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Model Dehydrogenase Reactions. Zinc Ion Catalyzed Reduction of Chelating Aldehydes by *N*-Propyl-1,4-dihydronicotinamides and Borohydride

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Abstract: Zinc ion catalyzes the reduction of 1,10-phenanthroline-2-carboxaldehyde by *N*-propyl-1,4-dihydronicotinamide in acetonitrile. This nonenzymic model reaction for alcohol dehydrogenase supports the view that the catalytic function of the zinc ion at the active site of alcohol dehydrogenase is to polarize the carbonyl group of the aldehyde substrates and to facilitate the deprotonation of the hydroxyl group of alcohol substrates. The model reaction proceeds by direct hydrogen transfer, is absolutely dependent on zinc ion, and is insensitive to free-radical quenching agents such as dihydroquinone. The disparity between the kinetic isotope effects and the isotopic partitioning ratio indicates that an intermediate forms during the course of the reaction. The zinc ion catalyzed reduction of 2- and 4-pyridinecarboxaldehyde by tetraethylammonium borohydride is consistent with coordination or proximity of the metal ion to the carbonyl group being primarily responsible for the absolute dependence on metal ion observed in the dihydronicotinamide reduction of 1,10-phenanthroline-2-carboxaldehyde. The metal ion complex of 2-pyridinecarboxaldehyde is reduced at least 700 000 times faster than the free aldehyde whereas the zinc complex of 4-pyridinecarboxaldehyde is reduced only 100 times faster than the corresponding free aldehyde. The rate of borohydride reduction of the zinc complex of the 2-isomer is independent of borohydride concentration but that of the 4-isomer is not. The mechanistic implications of this observation are discussed.

The NAD^+-dependent alcohol dehydrogenase from horse liver contains one catalytically essential zinc ion at each of its two active sites.[2,3] Solution studies using bipyridine as a probe for interactions taking place at the active site zinc ion have indicated that the metal ion is at or near the substrate binding site and in close proximity to the binding site of the nicotinamide moiety of the coenzyme.[2b] X-Ray crystallographic studies have supported this location of the zinc ion within the active site.[4,5]

These results are consistent with the view that an essential feature of the enzymic catalysis is direct coordination of the enzyme-bound zinc ion by the carbonyl and hydroxyl groups of the aldehyde and alcohol substrates. Such interactions could promote hydrogen transfer between coenzyme and substrate by polarizing the carbonyl group of the aldehyde substrates and by facilitating deprotonation of the hydroxyl group of alcohol substrates.[6-8] In a previous communication, we reported that zinc ion catalyzed the reduction of 1,10-phenanthroline-2-carboxaldehyde (I) to 1,10-phenanthroline-2-carbinol (II) by *N*-propyl-1,4-dihydronicotinamide (III) in anhydrous acetonitrile (eq 1).[9] These studies provided the first chemical precedent for metal ion activation of a carbonyl group for reduction by a dihydronicotinamide and therefore supported the suggested function of the zinc ion at the active site of alcohol dehydrogenase. Subsequently the metal ion catalyzed reduction of pyridoxal phosphate and its derivatives by the Hantzsch ester, 2,6-dimethyl-3,5-dicarbethoxy-1,4-dihydropyridine, was reported in aqueous solution.[10] These studies also demonstrated that dihydropyridine reductions of aldehydes are facilitated by general acid catalysis.[10]

In the present communication, we wish to present further experimental and mechanistic details of the reaction summarized in eq 1. We also report kinetic studies on the zinc

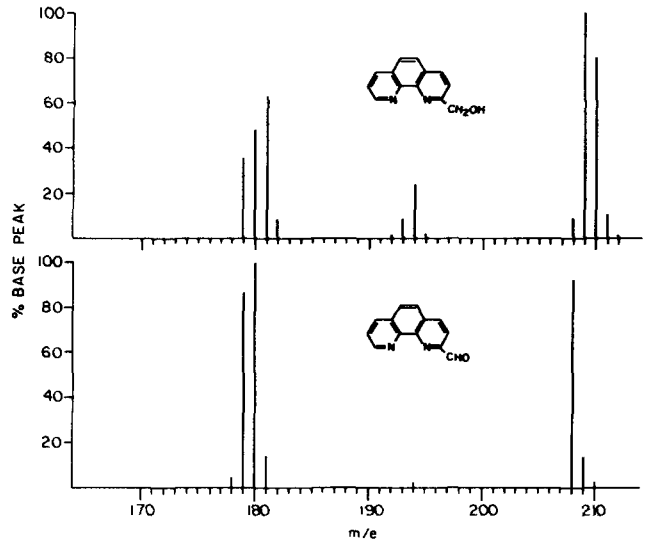
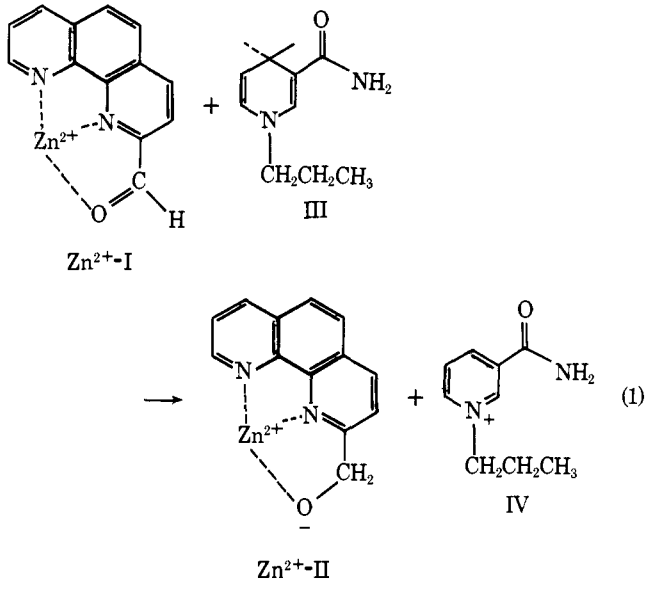


Figure 1. The 70-eV mass spectra of 1,10-phenanthroline-2-carbinol (top) and 1,10-phenanthroline-2-carboxaldehyde (bottom).



ion catalyzed reduction of 2- and 4-pyridinecarboxaldehyde. These studies indicate that proximity or direct coordination of the carbonyl group to the zinc ion is primarily responsible for the metal ion catalyzed reductions of chelating aldehydes and not electronic effects exerted through the aromatic ring.

