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COMMUNICATION

Reduction of 2-Pyridinecarboxaldehyde by a Dehydrogenase-NADH Model

Zinc 1-(α-2-methyl-1,10-phenanthroline)-1,4-dihydronicotinamide has been synthetized as a model for the enzyme–coenzyme complex of NADH–alcohol dehydrogenase. The model forms with 2-pyridinecarboxaldehyde a ternary complex in which the phenanthroline-bound zinc ion activates the aldehyde group for reduction. Transfer of a hydride equivalent in this complex occurs in an orientation in which the dihydronicotinamide ring lies over the carbonyl group with the carbonyl oxygen pointing to the ring nitrogen. Kinetic data for the reaction are given.

Metal ion catalysis in biomimetic reduction of carbonyl compounds by 1,4-dihydropyridines is of considerable interest in view of the catalytic activity of zinc ion in a number of dehydrogenases (*1*). However, the model reactions studied thus far give a miscellaneous and sometimes contradictory view about the role of metal ions (*2, 3*). Mechanistic interpretation of observed metal ion effects is frequently hampered by complicated kinetics and the fact that relative orientations of substrate, NADH model, and metal ion are not known. We have synthetized a dehydrogenase–NADH model, zinc 1-(α-2-methyl-1,10-phenanthroline)-1,4-dihydronicotinamide (**6**-Zn^{2+}), which evades this problem. In this model the 1,4-dihydronicotinamide group is connected via a methylene group to a 1,10-phenanthroline group which coordinates a zinc ion. This enzyme–coenzyme model combines both the metal-ion-activation characteristic of the enzyme and the reducing property of the coenzyme. Moreover, it has the advantage that substrate receptor site and dihydronicotinamide reactive site are in a fixed position to each other.

Structure **6** was prepared by conversion of 1,10-phenanthroline, **1,** to 2-aminomethyl-1,10-phenanthroline, **4** (*4*), followed by reaction of this compound with 1-(2,4-dinitrophenyl)-3-aminocarbonylpyridinium chloride (*5*) and reduction with sodium dithionite (*6*) (Scheme I). Spectrophotometric titration of **6** with zinc perchlorate in acetonitrile indicates that 1 equivalent of zinc is quantitatively bound to the phenanthroline moiety. The observed shift in absorption maximum of the phenanthroline ring from 266 to 276 nm and the appearance of a shoulder at 296.5 nm are characteristic for binding of zinc ion to the phenanthroline nucleus (*7*). It should be noted that the phenanthroline-bound zinc ion in this model is able to approach close to the dihydronicotinamide moiety, analogous to the ternary complex of NAD–alcohol dehydrogenase for which van der Waals contact between the nicotinamide ring and the active site zinc ion has been established (*1a*).

It can be expected that a zinc ion bound to phenanthroline stabilizes the dihydronicotinamide moiety by inductive electron withdrawal effects. This is apparent

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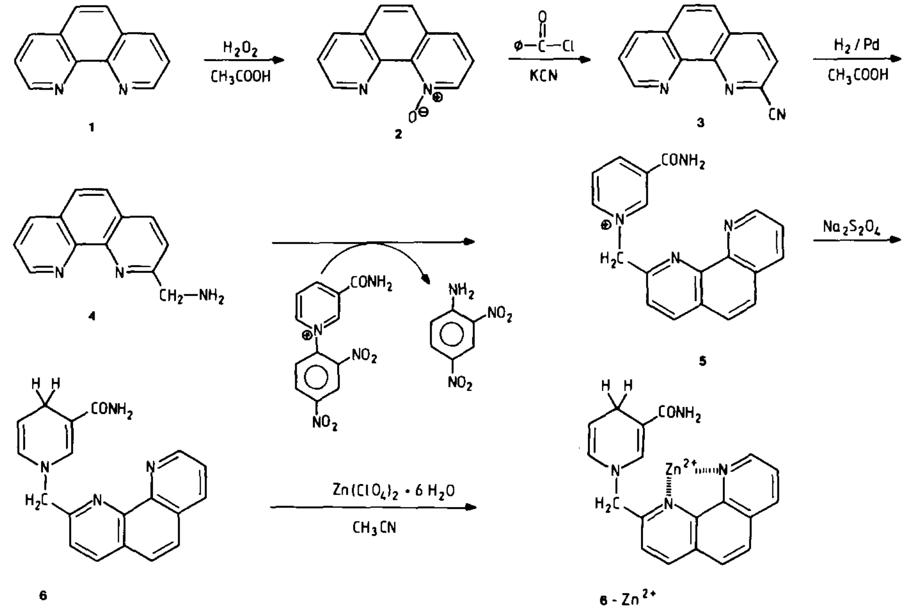

