2-type nucleophilic reaction,[7,8] although the detailed mechanism is still a matter of some controversy.[9–12] As for the S_N2-type nucleophilic reaction, Buckingham[13,14] and others[15–17] have reported many examples of metal catalysis in aqueous solutions. For example, metal ions such as Cu^{2+}, Co^{2+}, Ni^{2+}, and Zn^{2+} are good catalysts for the ester hydrolysis of α-amino acids, the rate augmentation amounting to 10^3—10^6 fold. The marked rate acceleration is explained by such that the metal ion can bind to the nitrogen and at the same time remain close to the ester carbonyl group. One would thus anticipate that the metal coordinated substrate may be considerably electrophilic and may serve as a good hydride (or its equivalent) acceptor. The object of the present investigation has been to search for the metal-catalyzed NADH model reduction of the C=O and C=N double bonds by an acid-stable NADH analogue, 1-benzyl-1,4-dihydro-3-quinolinecarboxamide (BzlQH).[18] We thus found that the reduction of Schiff base is remarkably facilitated by added metal ions (Ni^{2+} and Zn^{2+}).

BzlQH;


Experimental

Materials. The preparation of BzlQH has been described elsewhere.[19] 2-Pyridylmethylamine dihydrochloride was prepared by Dr. M. Ohnishi (Nagasaki University). Other reagents (trifluoroacetophenone, ethyl benzoylformate, 2-pyridinecarbaldehyde, and benzil) were purified by distillation or recrystallization before use.

Kinetics. Kinetic measurements were carried out at 50±0.1 °C in a 2.2%(v/v) aqueous acetonitrile solution with an ionic strength of 0.2(KCl). The progress of the reaction was followed spectrophotometrically by monitoring the decrease of BzlQH at λ_{max} (343 nm). The details of the method were described in a previous paper of this series.[19] Since excess substrate was present in all the cases, the pseudo first-order behavior was observed for up to 3-half-lives. In the case where the reaction rate was extremely slow, the rate constans were estimated from the initial slope of the reaction. Here, the overall first-order rate constants (k_{obsd}) is expressed by Eq. 1,

$$k_{obsd} = k_d + k_r \tag{1}$$

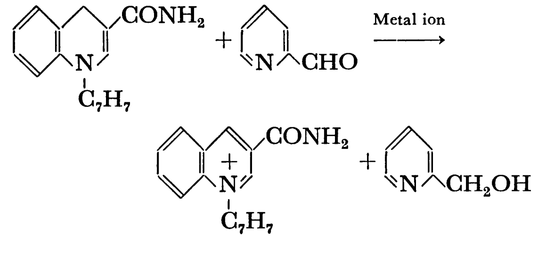
where k_d is the acid-catalyzed (spontaneous) decomposition of BzlQH and k_r is the pseudo first-order rate constants for the reduction reaction. The k_d value was determined separately from the disapperance of the absorption of BzlQH in the absence of substrate, and the k_r value was determined from Eq. 1. Since the k_r value is linearly correlated to substrate concentrations ([substrate]=(1—5)$\times 10^{-3}$ M, $[Zn^{2+}]$=(0—5)$\times 10^{-3}$M), the apparent second-order rate constants ($k_{2'}$) are given by $k_{2'}=k_r/[\text{substrate}]$.

Results and Discussion

Suppressed Catalytic Activity of Metal Ions in Aqueous Systems. Dihydronicotinamides in dry acetonitrile interact strongly with Mg^{2+} ion.[20,21] The interaction is substantiated by bathochromic shifts of the absorption maxima. The absorption maximum of BzlQH in acetonitrile (335 nm, ε_{max}=11900 $M^{-1}\,cm^{-1}$) also shifted to longer wavelength on the addition of Mg^{2+} ion (346 nm, ε_{max}=14700 $M^{-1}\,cm^{-1}$, $[Mg(ClO_4)_2]$=1.0×10^{-2} M). On the other hand, the spectrum of BzlQH in an aqueous system (λ_{max} 343 nm, ε_{max}=13100 $M^{-1}\,cm^{-1}$) was hardly affected by added metal ions ($[Ni^{2+}]=[Zn^{2+}]$=(2.5—5.0)$\times10^{-3}$ M at pH 4—9). The contrasting results indicate that the metal ion in aqueous solutions is deactivated due to strong solvation.

