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A Model for Catalytically Active Zinc(II) Ion in Liver Alcohol Dehydrogenase: A Novel "Hydride Transfer"[1] Reaction Catalyzed by Zinc(II)-Macrocyclic Polyamine Complexes

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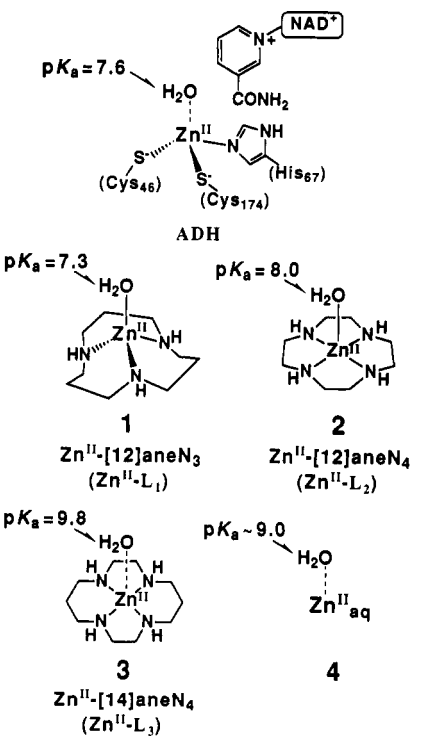
Abstract: The role of Zn^{II} ion at the active center of liver alcohol dehydrogenase has been well-defined for the first time by the comparative studies of Zn^{II}[12]aneN$_3$, **1** ([12]aneN$_3$ = 1,5,9-triazacyclododecane, L_1), Zn^{II}[12]aneN$_4$, **2** ([12]aneN$_4$ = 1,4,7,10-tetraazacyclododecane, L_2), Zn^{II}[14]aneN$_4$, **3** ([14]aneN$_4$ = 1,4,8,11-tetraazacyclotetradecane, L_3), and free Zn^{II} salts, **4**. Variations in Zn^{II} acidity and coordination environment in these complexes result in varying degrees of catalytic activity in the reduction of *p*-nitrobenzaldehyde (**9**) and an NAD^+ model compound (**18**) with alcohols as the "hydride" sources (e.g., 2-PrOH) to *p*-nitrobenzyl alcohol (**10**) and the corresponding NADH model compounds (**19** and **20**), respectively. Among Zn^{II} species tested, the Zn^{II} complex of macrocyclic triamine [12]aneN$_3$, **5** (L_1-Zn^{II}-OH)$_3$·(TfO)$_3$·TfOH (TfO = $CF_3SO_3^-$), was by far the most effective catalyst: **10** was obtained from **9** in 7820% yield (based on the concentration of Zn^{II}) in the presence of **5** (0.8 mol %) in refluxing 2-PrOH for 24 h. The Zn^{II} complex **5**, also promotes the "hydride transfer" from 2-PrOH to an NAD^+ model compound, *N*-benzylnicotinamide chloride (**18**), to yield the 1,4-adduct, *N*-benzyl-1,4-dihydronicotinamide (**19**), almost exclusively. It is concluded, from the comparison of **5** with other Zn^{II} complexes of [12]aneN$_4$ and [14]aneN$_4$, that the most acidic and coordinatively least saturated Zn^{II} in L_1 catalytically generates zinc(II)-alkoxide complex to facilitate the hydride transfer to the hydride acceptor on the Zn^{II} coordination sphere. The present study provides the first chemical model illustrating the significance of the Zn^{II} acidity and the steric requirement around Zn^{II} coordination sphere in the hydride transfer reaction (from alcohol) catalyzed by Zn^{II}-containing alcohol dehydrogenases (ADH).

Introduction

Zn^{II}-containing alcohol dehydrogenases (ADH) catalyze the hydride transfer[1] from alcohols to NAD^+. The X-ray structure of horse liver alcohol dehydrogenase reveals that the active site of the enzyme contains a Zn^{II} ion tetrahedrally coordinated with two cystein sulfurs (Cys-46 and Cys-174), one histidine nitrogen (His-67), and a water.[2] Studies performed with the enzyme suggest that the acidic Zn^{II} ion may generate alkoxide ions from alcohols with simultaneous coordination.[3] However, the question has been raised whether the pK_a values of alcohols (normally ~16) can be reduced by about 9 units upon coordination to Zn^{II}.[4] Meanwhile, Kvassman and Pettersson have shown that in ADH the pK_a values of the H_2O bound to Zn^{II} dramatically vary from 9.2 in the free form, 11.2 upon binding of NADH, and 7.6 in the binary complex with NAD^+.[5]

Few chemical models of the active site of ADH have been designed, mostly attempting to reconstruct the coordination environment around Zn^{II}.[6] In addition, the (thermodynamically

Chart I



(1) The word hydride as used in the text denotes only the entity ultimately transferred, not the mechanism.

(2) Eklund, H.; Branden, C.-I. In *Biological Macromolecules and Assemblies*; Wiley: New York, 1985.