SCHEME I

for the reduction of 2,4,6-trinitrobenzene sulfonate (*8*) with **6**-Zn^{2+} which proceeds nearly two times slower than that with **6** with second-order rate constants of 0.484 and 0.897 M^{-1} s^{-1}, respectively (H_2O, pH 6.40, 25°C). In addition, the rate of formation of **6**-Zn^{2+} from **5**-Zn^{2+} is 20 times faster than that of **6** from **5** in the transhydrogenation with 1-propyl-1,4-dihydronicotinamide (*9*). On the other hand, zinc ion bound to the phenanthroline nucleus has the potentiality to serve as a template for substrate binding, thereby activating and orienting the substrate properly for reduction inside a ternary **6**-Zn^{2+}–substrate complex. In order to investigate this possibility 2-pyridinecarboxaldehyde (PyCHO)[1] was selected as a suitable substrate. PyCHO is known to undergo smooth reduction by dihydronicotinamides in the presence of zinc ion and it has been established that binding of zinc to pyridine nitrogen and carbonyl oxygen (at least in the transition state) is necessary for reduction of the carbonyl group (*2c*, *2f*).

When equimolar amounts of **6**-Zn^{2+} and PyCHO (6.0×10^{-4} M) are mixed in acetonitrile–chloroform 95 : 5 (v/v)[2] at 25°C a smooth reaction takes place which can be followed by observation of the decrease in absorbance of the dihydronicotinamide group at 365 nm. The reaction is first order in both [**6**-Zn^{2+}] and [PyCHO] and proceeds with an apparent second-order rate constant $k_2^{\text{app}} = 49.8$ M^{-1} s^{-1}. This rate is four times faster than the rate of reduction of PyCHO with 1-

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[1] Abbreviation used: PyCHO, 2-pyridinecarboxaldehyde.

[2] PyCHO is added to the sample solution from a stock solution in chloroform. This is necessary because PyCHO slowly decomposes in acetonitrile solution. The chloroform which is added shows no reactivity toward any of the compounds present in the sample solution.

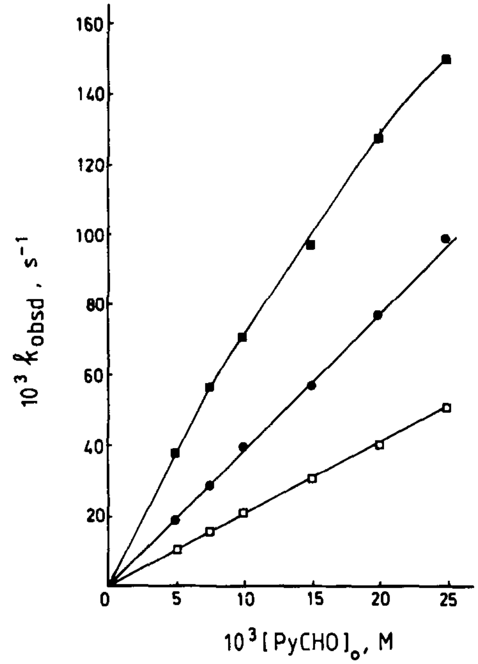

FIG. 1. Rate of reduction of PyCHO by 6-Zn^{2+} in $CH_3CN/CHCl_3$ 95:5 (v/v) at 25°C. [$Zn(ClO_4)_2 \cdot 6H_2O$] = 4.0 × 10^{-2} (□), 2.0 × 10^{-2} (●), and 1.0 × 10^{-2} M (■).

benzyl-1,4-dihydronicotinamide in the presence of an equimolar amount of zinc perchlorate (k_2 = 12.3 M^{-1} s^{-1}).

In a preparative experiment (10-mmol scale) TLC, NMR, and GC–MS analyses established that the reaction products were **5** and 2-pyridinemethanol. No reaction of **6** with PyCHO occurs in the absence of zinc ion. Furthermore, the isomeric 3- and 4-pyridinecarboxaldehydes exhibited no reactivity toward **6**-Zn^{2+}. Reaction of PyCHO with monodeutero-**6**-Zn^{2+}, in which one of the hydrogens at C-4 of the dihydronicotinamide group is replaced by deuterium, yields a kinetic isotope effect k_{HH}/k_{HD} = 1.62 indicating that hydrogen transfer is part of the rate-determining step of the process.[3] In order to investigate how far reduction of PyCHO proceeds inside a ternary **6**-Zn^{2+}–PyCHO complex, effects of variation of PyCHO and zinc perchlorate concentration on the reaction rate have been studied. When PyCHO is added in a 10-fold excess to a 3 × 10^{-4} M solution of **6**-Zn^{2+} the initially observed reaction rate rapidly decreases due to the loss of zinc ion from **6**-Zn^{2+} to the excess of PyCHO. This observation points out that zinc ion is primarily catalytically active when it is located to the phenanthroline moiety. To prevent loss by **6**-Zn^{2+} upon addition of excess PyCHO, the effect of [PyCHO] variation was studied in $ZnClO_4 \cdot 6H_2O$ buffer solutions of 1.0 × 10^{-2}, 2.0 × 10^{-2}, and 4.0 × 10^{-2} M. Figure 1 shows that an increase of [PyCHO] results in a nearly proportional increase of k_{obsd}.