Benzil, ethyl benzylformate, and trifluoroacetophenone are slowly reduced by BzlQH in an acidic aqueous solution (pH 4.0). The dihydronicotinamide reduction of these substrates in aprotic solvents is efficiently catalyzed by Mg^{2+} ion,[2,4,25,26] whereas the aqueous BzlQH reduction was uncatalyzed by added metal ion ([metal ion (Ni^{2+} or Zn^{2+})]=*ca.* 5×10^{-3} M, pH 4—9). It is presumed that neither BzlQH nor substrate interacts with metal ions during the reduction process. Supposedly, one should employ more coordinative substrates to substantiate the metal catalysis in an aqueous solution.

Reduction of 2-Pyridinecarbaldehyde(2-PyCHO). 2-Acylpyridines are frequently employed in the model reduction in aprotic solvents.[4,27] This is probably due to the high coordination ability of 2-acylpyridines. 2-Acylpyridines are presumed to form the relatively stable complexes with metal ions in an aqueous system,[15] so that it would meet the requirement of the present study. Tagaki and coworkers[28] previously reported the reaction of 1,4-dihydronicotinamide with 2-PyCHO. They found, contrary to their expectation, that the isolated is a covalent adduct which is formed by a nucleophilic attack of 5-position of 1,4-dihydronicotinamide on the carbonyl carbon of 2-PyCHO. BzlQH has within the molecule the basic structure of 1,4-dihydronicotinamide, but the adduct-forming 5-position is involved in the fused ring. Thus, the formation of the adduct is almost unconceivable. Spectral examination of the reaction mixture([2-PyCHO]=1.0×10^{-4} M, [BzlQH]=5.0×10^{-5} M, [Zn^{2+}]=(0—1) $\times10^{-2}$ M, pH 4.7) established that the decrease of the absorption band of BzlQH (343 nm) is accompanied by the appearance of products with 215 and 250 nm which may be compared with the spectrum of a mixture of BzlQ$^+$ and 2-(hydroxymethyl)pyridine (209 and 244 nm). In fact, we have found that 2-PyCHO is reduced by BzlQH in 58—65% yield to corresponding alcohol.[29]



(2)

The apparent second-order rate constants (k_2') for BzlQH reduction of 2-PyCHO are plotted against metal concentrations (Fig. 1). In the alkaline pH region (pH 8.7), the rate constants could not be determined due to the ready precipitation of metal ions. The precipitation was not observed in the acidic pH region (pH 4.7), but the reaction was rather retarded by added metal ions. According to Pocker and Meany,[30] divalent metal ions such as Zn^{2+} and Co^{2+} exhibits a dramatic catalytic effect on the hydration of 2-PyCHO. It is expected, therefore, that the rate suppression may be rationalized in terms of the decrease in the fraction of free ketone. Since the hydration causes the diminution of the absorbancy of 2-PyCHO at around 260 nm,[30] the optical density(OD) was measured as a function of metal ion concentrations. As shown in Fig. 2, however, the absorption band is affected to a small extent (less than 10%) by added metal ions, indicating that the fraction of free ketone is maintained almost constant. Thus, the rate retardation cannot be attributed wholly to the hydration of 2-PyCHO.

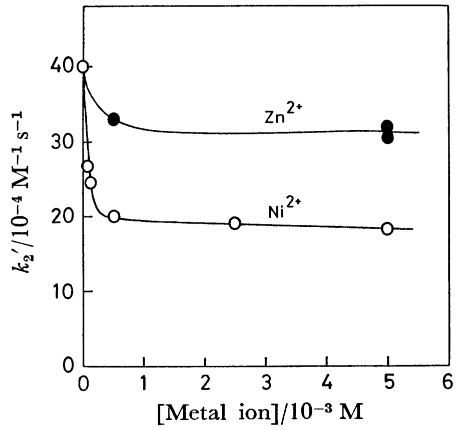


Fig. 1. Metal ion catalysis of the BzlQH reduction of 2-pyridinecarbaldehyde. [2-PyCHO]=5.00×10^{-3} M, [BzlQH]=5.0×10^{-5} M. The pH of the reaction media was maintained with 0.02 M acetate (pH 4.7).