(3) For example: (a) Branden, C.-I.; Jornvall, H.; Eklund, H.; Furugren, B. In *The Enzymes*, 3rd ed.; Boyer, P. D., Ed.; Academic Press: New York, 1975; Vol. XI, pp 104-190. (b) Eklund, H.; Nordstrom, B.; Zeppezauer, E.; Soderlund, G.; Ohlsson, I.; Boiwe, T.; Soderberg, B.-O.; Tapia, O.; Branden, C.-I.; Akeson, A. *J. Mol. Biol.* **1976**, *102*, 27. (c) Branden, C.-I. In *The Pyridine Nucleotide-Dependent Dehydrogenases*; Sund, H., Ed.; W. de Gruiter: Berlin, 1977; pp 325-338. (d) Dowarschack, R. T.; Plapp, B. V. *Biochemistry* **1977**, *16*, 2716. (e) Drysdale, B.-E.; Hollis, D. P. *Arch. Biochem. Biophys.* **1980**, *205*, 267. (f) Dutler, H.; Branden, C.-I. *Bioorg. Chem.* **1981**, *10*, 1. (g) Dutler, H.; Ambar, A. *NATO Adv. Study Inst. Ser. C* **1983**, *100*, 135. (h) Makinen, M. W.; Kuo, L. C.; Yim, M. B.; Wells, G. B.; Fukuyama, J. M.; Kim, J. E. *J. Am. Chem. Soc.* **1985**, *107*, 5245. (i) Eklund, H.; Jones, A.; Schneider, G. In *Zinc Enzymes*; Bertini, I., Luchinat, C., Maret, W., Zeppezauer, M., Eds.; Birkhauser: Boston, 1986; pp 377-392. (j) Cedergren-Zeppezauer, E. S. In *Zinc Enzymes*; 1986; pp 393-415. (k) Hansch, C.; Klein, T.; McClarin, J.; Landridge, R.; Cornell, N. W. *J. Med. Chem.* **1986**, *29*, 615. (l) Hansch, C.; Klein, T. *Acc. Chem. Res.* **1986**, *19*, 392. (m) Laurie, D.; Lucas, E.; Nonhebel, D. C.; Suckling, C. J. *Tetrahedron* **1986**, *42*, 1035. (n) Verhoeven, J. W.; Van Gerreheim, W.; Martens, F. M.; Van der Kerk, S. M. *Tetrahedron* **1986**, *42*, 975. (o) Westheimer, F. H. In *Coenzymes and Cofactors*; Pyridine Nucleotide Coenzymes, Part A. Dolphin, D., Poulson, R., Avramovic, O., Eds.; J. Wiley and Sons: New York, 1987; Vol. II, pp 253-322. (p) Eklund, H.; Branden, C.-I. in ref 3o, pp 52-98. (q) Ohno, A.; Ushio, K. in ref 3o, Part B, pp 276-331. (r) Callender, R.; Chen, D.; Lugtenburg, J.; Martin, C.; Rhee, K. W.; Sloan, D.; Verdersteen, R.; Yue, K. T. *Biochemistry* **1988**, *27*, 3672. (s) Sekher, V. C.; Plapp, B. V. *Biochemistry* **1988**, *27*, 5082. (t) Tanner, D. D.; Stein, A. R. *J. Org. Chem.* **1988**, *53*, 1642, 1646.

(4) Makinen, M. W.; Maret, W. In *Zinc Enzymes*; Bertini, I., Luchinat, C., Maret, W., Zeppezauer, M., Eds.; Birkhauser: Boston, 1986; pp 465-470.

(5) (a) Kvassman, J.; Pettersson, G. *Eur. J. Biochem.* **1980**, *103*, 565. (b) Pettersson, G. In *Zinc Enzymes*; Bertini, I., Luchinat, C., Maret, W., Zeppezauer, M., Eds.; Birkhauser: Boston, 1986; pp 451-505.

(6) (a) Kaptein, B.; Wang-Griffin, L.; Barf, G.; Kellogg, R. M. *J. Chem. Soc., Chem. Commun.* **1987**, 1457. (b) Curtis, N. J.; Brown, R. S. *Can. J. Chem.* **1981**, *59*, 5965. (c) Kaptein, B.; Barf, G.; Kellogg, R. M.; Bolhuis, F. V. *J. Org. Chem.* **1990**, *55*, 1890 and references cited therein.

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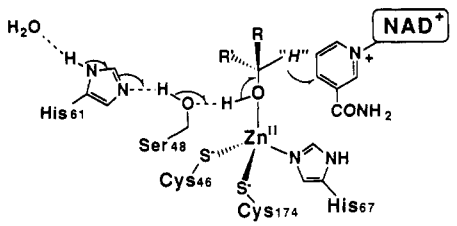
unfavorable) forward reaction, hydride transfer from alcohols to pyridinium salts such as NAD^+ or its homologues (e.g., carbonyl compounds), has not been studied in sufficient detail.[7] Roles of metal ions have been partially elucidated for the reverse (energetically feasible) "hydride donation", i.e., the reduction of activated carbonyl compounds (e.g., α-ketoesters, etc.) by NADH or its model compounds.[8,9]