Experimental Section

Materials. The preparation of 1,10-phenanthroline-2-carbinol (II) has been previously reported.[11]

To prepare 1,10-phenanthroline-2-carboxaldehyde (I), selenium dioxide (Matheson), 0.264 g (9.52 mequiv), was added to a solution of 1 g (9.52 mequiv) of II in 250 ml of pyridine with constant stirring. The mixture was then refluxed until the color changed from a light yellow to a dirty brown, characteristic of selenium metal precipitation. After cooling to room temperature, the bulk of the selenium metal precipitate was removed by filtration. After the addition of 189 ml of water to the filtrate, the solvent was removed on a rotatory evaporator. The red solid residue was dissolved in 100 ml of absolute methanol and filtered through a 1:1 (by weight) mixture of precipitated silver metal and basic alumina (AG-10, 100-200 mesh, pH 10.0-10.5) to remove the last traces of selenium metal. The methanol filtrate was evaporated to dryness and the resulting light yellow oil dissolved in 50 ml of chloroform. The chloroform solution was then extracted with five 50-ml aliquots of a 1% aqueous disodium EDTA solution. The aqueous EDTA solution was in turn extracted with additional chloroform. All the chloroform phases were pooled, dried over sodium sulfate, and reduced to a volume of 7 ml. Petroleum ether was added to the chloroform and the product allowed to crystallize at 0°. Recrystallization from acetonitrile gave 0.469 g (47% yield) of colorless needles, mp 152-153° dec. Anal. Calcd for $C_{13}H_8N_2O$: C, 74.90; H, 3.84; N, 13.45. Found: C, 74.85; H, 4.12; N, 13.33. The ir spectrum contained a strong band at 1700 cm^{-1}; the NMR spectrum exhibited a singlet at δ 10.58 (one aldehydic proton) with a complex splitting pattern for the aromatic protons in the δ 7.2-9.2 range. The mass spectrum of the product shows an intense molecular ion at *m/e* 208 (Figure 1). The isotope abundance peaks at *m/e* 209 (P + 1) and *m/e* 210 (P + 2) are 15.55 and 2.22% of the parent peaks, close to the values expected from the molecular formula of I.

N-Propyl-1,4-dihydronicotinamide (III) was prepared by the method of Karrer.[12] The monodeuterated form, 4-deuterio-*N*-propyl-1,4-dihydronicotinamide (III-d_1), was prepared by the same method but using 99.8% D_2O (Diaprep. Industries) as a solvent. The NMR revealed 47.49 ± 3.9% of incorporation of deuterium at C_4 of the nicotinamide ring. Substantial incorporation (50 ± 3.9%) of deuterium was detected in the nontransferring C-2 position of the nicotinamide ring.

The synthesis of 4,4-dideuterio-*N*-propyl-1,4-dihydronicotinamide (III-d_2) was carried out by oxidizing III-d_1 with *N*-methylacridinium chloride to give 4-deuterio-*N*-propylnicotinamide chloride as the major isotopic species. Subsequent dithionite reduction of this compound in D_2O yielded III-d_2 with an isotopic purity of 89% at C_4 by analysis with NMR and mass spectrometry. Previous attempts at preparing 4-deuterio-*N*-propylnicotinamide chloride by oxidation of III-d_1 with chloranil in dimethylformamide[13] or by incubating the oxidized nicotinamide in D_2O in the presence of cyanide ion[14] were cumbersome and characteristically resulted in low yields of product (<30%). The use of *N*-methylacridinium chloride to oxidize III-d_1 to 4-deuterio-*N*-propylnicotinamide chloride is favored here because it gave high yields of product (>95%). A detailed discussion of this reaction may be found elsewhere.[15]

A solution of 1.73 g of *N*-methylacridinium chloride, synthesized according to Mooser et al.,[16] in 78 ml of anhydrous methanol was added to a solution of 1.28 g of III-d_1 in 78 ml of anhydrous methanol. The reaction mixture was stirred at room temperature, in the dark, for 3 h. The solvent was removed in vacuo and the residue dissolved in a mixture of 30 ml of reagent chloroform plus 20 ml of water. After separating the two phases, the chloroform solution was extracted with six 20-ml aliquots of water. The aqueous washes were combined and extracted once with 20 ml of chloroform. Nitrogen was passed through the aqueous phase to remove residual chloroform and then the solution was lyophilized to give approximately 1.6 g of 4-deuterio-*N*-propylnicotinamide chloride as a glassy product. The oxidized nicotinamide was reduced in D_2O (99.8%) with dithionite according to Karrer[12] to give III-d_2 as light yellow needles, mp 88-90°. The overall yield from III-d_1 was 40%.

The extent of deuterium incorporation was determined by NMR and mass spectrometry. The NMR spectrum in deuteriochloroform revealed 89% incorporation of deuterium in the C-4 position. In addition, deuterium incorporation at the nontransferring C-2 position (45-50%) was evident. The base peak of the 70 eV mass spectrum is the P −1 (*m/e* 165) peak. Deuterium content was quantitated by using the mass spectrum obtained at the appearance potential of the P − 1 peak. The relative peak intensities were corrected for overlapping natural isotope abundance according to Biemann.[17] For the preparation of III-d_2 employed, the mole percents of species with *m/e* 166, 167, 168, 169, and 170 are 0, 10.18, 47.54, 39.30, and 2.95%, respectively. The NMR and mass spectra are internally consistent if the 89% net deuteration at C-4 is the result of 78% of the molecules dideuterated at C-4 and 22% monodeuterated at C-4. Assuming 50% of the C-4 dideuterated and C-4 monodeuterated species contain deuterium at C-2 as suggested from the NMR spectra, the distribution pattern in the appearance potential spectrum presented above can be predicted.