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[3] Assuming the absence of a secondary isotope effect, a primary isotope effect k_H/k_D = 4.3 can be calculated. However, it must be realized that this value is very sensitive to small deviations in secondary isotope effect and percentage of deuteration in the model (*10*).

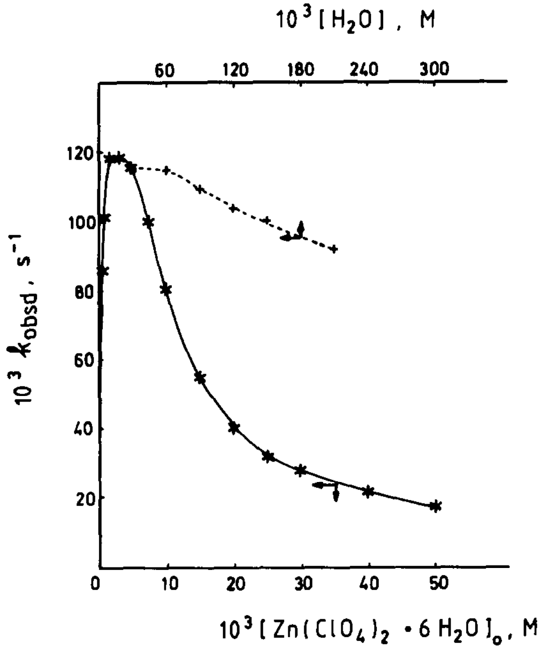


FIG. 2. Rate of reduction of PyCHO (10^{-2} M) by **6**-Zn^{2+} in $CH_3CN/CHCl_3$ 95:5 (v/v) at 25°C as function of the $Zn(ClO_4)_2 \cdot 6H_2O$ concentration (*). Dashed line gives the observed rate constant at a fixed $Zn(ClO_4)_2 \cdot 6H_2O$ concentration of 5×10^{-3} M and increasing H_2O concentration (+).

Remarkably, increment of the zinc perchlorate concentration has an inhibitory effect on the reaction. This is a strong indication that free PyCHO and not PyCHO·Zn^{2+} is the substrate for reaction with **6**-Zn^{2+}.

The inhibitory effect of zinc perchlorate is further illustrated by the rate profile at zinc perchlorate concentrations above 5×10^{-3} M ($[PyCHO]_0 = 10^{-2}$ M) as has been depicted in Fig. 2.

The increase in reaction rate at very low concentrations of zinc perchlorate has to be ascribed to the diminished loss of zinc ion from **6**-Zn^{2+} when excess of PyCHO is added. The amount of water which is present concomitantly with the zinc perchlorate in the solution has only a moderate effect on the reaction rate. This is illustrated by the dashed line in Fig. 2, where the water concentration is increased at a fixed zinc perchlorate concentration of 5×10^{-3} M.

The most simple reaction scheme which accounts for the observed effects is depicted in Scheme II.

$$\mathbf{6}\text{-}Zn^{2+} + PyCHO \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} \mathbf{6}\text{-}Zn^{2+}\text{–}PyCHO \xrightarrow{k_c} \mathbf{5} + PyCH_2O^{\theta} \cdot Zn^{2+} \qquad [1]$$

$$PyCHO + Zn^{2+} \overset{K_i^1}{\rightleftarrows} PyCHO \cdot Zn^{2+} \xrightleftharpoons{K_i^2[PyCHO]} (PyCHO)_2Zn^{2+}. \qquad [2]$$

$$\mathbf{6}\text{-}Zn^{2+} + 2PyCHO \xrightarrow{K_d} \mathbf{6} + (PyCHO)_2Zn^{2+}. \qquad [3]$$