Earlier, we demonstrated that the enhanced acidity of Zn^{II} ions in macrocyclic polyamines **1** (L_1-Zn^{II}-OH_2, L_1 = [12]aneN$_3$ = 1,5,9-triazacyclododecane) and **2** (L_2-Zn^{II}-OH_2, L_2 = [12]aneN$_4$ = 1,4,7,10-tetraazacyclododecane) renders the pK_a values of the bound H_2O to 7.3 and 8.0, respectively, at 25 °C (Chart I).[10-14] The Zn^{II}-OH^- species at near neutral pH acts dynamically as a nucleophile to carbonyls (aldehydes and esters),[10] as a bifunctional nucleophile to phosphates,[11] and as a base toward amides[13] and sulfonamides.[14] We now turn our attention to the similar pK_a values for Zn^{II}-OH_2 in ADH[5] and **1**. In this report we show that L_1-Zn^{II}-OH^- is indeed a very good catalyst for the forward reaction, hydride transfer from an alcohol to an aldehyde (reaction 1) and an NAD^+ model compound **18** (reaction 4).

Experimental Section

General Information. ^{1}H NMR spectra were obtained on a JEOL GX-400 spectrometer (400 MHz, 27 °C). IR and UV spectra were recorded on Shimadzu FTIR-4200 and Hitachi U-3200 spectrophotometers, respectively. All reactions were routinely carried out under an inert atmosphere of argon. Product analysis was performed on a Shimadzu LC-6A Liquid Chromatograph. Elemental analysis was performed on a YANAKO CHN Corder MT-3.

Materials. Commercial reagents of analytical grade were used without further purification. Solvents were generally purified and dried by standard methods before use.[15] Macrocyclic polyamine ligands, 1,5,9-triazacyclododecane (L_1, [12]aneN$_3$), 1,4,7,10-tetraazacyclododecane (L_2, [12]aneN$_4$), and 1,4,8,11-tetraazacyclotetradecane (L_3, [14]aneN$_4$) were purchased from Aldrich Chemical or Tokyo Kasei Company Ltd. *N*-Benzylnicotinamide chloride (**18**) and the corresponding dihydronicotinamides, **19** and **20**, were prepared according to the literature procedure.[16]

Preparation of Zn^{II} Complexes with Macrocyclic Polyamines: (L_1-Zn^{II}-OH)$_3$·(TfO)$_3$·TfOH (TfO = $CF_3SO_3^-$), 5. Crystalline of the Zn^{II} complex **5** was prepared by a method similar to that for (L_1-Zn^{II}-OH)$_3$·(ClO$_4$)$_3$·HClO$_4$.[10] A solution of L_1 (103 mg, 0.55 mmol) and $Zn^{II}(TfO)_2$ (180 mg, 0.50 mmol) in 10 mL of 99.5% EtOH was stirred for 3 h at room temperature. The solution was concentrated under reduced pressure to ca. 1 mL and kept standing. Colorless prisms were obtained as **5** in 27% yield. Its ^{1}H NMR spectrum in D_2O at pD 10 (3-(trimethylsilyl)propionic-*2,2,3,3-d*$_4$ acid sodium salt (Merck) as the reference) was identical with that of (L_1-Zn^{II}-OH)$_3$·(ClO$_4$)$_3$·HClO$_4$:[10] IR (KBr pellet) 3569 (br), 3230 (s), 2950, 2940, 1489 (s), 1451 (s), 1283 (s), 1260 (s), 1224 (s), 1167 (s), 1032 (s), 1032 (s), 976, 912, 891, 831, 760, 637 (s), 575, 517 cm^{-1}. Anal. Calcd for $C_{27}H_{66}N_9O_3Zn_3$·(CF$_3$SO$_3$)$_3$·CF$_3$SO$_3$H: C, 27.41; H, 4.97; N, 9.28. Found: C, 27.94; H, 4.73; N, 9.18.

Its potentiometric titration also suggests the formula (L_1-Zn^{II}-OH)$_3$·(TfO)$_3$·TfOH. Crystalline **5** (13.6 mg, 0.01 mmol) was dissolved in 100 mL of H_2O (at 25 °C, I = 0.10 (NaClO$_4$)) and titrated with 0.10 M NaOH.[10] An inflection (pH ca. 8) was observed at a titration point of 1 equiv of OH^- consumption (0.01 mmol), supporting the [3(Zn^{II}-OH) (this is inert to NaOH) + TfOH] form in each Zn^{II} trimer. The X-ray crystal structure of trimeric (L_1-Zn^{II}-OH)$_3$·(ClO$_4$)$_3$·HClO$_4$ was previously reported.[10]

[L_2-Zn^{II}-OH_2](ClO$_4$)$_2$, 6, and [L_2-Zn^{II}-OH]$_2$(ClO$_4$)$_2$·HClO$_4$, 7. The former complex **6** was prepared as follows. To a solution of L_2 (300 mg, 1.74 mmol) in 10 mL of 99.5% EtOH was slowly added a solution of Zn^{II}(ClO$_4$)·6H$_2$O (649 mg, 1.74 mmol) in 10 mL of EtOH at 50–60 °C. Upon cooling to room temperature, the resulting colorless precipitate was collected and then recrystallized from hot water to obtain 409 mg of colorless prisms (52 % yield). ^{1}H NMR (D_2O at pD 6, 3-(trimethylsilyl)propionic-*2,2,3,3-d*$_4$ acid sodium salt as the reference): δ 2.72–2.82 (8 H, m), 2.86–2.96 (8 H, m); IR (KBr pellet) 3420 (br), 3177, 2918, 2870, 1481, 1444, 1278, 1143 (s), 1113 (s), 1091 (s), 1010, 993, 964, 806, 626 cm^{-1}. Anal. Calcd for $C_8H_{20}N_4Zn$·(ClO$_4$)$_2$·H$_2$O: C, 21.14; H, 4.88; N, 12.33. Found: C, 21.41, H, 4.89; N, 12.65. The latter complex **7** was prepared as previously described.[17]