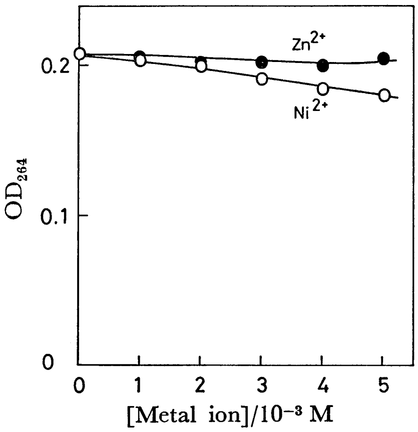
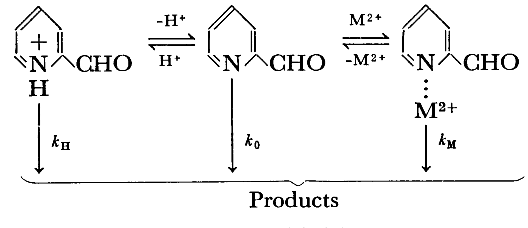


Fig. 2. Hydration equilibrium of 2-pyridinecarbaldehyde: optical density at 264 nm plotted against the concentration of metal ions. pH 4.7 with 0.02 M acetate buffer, [2-PyCHO]=5.0×10^{-5} M.

As mentioned above, 2-PyCHO(pK_a 3.8)[31] in acetate buffer is reducible by BzlQH in good yields. If *N*-protonated 2-PyCHO which is presumed to be the active species in the acidic pH region exhibits the reactivity greater than metal-coordinated 2-PyCHO (*i.e.*, $k_H > k_M \gg k_0$ in Eq. 3), added metal ions would suppress the observed reduction rates.



(3)

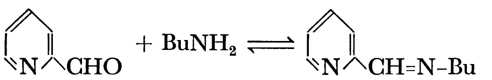
Ni^{2+} ion caused the inhibition to a greater extent than Zn^{2+} ion. The trend is compatible with the general order of the stability constants.[17,32] Thus, the above explanation seems most likely at present.

Here, one may notice a dilemma occurring in the metal catalysis in aqueous systems. As mentioned above, stability constants of carbonyl substrates such as ethyl benzoylformate and benzil are extremely small.[32] Although 2-PyCHO in which a nitrogen base is closely fixed near the carbonyl group captures the metal ion to a significant extent, *N*-protonation is more effective than metal coordination.

The above summary suggests that the metal ion catalysis would become effective only for the substrate the C=X double bond of which is able to bind metal ions strongly. Schiff base is the case.[32]

Reduction of Schiff Bases of 2-PyCHO. NADH model reduction of Schiff bases in organic solvents was reported earlier by Pandit *et al.*[33] and Shinkai *et al.*,[8] but there is no precedent for the reduction in an aqueous system.

The spectroscopic measurements indicated that the addition of butylamine ($BuNH_2$) to the basic solution of PyCHO (pH 8.9) enhances the absorption band near 350 nm which is characteristic of the Schiff base.[34]



(4)

For example, the OD increase of 0.082 was recorded when butylamine (1.0×10^{-2} M) was added to the solution of 2-PyCHO (5.0×10^{-3} M) at pH 8.9. On the other hand, the formation of the Schiff base was hardly detected in an acetate buffer solution (pH 4.7). In Fig. 3, the OD value at 350 nm was plotted against the concentration of metal ions. The OD value was enhanced gradually with increasing metal ion concentrations and finally reached a plateau. In case 2-pyridylmethylamine ($PyCH_2NH_2$) was employed as an amine counterpart the absorption band was enhanced at the very low concentration of metal ions, and the solution containing Ni^{2+} ion became yellow (or orange at the higher concentrations). The result endorses that the Schiff base (particularly, *N*-(2-pyridylmethylene)-2-pyridylmethylamine) efficiently interacts with metal ions in an aqueous solution.