Anhydrous acetonitrile was prepared by distilling reagent grade acetonitrile (Baker), 400 ml, under nitrogen three times from three 1-g portions of P_2O_5 and twice from two 1.5-g portions of anhydrous Na_2CO_3. The dried solvent was stored over 2 g of Linde 4A molecular sieves and distilled under nitrogen just before use.

Anhydrous zinc chloride was prepared by refluxing 14 g of

Table I. Second-Order Rate Constants for Reduction of 1,10-Phenanthroline-2-carboxaldehyde[a]

NADH analogue	Concn, M	OP-aldehyde, M	Other components	k_2, M^{-1} min^{-1}
III	5.00×10^{-5}	5.00×10^{-5}		0.0
	5.11×10^{-5}	4.70×10^{-5}	$ZnCl_2$, 10.0×10^{-5}	19.0 ± 4.0
	3.94×10^{-5}	3.01×10^{-5}	$ZnCl_2$, 9.74×10^{-5}; H_2Q,[b] 4.87×10^{-5}	16.0 ± 2.0
III-d_2[c]	4.63×10^{-5}	4.67×10^{-5}	$ZnCl_2$, 10.0×10^{-5}	11.8 ± 2.0

[a] $T = 25°$; anhydrous acetonitrile. [b] Dihydroquinone. [c] 89% deuterium substituted at C-4.

$ZnCl_2$ (Mallinckrodt) in 140 ml of dioxane with 1 g of powdered zinc metal for 24 h. The mixture was then filtered by suction through a sintered glass filter and allowed to cool to room temperature, giving colorless needles of $ZnCl_2$. The supernatant was decanted and the $ZnCl_2$ recrystallized twice more from dioxane. Exposure to atmospheric moisture was rigorously avoided. The purified $ZnCl_2$ was stored under dioxane at 4° until use. Just before use the crystals were collected and heated under a stream of dry nitrogen at 250–300° for 3 h to give $ZnCl_2$ as an anhydrous white powder.

The 2- and 4-pyridinecarboxaldehyde isomers (Aldrich Chemical Co.) were purified by fractional distillation under reduced pressure just before use. Dihydroquinone was purchased from J. T. Baker and recrystallized once from ethanol, mp 173–174°.

Methods. The kinetics of the reduction of Zn^{2+}-I by III and III-d_2 were carried out in anhydrous acetonitrile under a dry nitrogen atmosphere. Anhydrous conditions were necessary to prevent the facile hydration of the carbonyl function of Zn^{2+}-I. Formation of the aldehydrol seriously complicates the kinetics of the reduction reaction. Stock solutions of reagents were prepared in anhydrous acetonitrile under a dry nitrogen atmosphere. The compounds used in preparing the stock solutions were dried in vacuo just before use in the following manner: I, 0.5 mm, 8 h, 40°; III and deuterated III, 0.5 mm, 4 h, 25°; dihydroquinone, 0.5 mm, 4 h, 25°. As previously described, $ZnCl_2$ was dried at 250–300° for 3 h prior to use. The reaction mixtures used for the kinetic measurements were prepared directly in cuvettes (1 cm) with ground glass stoppers under a nitrogen atmosphere and transferred to the thermostated (25°) cuvette carriage of a Zeiss PMQ II spectrophotometer.

Anhydrous conditions were also required for measuring the rates of reduction by tetraethylammonium borohydride of the zinc complexes of 2- and 4-pyridinecarboxaldehyde in order to prevent the metal ion catalyzed hydration.[18,19] The zinc ion catalyzed rates of reduction of 2-pyridinecarboxaldehyde were measured on a Durrum-Gibson stopped-flow spectrophotometer. The decrease in the characteristic absorption spectrum of the aldehyde was used to follow the course of the reaction. The titrimetric assay used for determining the normality of tetraethylammonium borohydride was adapted from a procedure used to assay sodium borohydride.[20]

To verify that 1,10-phenanthroline-2-carbinol was formed, product was isolated from 200-ml reaction mixtures composed of I (4.0×10^{-5} M), III (5.0×10^{-5} M), and $ZnCl_2$ (10^{-4} M) prepared in anhydrous acetonitrile under a dry nitrogen atmosphere and incubated for 8 days at room temperature in the dark in sealed air-tight flasks. The progress of the reaction was assayed spectrophotometrically at 350, 292, and 272.5 nm. When the reaction was complete (ca. 8 days), the reaction mixture was evaporated to dryness (50°, 17 mm) and the residue redissolved in 5 ml of chloroform. The chloroform was then extracted with five 5-ml portions of aqueous $ZnCl_2$ (8.0×10^{-3} M). The aqueous $ZnCl_2$ washes were collected and, in turn, extracted twice with 5 ml of chloroform. The chloroform layers, containing any unreacted III, were discarded. A 4 molar excess of disodium EDTA (with respect to $ZnCl_2$) was added to the aqueous $ZnCl_2$ solution which contained nicotinamide salts and 1,10-phenanthroline derivatives coordinated to zinc ion. The zinc free, 1,10-phenanthroline derivatives were then extracted from the aqueous EDTA solution with five 4-ml portions of chloroform. The chloroform extracts were washed twice with 5 ml of distilled water, dried over sodium sulfate, and evaporated to dryness with a stream of dry nitrogen. Product analysis was then carried out on the remaining residue by ultraviolet, infrared, and mass spectrometry as well as paper chromatography. For ultraviolet spectrophotometric analysis, a small portion of the reaction product was dissolved in anhydrous acetonitrile. The ultraviolet spectrum of this solution gave a maximum absorbance at 266 nm and the shape of the spectrum corresponded closely to that of a solution of synthetic II dissolved in acetonitrile. The addition of $ZnCl_2$ to this solution resulted in a red shifted spectrum with a maximum at 272.5 nm and a smaller absorbance peak at 292 nm, characteristic of the Zn^{2+}-II complex. The ratio of absorbances at 272.5 and 292 nm for the product was 2.57, close to the ratio of 2.82 found for authentic Zn^{2+}-II. However, because these ratios were not identical, the presence of other uv-absorbing materials in the product was indicated. On the basis of the absorption spectrum, II was isolated in 26% yield. However, the absorbance changes at 272.5 and 292 nm observed in the reaction mixture indicate the reaction actually proceeds with greater than 80% yield.