[L_3-Zn^{II}](TfO)$_2$, 8. This complex was prepared in a similar method described before.[18] To a solution of $Zn^{II}(TfO)_2$ (112 mg, 0.56 mmol) in 6 mL of MeOH was added dropwise a solution of L_3 (205 mg, 0.56 mmol) in 4 mL of MeOH. The reaction mixture was heated at reflux for 1 h. After cooling, the solution was evaporated to ca. 1 mL and kept standing. Colorless prisms of **8** (68 mg, 21 % yield) were obtained: IR (KBr pellet) 3449 (br), 3214 (s), 2930 (s), 2907 (s), 1478, 1458, 1429, 1292 (s), 1279 (s), 1254 (s), 1237 (s), 1165 (s), 1101 (s), 1049 (s), 1036 (s), 938 (s), 874 (s) 766, 656 (s), 579, 523 cm^{-1}. Anal. Calcd for $C_{10}H_{24}N_4Zn(CF_3SO_3)_2$: C, 25.56; H, 4.29; N, 9.94. Found: C, 25.53; H, 4.34; N, 9.97.

Reactions of *p*-Nitrobenzaldehyde (9) with Alcohols Catalyzed by Various Zn^{II} Species. *p*-Nitrobenzaldehyde (**9**, 0.125 mmol) was added in one portion to a 1.0 mL alcohol solution of a Zn^{II} catalyst (total [Zn^{II}] = 1 mM). The mixture was heated at gently reflux for 24 h under argon. The reaction was quenched with water and immediately extracted with several portions of CH_2Cl_2. The combined organic layer was analyzed for **9**, *p*-nitrobenzyl alcohol (**10**), and *p*-nitrobenzaldehyde dialkyl acetal (**11**). Identification of reaction products was performed by coinjection of reaction mixtures with standards onto HPLC column and/or by ^{1}H NMR measurements of isolated products. HPLC analysis was performed by a 5 μm Lichrospher Si60 column (250 × φ*50* mm) eluted with 1:1 *n*-hexane/ethyl acetate at 1.5 mL/min with detection at 258 nm. For analysis, a Shimadzu SPD-6A UV spectrophotometric detector and a Shimadzu C-R6A Chromato PAC were used. Product quantitation was determined from HPLC analysis by integration after determining response factors for authentic standards. Quantitation must proceed immediately after workup for consistent results. Retention times were 9.9 and 1.8 min for products **10** and **11** (diisopropyl acetal), respectively. The results are summarized in Table I.

Reductions of *N*-Benzylnicotinamide Chloride (18) with 2-PrOH Catalyzed by Various Zn^{II} Species. *N*-Benzylnicotinamide chloride (**18**, 124 mg, 0.5 mmol) and Zn^{II} species (0.05 mmol) were added to 20 mL of 2-PrOH degassed thoroughly with argon bubbling. The reaction

(7) For example: (a) Overman, L. E. *J. Org. Chem.* **1972**, *37*, 4214. (b) Shirra, A.; Suckling, C.-J. *Tetrahedron Lett.* **1975**, 3323. (c) Shirra, A.; Suckling, C.-J. *J. Chem. Soc., Perkin Trans. II* **1977**, 759. (d) Ohnishi, Y.; Minato, H.; Okuma, K.; Kobayashi, M. *Chem. Lett.* **1977**, 525. (e) Ohnishi, Y.; Kitami, M. *Tetrahedron Lett.* **1978**, 4035. (f) Ohnishi, Y.; Tanimoto, S. *Tetrahedron Lett.* **1977**, 1909. (g) Ohnishi, Y.; Kikuchi, Y.; Kitami, M. *Tetrahedron Lett.* **1979**, 3005. (h) Dittmer, D. C.; Blidner, B. B. *J. Org. Chem.* **1978**, *38*, 2873.

(8) Magnesium ion is the most successful of many metal ions tested for activation of carbonyl compounds toward reduction by NADH model compounds. See, for example: (a) Ohnishi, Y.; Numakunai, M.; Ohno, A. *Tetrahedron Lett.* **1975**, 3813. (b) Ohno, A.; Ikeguchi, M.; Kimura, T.; Oka, S. *J. Chem. Soc., Chem. Commun.* **1978**, 328. (c) Ohnishi, Y.; Kagami, M.; Ohno, A. *J. Am. Chem. Soc.* **1975**, *97*, 4766.