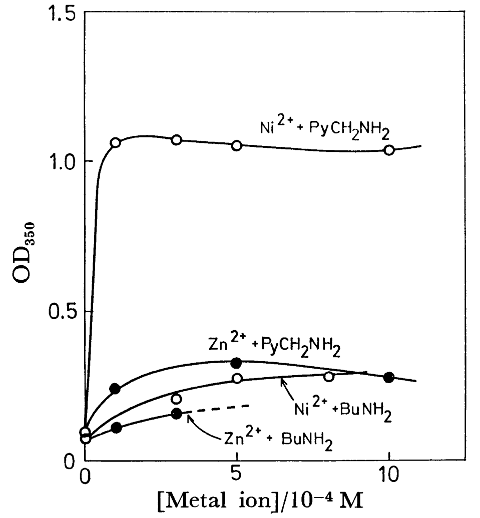


Fig. 3. Interaction of Schiff bases with metal ions: optical density at 350 nm plotted against the concentration of metal ions. pH 8.9 with 0.02 M borate buffer, [2-PyCHO]=5.00×10^{-3} M, [$BuNH_2$]=[$PyCH_2NH_2$]=1.00×10^{-2} M. The dotted line indicates the precipitation.

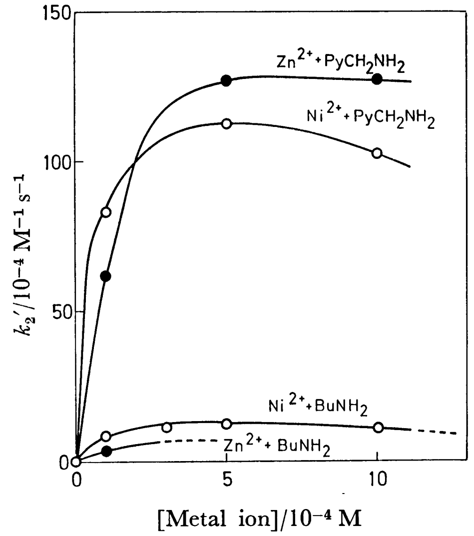
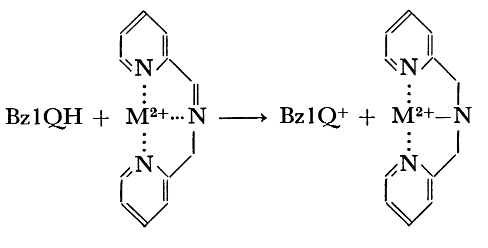


Fig. 4. Metal ion catalysis of the BzlQH reduction of Schiff bases. pH 8.9 with 0.02 M borate buffer, [2-PyCHO]=5.00×10^{-5} M, [$BuNH_2$]=[$PyCH_2NH_2$]=1.00×10^{-2} M. The dotted line indicates the precipitation.

The metal-catalyzed reduction was carried out in basic pH region (pH 8.9). The resultant second-order rate constants (k_2') are plotted as a functions of metal ion concentrations (Fig. 4). The BzlQH reduction was undetectable in the absence of metal ions, indicating that the Schiff base itself is insensitive to the BzlQH reduction. As seen in Fig. 4, metal ions efficiently catalyzed the BzlQH reduction. The plots showed rapid increase at low metal concentrations (1×10^{-4} M) followed by a gradual rate saturation. In particular, the reduction of *N*-(2-pyridylmethylene)-2-pyridylmethylamine, the Schiff base from 2-pyridinecarbaldehyde and 2-pyridylmethylamine, was remarkably accelerated. Since the rate constant in no metal system is estimated to be less than $5\times10^{-5}\ M^{-1}\ s^{-1}$, the rate augmentation of more than 200-fold is achieved. The marked rate acceleration is rationalized in terms of efficient binding of metal ions and efficient metal-induced polarization of the C=N double bond. Probably, the curvature in Fig. 4 reflects the progressive increase in the fraction of metal-bound species of the Schiff base, because the OD_{350} in Fig. 3 shows the dependence similar to that in Fig. 4. Thus, the reaction may be written as Eq. 5.



(5)

Figure 3 shows that Ni^{2+} ion forms the complex more efficiently than Zn^{2+} ion. On the other hand, the catalytic efficiency of Zn^{2+} ion is comparable with that of Ni^{2+} ion (Fig. 4). This endorses that, provided the rate constants are calculated based on the complexed substrate concentration, Zn^{2+} ion would give the greater rate constants than Ni^{2+} ion.