The product from the reaction mixture using III as a reducing agent was also analyzed by paper chromatography. A portion of the residue corresponding to approximately 2.5 μmol of II was dissolved in 0.5 ml of dimedone reagent (0.04 M in 10% ethanolic water) and incubated for 10 min at 40°. A portion of this mixture was withdrawn and spotted on Whatman No. 3M paper along with authentic samples of I, II, and III which had been treated with dimedone reagent in the same way. The paper was eluted with a solvent system of isopropyl alcohol-water-trichloroacetic acid-ammonia (75 ml:25 ml:5 g:0.2 ml). Authentic II and the product isolated from the reaction mixture each yielded a component with a R_f value of 0.75. Both spots were of approximately equal intensity. No dimedone positive component (R_f 0.93) was present in the reaction product or in an authentic sample of II. The dimedone treatment is absolutely essential in order to distinguish I from II in this chromatography system. In the absence of dimedone, I will migrate (R_f 0.75) to the same extent as II. The greater R_f value of I in the presence of dimedone is presumably caused by derivatization of I to the alkylidene dimethone.

Additional evidence confirming that II is the primary constituent of the product isolated from the reaction mixture was obtained from an infrared analysis of product. Approximately 1 μmol of product and authentic II was dissolved in chloroform and layered on silver chloride plates. After evaporation, the spectrum of authentic II and that of the isolated product was obtained and was identical with respect to the position of peaks and their intensities from 750 to 1625 cm^{-1}. The intense bands at 1390 and 1055 cm^{-1} in the spectrum of the product are particularly characteristic of II. As final product proof, the 70-eV mass spectrum of the product isolated from the reaction mixture composed of Zn^{2+}-I and III was found to be identical with the spectrum of authentic II presented in Figure 1.

Results

Reduction of 1,10-Phenanthroline-2-carboxaldehyde (I) by *N*-Propyl-1,4-dihydronicotinamide (III). The second-order rate constants for the zinc ion catalyzed reduction of I by III and by its doubly deuterated analogue, III-d_2, in anhydrous acetonitrile are reported in Table I. The concentration of Zn^{2+} in these kinetic systems was sufficient to completely saturate all the aldehyde present. The absorbance changes which occurred in a typical complete reaction system at room temperature over a 17-h period are summarized in Figure 2. The decrease at 350 nm corresponds to the oxidation of the dihydronicotinamide ring of III. The absorption spectra of the zinc complexes of I and II, determined independently, permit the assignment of the absorp-

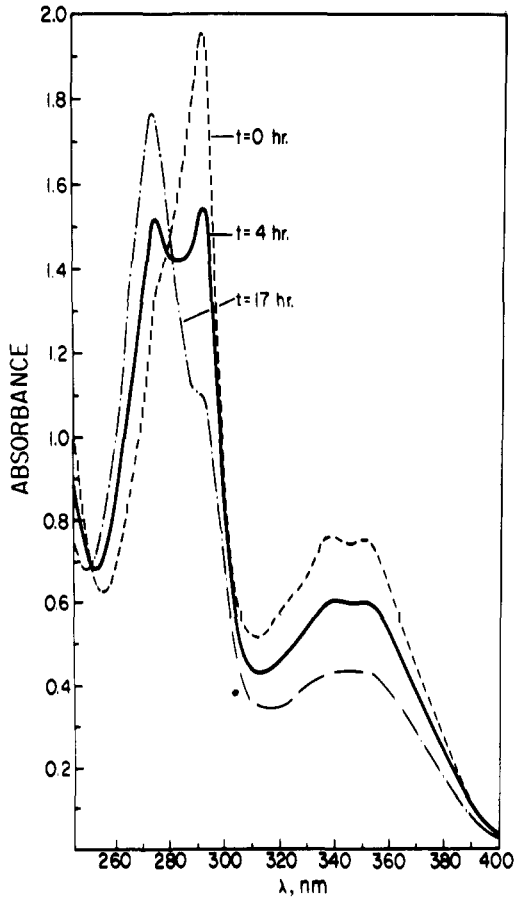


Figure 2. The time-dependent change in the absorption spectrum of a solution of I (6.64×10^{-5} M), III (8.5×10^{-5} M), and $ZnCl_2$ (10.0×10^{-5} M) in anhydrous acetonitrile, at 25°.