(9) For Zn^{II} ion catalysis, for example: (a) Creighton, D. J.; Sigman, D. S. *J. Am. Chem. Soc.* **1971**, *93*, 6314. (b) Creighton, D. J.; Hajdu, J.; Mooser, G.; Sigman, D. S. **1973**, *95*, 6855. (c) Creighton, D. J.; Hajdu, J.; Sigman, D. S. *J. Am. Chem. Soc.* **1976**, *98*, 4619. (d) Shinkai, S.; Bruice, T. C. *J. Am. Chem. Soc.* **1972**, *94*, 8258. (e) Shinkai, S.; Bruice, T. C. *Biochemistry* **1973**, *12*, 1750. (f) Gase, R. A.; Boxhoorn, G.; Pandit, U. K. *Tetrahedron Lett.* **1976**, 2889. (g) Gase, R. A.; Pandit, U. K. *J. Am. Chem. Soc.* **1979**, *101*, 7159. (h) Ohno, A.; Kimura, T.; Kim, S. G.; Yamamoto, H.; Oka, S.; Ohnish, Y. *Bioorg. Chem.* **1977**, *6*, 21. (i) Ohno, A.; Yasui, S.; Oka, S. *Bull. Chem. Soc. Jpn.* **1980**, *53*, 2651, 3244. (j) Shinkai, S.; Hamada, H.; Kusano, Y.; Manabe, O. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 2600. (k) Hood, R. A.; Prince, R. H. *J. Chem. Soc., Chem. Commun.* **1979**, 163.

(10) Kimura, E.; Shiota, T.; Koike, T.; Shiro, M.; Kodama, M. *J. Am. Chem. Soc.* **1990**, *112*, 5805.

(11) Koike, T.; Kimura, E. *J. Am. Chem. Soc.* **1991**, *113*, 8935.

(12) Kimura, E.; Koike, T. *Comments Inorg. Chem.* **1991**, *11*, 285.

(13) Kimura, E.; Koike, T.; Shiota, T.; Iitaka, Y. *Inorg. Chem.* **1990**, *29*, 4621.

(14) Koike, T.; Kimura, E.; Nakamura, I.; Hashimoto, Y.; Shiro, M. *J. Am. Chem. Soc.* **1992**, *114*, 7338.

(15) Perrin, D. D.; Armarego, W. F.; Perrin, D. R. *Purification of Laboratory Chemicals*; Pergamon: Oxford, 1980.

(16) Mauzerall, D.; Westheimer, F. H. *J. Am. Chem. Soc.* **1955**, *77*, 2261.

(17) (a) Norman, P. R. *Inorg. Chim. Acta* **1987**, *130*, 1. (b) Norman, P. R.; Tate, A.; Rich, P. *Inorg. Chim. Acta* **1988**, *145*, 211.

(18) Kate, M.; Ito, T. *Inorg. Chem.* **1985**, *24*, 504.

Table I. Products Yield of the Reaction of *p*-Nitrobenzaldehyde (**9**) with Alcohols in the Presence of Various Zn^{II} Species at Reflux after 24 h[a]

catalyst	alcohol	product yield[b] (%) 10	11
$[L_1\text{-}Zn^{II}\text{-}OH]_3\cdot(TfO)_3\cdot TfOH$ (**5**)	2-PrOH	7820	370
	EtOH	5900	*c*
	MeOH	(55)[d]	*c*
	CF_3CH_2OH	3	*c*
$[L_2\text{-}Zn^{II}\text{-}OH_2](ClO_4)_2$ (**6**)	2-PrOH	12	1350
$[L_2\text{-}Zn^{II}\text{-}OH]_2(ClO_4)_2\cdot HClO_4$ (**7**)	2-PrOH	37	26
$[L_3\text{-}Zn^{II}](TfO)_2$ (**8**)	2-PrOH	13	12
$Zn^{II}(TfO)_2$	2-PrOH	460	3180
$Zn^{II}(ClO_4)_2\cdot 6H_2O$	2-PrOH	460	3450
$[12]aneN_3$[e]	2-PrOH	4[f]	<1[f]
none	2-PrOH	~0[f]	3[f]

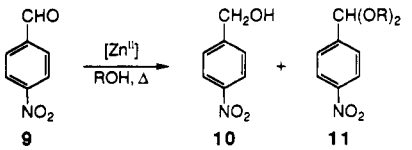
[a] $[Zn^{II}]$ = 1 mM (0.8 mol %), [**9**] = 125 mM. All reaction mixtures were gently refluxed. [b] Based on the total amount of Zn^{II} species, ([product]/$[Zn^{II}]$) × 100 (%). Yields of the dialkyl acetal product (**11**) fluctuate depending on time and temperature because of its equilibrium. Yields described here were determined after general workup at room temperature. [c] Not determined [d] Unidentified precipitate was formed during the reaction. [e] $[[12]aneN_3]$ = 1 mM. [f] Yield after 11 h.

mixture was heated at reflux for 24 h under argon in the dark. The reaction was quenched with water and immediately extracted with several portions of CH_2Cl_2. The combined organic layer was dried over anhydrous Na_2SO_4. After filtration, the filtrate was evaporated to dryness, and the crude residue was analyzed by ^1H NMR ($CDCl_3$, Me_4Si as the reference) with *p*-dinitrobenzene as the internal standard. The yields of the reduced products, "1,4-adduct" **19** and "1,6-adduct" **20**, were determined by using the peak integration of the benzylic proton (δ 4.24, s for **19**, and δ 4.16, s for **20**) and of the C_5 proton (δ 4.72, dt, J = 3.5, 8.1 Hz for **19**, and δ 4.94, dt, J = 3.5, 10.0 Hz for **20**). The results are summarized in Table II.