SCHEME II

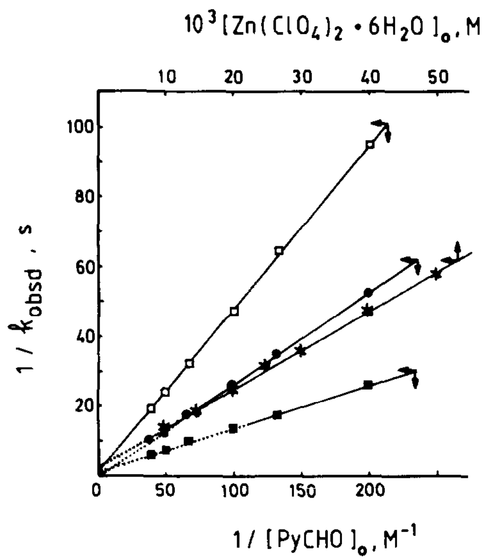


FIG. 3. Plots of $1/k_{obsd}$ vs $1/[PyCHO]_o$ with $[Zn(ClO_4)_2 \cdot 6H_2O] = 4.0 \times 10^{-2}$ (□), 2.0×10^{-2} (●), and 1.0×10^{-2} M (■) and of $1/k_{obsd}$ vs $[Zn(ClO_4)_2 \cdot 6H_2O]_o$ with $[PyCHO]_o = 10^{-2}$ M (*). The solid part of the line represents data in the concentration range $[PyCHO]_o < [Zn^{2+}]_o$; the dotted part of the line gives the region where $[PyCHO]_o \geq [Zn^{2+}]_o$.

According to this scheme, the rate of reduction of PyCHO will be determined by the concentration of the ternary 6-Zn^{2+}–PyCHO complex, which is dependent on the concentration of *free* PyCHO in solution according to

$$k_{obsd} = \frac{k_1 k_c[PyCHO]}{k_{-1} + k_c + k_1[PyCHO]}. \qquad [4]$$

Although the concentration of free PyCHO in solution cannot be determined directly, due to the formation of $(PyCHO)_2Zn^{2+}$ species at concentrations where $[Zn^{2+}]_o < [PyCHO]_o$ (Eq. [2]), spectroscopic measurements indicate that the complexation constant for Zn^{2+} with PyCHO is large (>4000) and this allows for the region where $[PyCHO]_o < [Zn^{2+}]_o$, the approximation written in

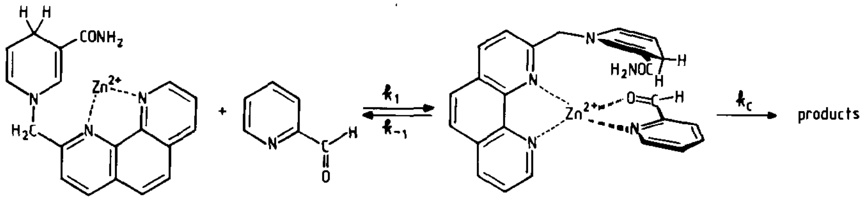
$$[PyCHO] = \frac{[PyCHO \cdot Zn^{2+}]}{K_i^1[Zn^{2+}]} = \frac{[PyCHO]_o}{K_i^1([Zn^{2+}]_o - [PyCHO]_o)}. \qquad [5]$$

Substitution of [5] into [4] and transformation yields

$$\frac{1}{k_{obsd}} = \left(\frac{k_{-1} + k_c}{k_1 k_c}\right) K_i^1 \frac{[Zn^{2+}]_o}{[PyCHO]_o} - \left(\frac{k_{-1} + k_c}{k_1 k_c}\right) K_i^1 + \frac{1}{k_c}. \qquad [6]$$

Equation [6] indicates that plots of $(k_{obsd})^{-1}$ vs $[Zn^{2+}]_o$ at constant $[PyCHO]_o$ and of k_{obsd}^{-1} vs $([PyCHO]_o)^{-1}$ at constant $[Zn^{2+}]_o$ should give straight lines for the region where $[PyCHO]_o < [Zn^{2+}]_o$. This is observed as is shown by the solid lines in Fig. 3. The slopes of the lines yield a value for $(k_{-1} + k_c)/(k_1 k_c)K_i^1$ of 11.0 (±1.0) s.

The intercept of the lines with the ordinate is approximately zero, indicating a



SCHEME III

value for k_c of approximately 0.1 s^{-1}. The dotted part of the lines in Fig. 3 gives the region where $[PyCHO]_0 \geq [Zn^{2+}]_0$. For this concentration range no simple relationship between k_{obsd} and $[PyCHO]_0$ can be derived due to significant formation of $(PyCHO)_2Zn^{2+}$ species.

A likely pathway for the reaction is given in Scheme III. CPK models demonstrate that transfer of a hydride equivalent inside the 6-Zn^{2+}–PyCHO complex necessarily have to proceed by an orientation in which the dihydronicotinamide ring lies over the carbonyl group with the carbonyl oxygen pointing to the ring nitrogen. This orientation is analogous to the orientation already suggested by Prelog for alcohol dehydrogenase-catalyzed carbonyl reductions (*11*) and has been frequently used to explain the stereochemistry of carbonyl reductions (*2d, 3d, 12*).

In summary, this model represents a first example of a model for the ternary NADH–enzyme–substrate complex in which relative orientation of dihydronicotinamide group, zinc ion, and carbonyl group is known. Variation of the position of the phenanthroline nucleus and synthesis of asymmetric analoga of this model are under current investigation.

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