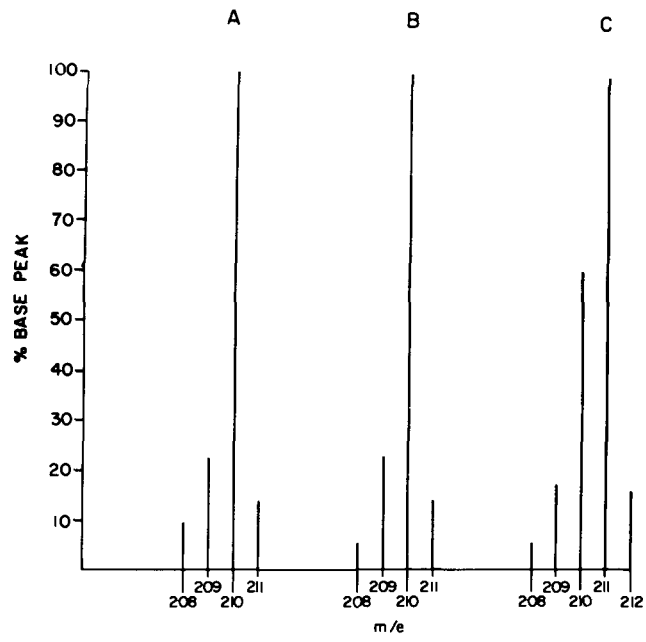


Figure 3. The appearance potential mass spectra of independently synthesized II (A), II from a reaction mixture using III (B), and II from a reaction mixture using III-d_2 (C).

tion changes at lower wavelengths. Since the Zn^{2+}-I complex absorbs more intensely than the zinc complex of II at 292 nm, there is a decrease in absorbance at 292 nm as a function of time. Correspondingly, since the Zn^{2+}-II complex absorbs more intensely at 272.5 nm than the Zn^{2+}-I complex, there is a net increase in absorbance at this wavelength as the reaction proceeds. The changes at 350 nm were usually used to calculate the second-order rate constants reported in Table I although comparable results were obtained using the data at shorter wavelengths.

Zinc ion is absolutely required for the reduction of I by III. The absorption spectra of incubation mixtures of I and III in the absence of the metal ion were stable over 4 days except for a slow decrease in the absorbance at 350 nm, which could be accounted for by the small intrinsic instability of III in anhydrous acetonitrile. The decrease in absorbance at 350 nm did not result in a corresponding increase in absorbance at 266 nm or decrease at 277 nm, as would be expected if reduction of I had taken place. Since dihydroquinone, a free radical quenching agent, does not affect the rate of the reduction, within experimental error, the formation of essential free radical intermediates during the course of the reaction is not indicated. The use of the doubly deuterated coenzyme analogue, III-d_2, as a reducing agent for Zn^{2+}-I results in a second-order rate constant that is significantly lower than that observed when using III, suggesting that the transfer of hydrogen is at least a partially rate-limiting step during the reaction. The primary kinetic deuterium isotope effect is 1.74 ± 0.6 if no significant secondary isotope effects exist and corrections are made for the isotopic purity of III-d_2.

Although the nature and rate of the spectrophotometric changes which take place are consistent with the production of II, the carbinol product was isolated from the reaction mixture for two reasons. First, final confirmation of the formation of II was required because zinc ion efficiently catalyzes the hydration of I in aqueous acetonitrile to yield the aldehydrol derivative whose absorption spectrum is similar to Zn-II. Therefore, it was possible that the spectral changes observed at 272.5 and 292 nm could be due to the traces of water in the reaction mixture and not the production of the desired alcohol. Second, deuterium analysis of the carbinol produced upon reduction by III-d_2 was required in order to establish direct hydrogen transfer. The procedure used to isolate the product from large-scale reaction mixtures is summarized in the Experimental Section. The formation of 1,10-phenanthroline-2-carbinol as the major product was verified by mass spectrometry and ultraviolet and infrared spectroscopy, as well as paper chromatography.

To establish that reduction of I by III involved direct hydrogen transfer from C_4 of the nicotinamide ring of III, product was isolated from an incubation mixture of Zn^{2+}-I and III-d_2 (89% D at C_4) and analyzed for deuterium by mass spectrometry. The number of gram atoms of deuterium incorporated into II was calculated from the mass spectrum obtained by running the mass spectrometer near the appearance potential for the P − 1 fragment ion of an authentic sample of undeuterated II. This procedure minimizes the contribution of the P − 1 fragment ion of monodeuterated II to the intensity of the molecular ion of any undeuterated carbinol in the sample. Figure 3 shows the appearance potential mass spectra of samples of authentic II, II isolated as the product from reducing Zn^{2+}-I with III, and II-d_1, obtained using III-d_2 as the reducing agent. Each of the spectra shown here is an average of at least seven runs of the sample. The intensity of the molecular ion for monodeuterated II (*m/e* 211) was corrected for the overlapping P + 1 peak from the undeuterated carbinol molecular ion. In like fashion, the molecular ion peak for the undeuterated carbinol in the sample (*m/e* 210) was corrected for the residual P − 1 ion from the monodeuterated II parent ion. The procedure for making these corrections has been outlined in detail by Biemann.[17] From the corrected relative

Table II. Rates of Reduction of 2- and 4-Pyridinecarboxaldehyde and Their Zinc Complexes by Tetraethylammonium Borohydride

Aldehyde	[Aldehyde], M	[BH_4^-], M	[$ZnCl_2$], M	k_{obsd}, M^{-1} s^{-1} [a]	k_{cor}, M^{-1} s^{-1} [b]
4-Pyridine	1.08×10^{-4}	4.98×10^{-3}	0	0.452	0.452
4-Pyridine	9.77×10^{-5}	1.22×10^{-3}	4.29×10^{-4}	10.50	47.10
2-Pyridine	1.37×10^{-4}	4.76×10^{-3}	0	0.406	0.406
2-Pyridine	9.81×10^{-5}	1.22×10^{-3}	4.29×10^{-4}	3900[c]	276 900[c]

[a] Calculated by dividing observed first-order rate constant by borohydride concentration. [b] Second-order rate constant corrected for concentration in zinc-aldehyde concentration using dissociation constants reported in the text. [c] This constant is not a valid second-order rate constant in view of the independence of the rate on borohydride concentration, but it does provide a direct comparison for the two aldehydes at 1.2×10^{-3} in BH_4^- when the correction is made for different amounts of complex present.

intensities of *m/e* 210 and 211, the gram-atom percent deuterium found for the deuterated carbinol sample was 70.0%. Since the natural isotopic abundance peak for monodeuterated II (*m/e* 212) was 15.8% of *m/e* 211, close to the theoretically expected value of 15.01%, essentially all the isotopic carbinol formed in the sample is in the monodeuterated form.