4-Pyridinecarbaldehyde(4-PyCHO) and butylamine also formed the corresponding Schiff base in a borate buffer solution (pH 8.9), the OD_{350} value (0.066) being comparable with that from 2-pyridinecarbaldehyde and butylamine. However, the BzlQH reduction was not catalyzed by added metal ions at all (Table 1). The low reactivity is solely ascribable to the difference in the affinity toward metal ions.

We previously reported the dihydronicotinamide reduction of Schiff bases in refluxing methanol.[8] Although the Schiff bases are totally unreactive toward dihydronicotinamide, the reduction reaction was surprisingly facilitated by the addition of small amounts of acid speices. It is summarized, therefore, that the Schiff base can be reduced with the aid of proton catalysis in the acidic pH region and with the aid of metal catalysis in the basic pH region.

Some Characteristics of NADH Model Reduction in Aqueous Systems. Recently, the mechanistic role of metal ions in non-enzymatic dihydronicotinamide reduction has been dissected in aprotic solvents.[2–6] The catalytic behavior may be roughly summarized as follows: (1) activation of substrate and/or dihydronicotinamide at the initial state and (2) facilitation of hydrogen transfer by "bridge" effect of metal ions at the transition state. In aqueous systems, the interaction of metal ions with dihydronicotinamide itself is hardly expected due to the solvation of metal ions. Although there is an effort to synthesize a NADH model system which has within a molecule both dihydronicotinamide and metal-binding site,[35] the catalytic efficiency seems still equivocal. At present, it seems most expeditious to employ the metal-binding substrates to substantiate the aqueous metal catalysis.

Table 1. Apparent second-order rate constants(k_2') for the BzlQH reduction[a)]

Substrate[b)]	Metal ion mM		$k_2' \times 10^4/M^{-1}\,s^{-1}$ at pH 4.7	at pH 8.9
2-PyCHO			40.3	ppt[c)]
2-PyCHO	Ni^{2+}	(5.0)	19.1	ppt[c)]
2-PyCHO	Zn^{2+}	(5.0)	32.2	ppt[c)]
2-PyCHO + $BuNH_2$			39.3	nd[d)]
2-PyCHO + $BuNH_2$	Ni^{2+}	(0.5)	20.2	12.2
2-PyCHO + $BuNH_2$	Zn^{2+}	(0.1)	28.9	3.7
4-PyCHO + $BuNH_2$				nd[d)]
4-PyCHO + $BuNH_2$	Ni^{2+}	(0.1)		nd[d)]
4-PyCHO + $BuNH_2$	Zn^{2+}	(0.1)		nd[d)]
2-PyCHO + $PyCH_2NH_2$				nd[d)]
2-PyCHO + $PyCH_2NH_2$	Ni^{2+}	(0.5)		113
2-PyCHO + $PyCH_2NH_2$	Zn^{2+}	(0.5)		126

a) 50 °C, $\mu = 0.2$ with KCl, 2.2%(v/v) acetonitrile. b) Abbreviations employed are: 2-PyCHO, 2-pyridinecarbaldehyde; 4-PyCHO, 4-pyridinecarbaldehyde; $BuNH_2$, butylamine; $PyCH_2NH_2$ 2-pyridylmethylamine. c) Precipitation was formed. d) The rate was not detected($k_2' < 5 \times 10^{-5}\ M^{-1}\,s^{-1}$).

The present study established that the reduction of C=O and C=N double bonds with adjacent nitrogen base(s) is subject to the metal-catalysis in aqueous systems. We noticed, however, that the proton catalysis is unexpectedly effective.

Another conclusion obtained in the present study is that the Schiff bases serve as good substrates for the metal-assisted BzlQH reduction in aqueous systems. The rate augmentation of two orders of magnitude is remarkable. It seems, therefore, that the interaction with NADH is not a prerequisite for the facile reduction, as proposed by Dunn and coworkers[36,37] in enzymatic systems. It is conceivable, however, that in the hydrophobic pocket of enzymes metal ions may be relatively desolvated and may act more "acidic" catalysts like metal ions in aprotic solvents. The influence of hydrophobic environments on the metal catalysis is now investigated.

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