Results and Discussion

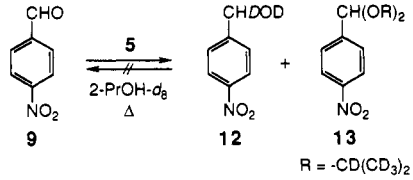
Reactions of *p*-Nitrobenzaldehyde (9) with Alcohols (ROH) Catalyzed by Zn^{II} Species (Reaction 1). In a typical experiment, *p*-nitrobenzaldehyde (**9**, 0.125 mmol)[19] was heated at reflux for 24 h under argon in 1.0 mL of an alcohol (2-PrOH, 2-PrOH-d_8, EtOH, MeOH, or CF_3CH_2OH) containing 0.8 mol % of a Zn^{II} catalyst (1 μmol): $[L_1\text{-}Zn^{II}\text{-}OH]_3\cdot(TfO)_3\cdot TfOH$ (**5**, TfO = $CF_3SO_3^-$), $[L_2\text{-}Zn^{II}\text{-}OH_2]_2\cdot(ClO_4)_2$ (**6**), $[L_2\text{-}Zn^{II}\text{-}OH]_2\cdot(ClO_4)_2\cdot HClO_4$ (**7**), $[L_3\text{-}Zn^{II}](TfO)_2$ (**8**, L_3 = 1,4,8,11-tetraazacyclotetradodecane), or other Zn^{II} salts. The reactions gave only two products, *p*-nitrobenzyl alcohol (**10**) and *p*-nitrobenzaldehyde dialkyl acetals (**11**), and the product mixtures were analyzed and followed by HPLC and ^1H NMR spectroscopy.

Comparison of the product distribution and yields with catalysts **5–8** (Table I) is highly instructive on the role of the Zn^{II} complex. Two types of nucleophilic reactions, i.e., the hydride transfer to carbonyl carbons to produce **10** (an ADH-like reaction) and "alkoxide transfer" to produce **11** (corresponding to the carbonyl hydration with carbonic anhydrase), apparently branch from a common intermediate.



9 **10** **11** (1)

Most significantly, *the reaction in 2-PrOH with 5 after 24 h gave the product 10 in 7820%* (corresponding to 62.4% consumption of the starting aldehyde), as illustrated in Figure 1. Other Zn^{II} species **6–8** were virtually noncatalytic for this ADH-mimic reaction. Reaction 2 in 2-PrOH-d_8 catalyzed by **5** was monitored by ^1H NMR for 40 h, which unequivocally proved *the quantitative "D^- transfer" to yield a monodeuterated p-nitrobenzyl alcohol (12) at the benzylic position.* The peak integration ratio for the benzylic proton (–CH*D*OD) was constant during this reaction, indicating no reverse hydride transfer from **12**. Indeed, starting from **10** and acetone the Zn^{II} complex **5** did not catalyze this reverse reaction, which should involve transfer of the less active hydride (due to *p*-nitro group) to the nonactivated carbonyl of acetone.



9 **12** **13** (2)

R = -CD(CD3)2

The outstanding yields of **10** by **5** over other Zn^{II} species (**6–8**) strongly suggest that the most acidic Zn^{II} in the $Zn^{II}\text{-}L_1$ complex (cf. pK_a values in Chart I) readily generates zinc(II)–alkoxide ion,[20] which then serves to labilize the α-C–H bond. In view of the low barrier for the four- ⇌ five-coordinate configurational interconversion with $Zn^{II}\text{-}L_1$ complex,[21] a mechanism comprising both four and five coordination is proposed (Figure 2). The hydroxide ion in **5** may act as a base to generate a pentacoordinate alkoxide complex. The electron-withdrawing substituents on CF_3CH_2OH would stabilize the developing negative charge on oxygen, thereby effectively reducing the reactivity (see Table I). By contrast, the electron-donating methyl groups on 2-PrOH lead to enhanced catalytic activity. The $Zn^{II}\text{-}L_1$ complex, which has the largest space available for the reaction site, facilitates the way

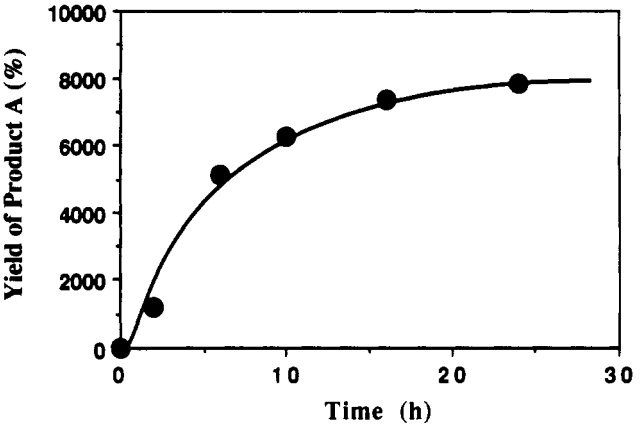


Figure 1. Time course of the reduction of *p*-nitrobenzaldehyde (**9**, 125 mmol) to the corresponding alcohol (product **10**) in refluxing 2-PrOH catalyzed by **5** (0.8 mol %). Yields are based on $[Zn^{II}]$.