The amount of deuterated carbinol formed is low considering the 89% isotopic purity of the III-d_2 used and the observed primary kinetic isotope effect of 1.74. The yield of monodeuterated carbinol should have been 82% instead of 70% assuming a strict second-order reaction and no substantial secondary isotope effects. Although a secondary isotope effect of 0.59 for the hydrogen transfer could account for the apparent divergence of the isotope partitioning ratio and the kinetic isotope effect in a bimolecular reaction mechanism, a secondary isotope effect of less than one is unlikely for reactions which involve the conversion from a sp^3 to an sp^2 hybridization. Therefore, the lack of correspondence of the isotope partitioning ratio and the kinetic isotope effect suggests that the reduction is not a simple bimolecular process. Similar isotope effects have been observed in other nonenzymic dihydronicotinamide reductions and are consistent with the partially rate-limiting formation of a noncovalent intermediate during the course of the reaction.[15]

Reduction of 2-Pyridinecarboxaldehyde and 4-Pyridinecarboxaldehyde by Tetraethylammonium Borohydride. The zinc ion catalyzed reduction of these isomeric aromatic aldehydes by tetraethylammonium borohydride was examined to test whether the primary catalytic effect of the metal ion in the reduction of 1,10-phenanthroline-2-carboxaldehyde (I) arose from direct coordination by or proximity to the carbonyl group or via an inductive effect exerted through the aromatic system. The most appropriate control would have been to use an isomer of I in which the carbonyl group was remote from the metal ion. However, since these derivatives were not synthetically accessible, the isomeric pyridinecarboxaldehydes were used.

The use of borohydride rather than III as reductant was desirable for two reasons. Since pyridinecarboxaldehydes are weaker chelating agents, an excess of zinc ion would have been required to generate sufficient complex. However, we have found that free zinc ion catalyzes the decomposition of III in acetonitrile. Therefore it would have been difficult to perform accurate kinetic measurements. Secondly, the absolute dependence of dihydronicotinamide reductions on zinc ion precludes estimation of the net rate enhancement caused by the metal ion. For borohydride, the rate of reduction in the absence of metal ion is easily measured. Strictly anhydrous acetonitrile was used in order to eliminate metal ion catalyzed aldehydrol formation.[18,19] Tetraethylammonium borohydride was employed in place of sodium borohydride because of its greater solubility in acetonitrile.

The dissociation constants of the two pyridinecarboxaldehyde-zinc complexes are essential in order to interpret the kinetic data. They could be readily determined spectrophotometrically because the spectrum of the zinc complex of each aldehyde is different from that of the free aldehyde. By measuring the absorbance changes as a function of zinc ion concentration at a constant concentration of aldehyde, the dissociation constants can be calculated using standard double reciprocal plots. Surprisingly, the dissociation constant of the 4-pyridinecarboxaldehyde-Zn^{2+} complex (1.5×10^{-3} M) is lower than that of the 2-pyridinecarboxaldehyde-Zn^{2+} complex (3.5×10^{-2} M). The NMR spectrum of 4-pyridinecarboxaldehyde and its zinc complex in deuterioacetonitrile indicates a more pronounced chemical shift of the C-2 and C-6 protons upon complex formation than on the C-3 and C-5 protons. These results are consistent with the zinc ion interacting with the heterocyclic nitrogen.[21] They suggest that the aldehyde group of the 2-isomer hinders the binding of the zinc ion to the nitrogen and that the stable complex of the 2-isomer does not involve coordination of the carbonyl to the zinc ion.

Nevertheless, the kinetic data presented in Table II indicate that tetraethylammonium borohydride (1.2×10^{-3} M) reduces the zinc complex of 2-pyridinecarboxaldehyde roughly 6000 times more rapidly than the zinc complex of 4-pyridinecarboxaldehyde when appropriate corrections are made for the differing amounts of the zinc complexes of the two aldehydes and both reactions are assumed to be first order in borohydride. Although 4-pyridinecarboxaldehyde is reduced 100 times more rapidly in its zinc complex than in its free form, tetraethylammonium borohydride reduces the pyridine-2-carboxaldehyde-zinc complex roughly 700 000 times more rapidly than the free aldehyde. The substantially greater activation by the zinc ion of 2-pyridinecarboxaldehyde than 4-pyridinecarboxaldehyde toward reduction by borohydride is certainly consistent with the view that the primary effect of zinc ion in the reduction of 1,10-phenanthroline-2-carboxaldehyde is the result of its proximity to or coordination by the carboxaldehyde moiety rather than a generalized inductive effect generated by the metal ion.

Additional kinetic studies on the zinc ion catalyzed reduction of 2-pyridinecarboxaldehyde by tetraethylammonium borohydride indicate that the rate enhancement produced by metal ions in the reduction of carbonyl groups by hydride donors has been underestimated by the second-order rate constants presented in Table II. The data summarized in Table III indicate that at a constant concentration of 2-pyridinecarboxaldehyde and zinc ion, the rate of reduction is independent of borohydride concentration. The zero-order dependence of the pseudo-first-order rate constant coupled with its linear dependence on zinc ion concentration (Table IV) is consistent with the view that the overall reaction rate is limited by a slow step which precedes borohydride reduction but follows the rapid formation of the

Table III. Independence of Rate of Reduction of Zn^{2+} 2-Pyridinecarboxaldehyde on Borohydride Concentration

[Aldehyde], M	[$ZnCl_2$], M	[$Et_4N^+EH_4$], M	k_{obsd}, s^{-1}
1.0×10^{-4}	4.9×10^{-4}	2.4×10^{-3}	5.8 ± 0.3
1.0×10^{-4}	4.9×10^{-4}	3.6×10^{-3}	6.1 ± 0.3
1.0×10^{-4}	4.9×10^{-4}	6.0×10^{-3}	6.0 ± 0.3
1.0×10^{-4}	2.1×10^{-3}	6.0×10^{-3}	31.5 ± 1.6
1.0×10^{-4}	2.1×10^{-3}	1.6×10^{-2}	28.9 ± 1.4
1.0×10^{-4}	2.1×10^{-3}	4.0×10^{-2}	30.1 ± 1.5

Table IV. Dependence of Reduction of 2-Pyridinecarboxaldehyde by Borohydride on Concentration of Zinc Ion

[Aldehyde], M	[$ZnCl_2$], M	[$Et_4N^+BH_4^-$], M	k_{obsd}, s^{-1}
2.65×10^{-4}	1.58×10^{-3}	5.6×10^{-3}	16.1 ± 0.8
2.65×10^{-4}	2.37×10^{-3}	5.6×10^{-3}	33.0 ± 1.7
2.65×10^{-4}	3.16×10^{-3}	5.6×10^{-3}	44.7 ± 2.2
2.65×10^{-4}	3.95×10^{-3}	5.6×10^{-3}	63.0 ± 3.2
1.0×10^{-4}	7.0×10^{-4}	6.0×10^{-3}	8.8 ± 0.4
1.0×10^{-4}	1.4×10^{-3}	6.0×10^{-3}	18.2 ± 0.9
1.0×10^{-4}	2.1×10^{-3}	6.0×10^{-3}	31.5 ± 1.6
1.0×10^{-4}	3.5×10^{-3}	4.0×10^{-2}	49.5 ± 2.5

zinc ion–2-pyridinecarboxaldehyde complex. Such a reaction scheme is summarized in eq 2

$$M + A \overset{K}{\rightleftharpoons} MA \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} MA^* \xrightarrow{k_3(BH_4^-)_t} \text{products} \quad (2)$$

where M is metal ion, A is aldehyde, M–A is the rapidly formed metal ion–aldehyde complex, K is the dissociation constant of this complex, and MA* is the form of the complex susceptible to rapid reduction by borohydride. The pseudo-first-order rate constant, k_{obsd}, for this reaction scheme under the conditions where the metal ion and borohydride are present in excess is given by eq 3

$$k_{obsd} = \frac{[k_2k_3(BH_4)_t(M_t)]/[k_2 + k_{-2} + k_3(BH_4)_t]}{[k_{-2} + k_3(BH_4)_t]/[k_2 + k_{-2} + k_3(BH_4)_t]K + M_t} \quad (3)$$

where $(M)_t$ and $(BH_4)_t$ represent the total concentration of zinc ion and borohydride present. The independence of the observed rate constants on borohydride concentration indicates $k_3(BH_4)_t \gg k_{-2}$ and the strict first-order dependence on metal ion concentration indicates $[k_{-2} + k_3(BH_4)_t]/[k_2 + k_{-2} + k_3(BH_4)_t]K \gg (M)_t$ Therefore, under the conditions of the present experiment, eq 3 reduces to eq 4.

$$k_{obsd} = (k_2/K)M_t \quad (4)$$

As expected from the relationship summarized in eq 4, a plot of k_{obsd} vs. total metal ion concentration is linear even when data obtained at several borohydride concentrations are employed. From these plots, the value determined for k_2/K is 14×10^3 M^{-1} s^{-1}. Using the previously determined dissociation constant of the zinc–2-pyridinecarboxaldehyde complex (3.9×10^{-2} M) the value of k_2 therefore is 546 s^{-1}. This first-order rate constant might represent the rate of dissociation of an acetonitrile molecule coordinated to the metal ion prior to the ligation of the carbonyl group.

In contrast to the results with 2-pyridinecarboxaldehyde, the observed first-order rate for the metal ion catalyzed reduction of 4-pyridinecarboxaldehyde depends linearly on the borohydride concentration. The reaction scheme summarized in eq 5a and 5b is consistent with the kinetic data for this reaction

$$M + A \rightleftharpoons MA \quad (5a)$$

$$MA + BH_4^- \rightarrow \text{product} \quad (5b)$$

when M is metal, A is aldehyde, and MA is the metal ion–aldehyde complex. The second-order rate constants summarized in Table I have been calculated assuming the equilibrium indicated in eq 5a is rapidly achieved relative to the overall rate of the reaction. The necessity for two different kinetic schemes to describe the reduction of the zinc complex of 2-pyridinecarboxaldehyde and 4-pyridinecarboxaldehyde is consistent with the hypothesis that the unimolecular process observed for the reduction of the former isomer relates to the interaction of the carbonyl group with the metal ion.

However, an alternative to the kinetic explanation for the zero-order dependence on BH_4^- for reduction of the 2-pyridinecarboxaldehyde is that BH_4^- forms a reversible complex at the zinc ion of the zinc–2-pyridinecarboxaldehyde complex prior to reducing the aldehyde in the rate-limiting step. A saturation effect at the zinc ion together with proximity of BH_4^- to the aldehydic carbon would account for the zero-order dependence on BH_4^- and the large rate enhancement in the presence of zinc ion. For the zinc–4-pyridinecarboxaldehyde complex, BH_4^- would be remote from the aldehydic group and a bimolecular reaction with BH_4^- from solution would account for the second-order kinetics.

In principal, this hypothesis could be tested from the magnitude of the primary kinetic deuterium isotope effect on the reduction of Zn^{2+}–2-pyridinecarboxaldehyde by borohydride and deuterioborohydride. A kinetic isotope effect in the presence of zinc indicating rate-limiting hydrogen transfer would suggest that a saturation effect at the zinc accounts for the zero-order dependence on BH_4^-. However, this approach was not feasible since a positive kinetic isotope effect could not be detected for reduction of the 2-pyridinecarboxaldehyde in the absence of zinc ion. This result is in accord with the findings of other workers that the primary kinetic deuterium isotope effect for reduction of most ketones with borohydride is in fact slightly inverse.[22] While this alternate mechanism cannot be excluded from the available data, the kinetic explanation advanced above is fully consistent with proximity or direct coordination to the carbonyl oxygen being the primary effect.