Table II. Products Yield of the Reaction of *N*-Benzylnicotinamide Chloride (**18**) with 2-PrOH in the Presence of Various Zn^{II} Species after 24 h[a]

catalyst	product yield[b] (%) 1,4-adduct (**19**)	1,6-adduct (**20**)	1,4-/1,6-
$[L_1\text{-}Zn^{II}\text{-}OH]_3\cdot(TfO)_3\cdot TfOH$ (**5**)	17	2.3	7.5
$[L_2\text{-}Zn^{II}\text{-}OH_2](ClO_4)_2$ (**6**)	0	0	
$[L_2\text{-}Zn^{II}\text{-}OH]_2(ClO_4)_2\cdot HClO_4$ (**7**)	0[c]	0	
$[L_3\text{-}Zn^{II}](TfO)_2$ (**8**)	0	0	
$Zn^{II}(TfO)_2$	0	0	

[a] $[Zn^{II}]$ = 2.5 mM (10 mol %), [**18**] = 25 mM. All reactions were carried out at reflux under argon in the dark. [b] Based on the total amount of Zn^{II} species, ([product]/$[Zn^{II}]$) × 100 (%). Yields were determined by ^1H NMR analysis using *p*-dinitrobenzene as the internal standard. [c] A trace amount of **19** was detected only by TLC.

(19) The reactions with other less activated carbonyl compounds (e.g., benzophenone, trifluoroacetophenone, acetone, benzaldehyde, etc.) as hydride acceptors proceed much slower than that with *p*-nitrobenzaldehyde (**9**).

(20) In support of this notion, with a recently synthesized *N*-hydroxyethyl-substituted $[12]aneN_3$ we observed the alcohol proton dissociation at pH 7~8 to yield a Zn^{II}–alkoxide anion coordinating complex. The details will be soon described elsewhere.

(21) Kimura, E.; Koike, T.; Shionoya, M.; Shiro, M. *Chem. Lett.* **1992**, 787.

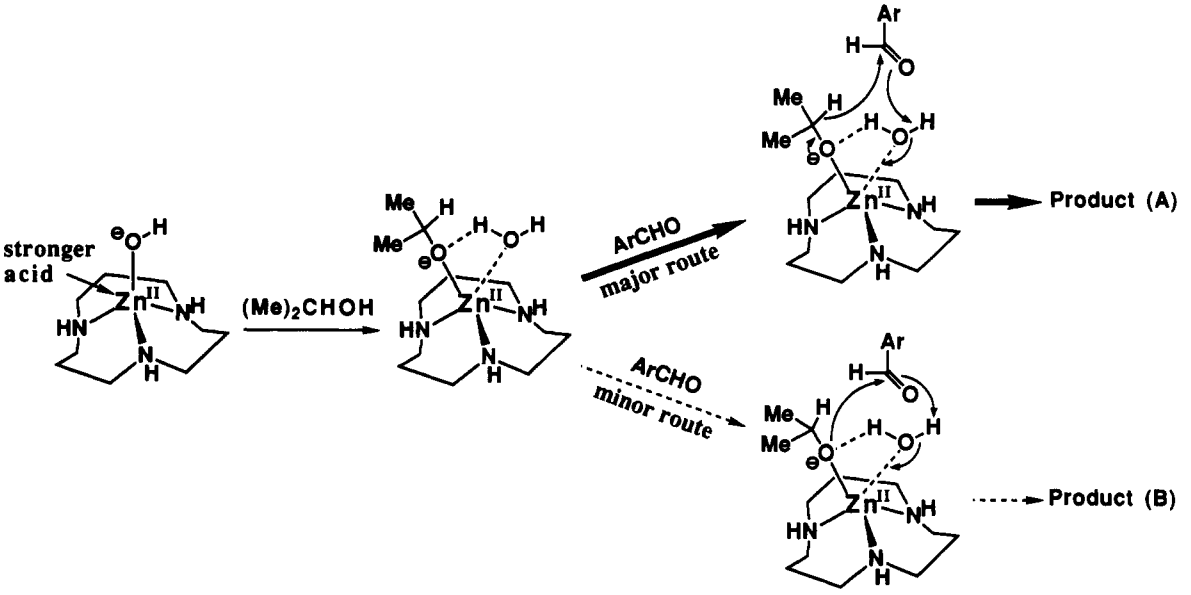
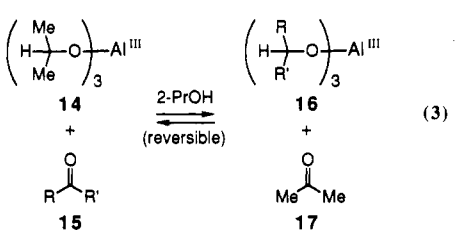


Figure 2. A proposed reaction mechanism for hydride transfer and alkoxide transfer on Zn^{II} species **5**.

for the smooth hydride transfer. A mechanism in Figure 2 is nearly the same as the Dworshack and Plapp's hydride transfer mechanism postulated for ADH.[22]