Discussion

The zinc ion catalyzed reduction of 1,10-phenanthroline-2-carboxaldehyde by *N*-propyldihydronicotinamide has provided the first example of the nonenzymatic reduction of an aldehyde by an NADH analogue. The inability to detect any reaction in the absence of the metal ion suggests that the catalytic effect of the metal ion on dihydronicotinamide reductions must be substantial. These observations, together with the spectroscopic, NMR, and x-ray crystallographic results[2-6] which indicate that substrates bind at or near the zinc ion, strongly suggest that coordination, or at the very least proximity, of the aldehyde substrates to the zinc ion at the active site is an important feature of the alcohol dehydrogenase reaction mechanism.

The zinc ion catalyzed reduction of 1,10-phenanthroline-2-carboxaldehyde in anhydrous acetonitrile proceeds by direct hydrogen transfer as it must in order to be considered an appropriate model for alcohol dehydrogenase. Although the mechanistic significance of this observation is diminished by lack of exchangeable protons in acetonitrile, all of the nonenzymic dihydronicotinamide reactions that have been carried out in protic solvents, which are not inhibited by free-radical quenching agents, exhibit direct hydrogen transfer. Since the zinc ion catalyzed reduction of 1,10-

phenanthroline-2-carboxaldehyde is not inhibited by the free-radical quenching agent dihydroquinone, this reaction most likely would proceed by direct hydrogen transfer independent of the nature of the solvent.

The insensitivity of the reaction rate to free-radical agents and the probable occurrence of direct hydrogen transfer is consistent with the reduction of 1,10-phenanthroline-2-carboxaldehyde proceeding by a hydride-transfer mechanism. However, the divergence of the isotope effect measured by kinetics and product analysis excludes a simple bimolecular reaction as would be anticipated in a hydride-transfer reaction.[15] The simplest reaction scheme consistent with the observed isotope effect is summarized in eq 6

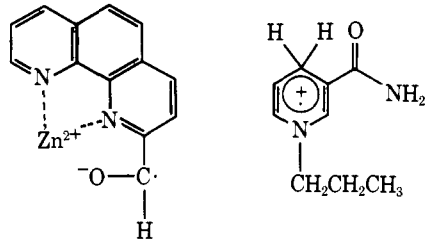
$$Zn^{2+}\text{-I} + \text{III} \rightleftharpoons Zn^{2+}\text{-I-III} \rightarrow Zn^{2+}\text{-II} + \text{IV} \quad (6)$$

where the complex, Zn-I-II, must be noncovalent in nature. This scheme is probably insufficient because it requires that the rate of formation of the noncovalent complex must be the same order of magnitude as the overall rate of the reaction. Generally, the rates of formation of noncovalent complexes of aromatic compounds approach the diffusion controlled limit.[23]

A more probable and complex alternative reaction scheme is presented in eq 7

$$Zn^{2+}\text{-I} + \text{III} \rightleftharpoons (\text{Zn-I-III})_i \rightleftharpoons (\text{Zn-I-III})_r \rightarrow \text{Zn-II} + \text{IV} \quad (7)$$

where $(\text{Zn-I-II})_i$ is the complex formed between the two aromatic molecules and $(\text{Zn-I-III})_r$ is a complex whose rate of formation is partially rate limiting and through which hydrogen atom transfer must proceed. At present, the only evidence for including a scheme with an additional intermediate complex, with a slow rate of formation, is that it is the simplest scheme which contains a noncovalent complex, as required by the isotope effect studies, but yet permits the rate of association of the complex to approach values characteristic of such species. Although no experimental evidence is available which specifies the chemical nature of $(\text{Zn-I-III})_r$, recent studies on dihydroflavins, which may share a common reduction mechanism with dihydronicotinamides, have emphasized the possible importance of radical pairs.[24] Perhaps a reasonable but highly speculative structure of $(\text{Zn-I-III})_r$ might be the radical pair represented below. An analogous radical pair has been identified in the reduction of thiobenzophenone by *N*-propyldihydronicotinamide by ESR but its kinetic competence has not been established.[25]



The kinetic studies on the metal ion catalyzed reduction of 2-pyridinecarboxaldehyde by borohydride are consistent with direct coordination or proximity to the metal ion to the carbonyl oxygen leading to large rate enhancements in hydride-transfer reactions. One kinetic explanation for the independence of the rate of reduction of the 2-pyridinecarboxaldehyde–zinc ion complex on borohydride concentration would involve a slow rate-controlling step prior to reduction. Consistent with other ligand substitution reactions, this limiting step may be the slow dissociation of an inner sphere acetonitrile molecule before the carbonyl can bind to the metal ion and be rapidly reduced.

The possible importance of radical pair intermediates in dihydronicotinamide reactions suggests that these reductions may proceed by different pathways than borohydride reactions which presumably proceed via hydride-transfer mechanisms. However, the difference between these two reaction schemes may be more semantic than real since there is a very low probability that two electrons and a hydrogen ion are transferred simultaneously[26] and a hydride-transfer mechanism may involve formation of a radical pair at low steady-state concentrations. The stabilization of negative charge on the carbonyl group is essential, independent of the steady-state concentration of the radical pair. The demonstration of large enhancements caused by zinc ion in two reactions which do not proceed via a free-radical mechanism indicates that the zinc ion of alcohol dehydrogenase can play a central role in catalysis whether or not the radical pair exists in any substantial steady concentration during the course of the enzymic reaction.

Acknowledgments. These studies were supported by U.S. Public Health Service Grant GM 21199-07 and the Alfred P. Sloan Foundation. We thank Professor G. J. Popjak for access to the mass spectrometer in his laboratory.

References and Notes

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