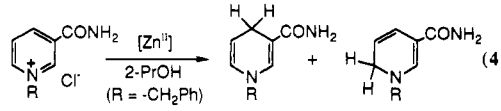
Furthermore, the same trend in catalytic dependence on alcohols is also seen in the "Meerwine–Pondorf–Verley" reaction[23] using aluminum isopropoxide (**14**), $(Me_2CHO)_3Al^{III}$, which involves a similar hydride transfer from 2-propoxide to carbonyl compounds RR'C=O (**15**) on Al^{III} (see reaction 3). In reaction 3, the reduced products ($RR'CHO^-$) are thought to be strongly bound to Al^{III} to form (**16**), since Al^{III} is an extremely strong acid. Accordingly, the reverse hydride transfer from **16** to acetone (**17**) can also occur as shown by the Oppenauer oxidation of a secondary alcohol to the corresponding ketone.[24] In reaction 3 it is usually practical to employ more than catalytic (i.e., a *stoichiometric* or more) amount of **14** and to remove acetone from the reaction mixture to drive the reaction. By contrast, in reaction 1 Zn^{II} species **5** is a milder acid and the interaction between Zn^{II} and alkoxide (either 2-PrO^- or $ArCH_2O^-$) is weaker, so that smooth ligand exchange can occur on Zn^{II}. Consequently, the *catalytic turnover* (with a trace amount of **5**) of the hydride transfer reaction become feasible with **5**.



14 + **15** ⇌ **16** + **17** (3)

Interestingly, in reaction 1 the RO^- nucleophilic reaction (to produce **11**) simultaneously occurred. However, its yield is much less with **5** with respect to free Zn^{II} salts (Table I). The reaction to yield **11** corresponds to the hydration of RR'C=O (or CO_2) in aqueous solution. The stronger acid nature of Zn^{II} in **5**, which is translated into the stronger $RO^- \rightarrow Zn^{II}$ withdrawing effect, is probably responsible for the greater labilization of the α-C–H bond, leading to the almost exclusive production of **10**. In addition, this reaction (to produce **10**) is strongly inhibited by the addition of two-thirds equivalent of anhydrous *p*-toluenesulfonic acid to **5** to convert Zn^{II}-OH^- to Zn^{II}-OH_2. Namely, the acidic form of **5**, L_1-Zn^{II}-OH_2, does not possess any catalytic activity in the forward hydride transfer reaction.

Reactions of *N*-Benzylnicotinamide Chloride (9) with 2-PrOH Catalyzed by Zn^{II} Species (Reaction 4). An NAD^+ model compound **18**, *N*-benzylnicotinamide chloride (0.50 mmol), was examined as a hydride transfer substrate in refluxing 2-PrOH in the presence of 10 mol % of Zn^{II} species under argon in the dark. The reaction was followed by TLC, UV, and ^1H NMR (reaction 4). The results are summarized in Table II. Most remarkably, in the reaction with **5** (up to 24 h) *we have observed almost exclusive formation of the 1,4-adduct* ***19****, N-benzyl-1,4-dihydronicotinamide (17% yield), as is the case in the real ADH reaction.* The reaction was neat, and no other product other than the minor 1,6-adduct **20** (**19**/**20** = 7.5) was detected. This fact implies that the electrostatic repulsion between Zn^{2+} and pyridinium$^+$ may have influence on the relatively slower rate (compared with the reaction 1) as well as on the exclusive reaction product **19** over **20**. As was seen in the reaction 1 with *p*-nitrobenzaldehyde, other Zn^{II} species **6**–**8** did not work as the catalyst at all (Table II). Interestingly, *a reverse reaction ("hydride donation") of (4) (e.g., treatment of* ***19*** *with an activated carbonyl, β-ketoester) was not catalyzed by* ***5*** *at all.* Interestingly, however, this reverse reaction occurred in the presence of weaker acids such as $Mg^{II}(ClO_4)_2$ and $Zn^{II}(TfO)_2$. More detailed studies of this backward reaction mechanism is underway.



18 (NAD$^+$ Model) → **19** (major) (NADH Model) + **20** (minor) (4)

In conclusion, the present study provides the first chemical model illustrating the significance of the strengthened acidity of Zn^{II} (pK_a 7.6)[5] and the resulting Zn^{II}-OH^- (conjugate base) formation (at neutral pH) and the steric requirement around Zn^{II} coordination sphere in the forward hydride transfer reaction catalyzed by ADH. It also demonstrates that for the catalysis of the reverse hydride donation reaction, a different function of Zn^{II} (e.g., the presence of Zn^{II}-OH_2 at neutral pH) may be required, as enzymologically shown by the weakened acidity of Zn^{II} (pK_a 11.2)[5] in ADH.

Acknowledgment. We are thankful to the Ministry of Education in Japan for the financial support by a Grant-in-Aid for Special Project of "Bioinorganic Chemistry" (No. 03241105).

(22) Dworschack, R. T.; Plapp, B. V. *Biochemistry* **1977**, *66*, 2716.
(23) (a) Wilds, A. L. *Org. React.* (NY) **1944**, *2*, 178. (b) Djerass, C. *Org. React.* (NY) **1951**, *6*, 207.
(24) Oppenauer, *Recl. Trav. Chim. Pays-Bas* **1937**, *56*, 137.