Modeling the Active Site Chemistry of Liver Alcohol Dehydrogenase: Mononuclear Zinc Methanol and *N*,*N*-Dimethylformamide Complexes of a Nitrogen/Sulfur Ligand Possessing an Internal Hydrogen Bond Donor

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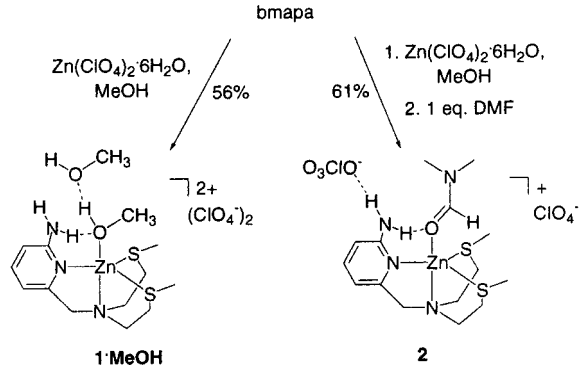
Liver alcohol dehydrogenase (LADH) catalyzes the biological oxidation of alcohols to aldehydes and ketones.[1] Within the active site of LADH is a mononuclear zinc center ligated in tetrahedral array by two cysteine thiolates, a histidine nitrogen, and a water molecule.[1] A mechanistic proposal for alcohol oxidation by LADH suggests a stepwise reaction pathway in which (1) NAD^+ binds to the enzyme; (2) the alcohol substrate binds to the enzyme via coordination to the Zn^{2+} center; (3) deprotonation of the alcohol occurs, producing a mononuclear nitrogen/sulfur-ligated zinc alkoxide species; (4) hydride transfer from the zinc-bound alkoxide to NAD^+ yields NADH and a zinc-bound aldehyde or ketone; (5) the product aldehyde or ketone is released; and (6) NADH dissociates.[2] Notably, a serine residue (Ser_{48}) in the active site of LADH plays a key role in several steps of this pathway. For example, X-ray crystallographic analysis of the $C_6F_5CH_2$-OH-bound form of LADH shows a strong hydrogen-bonding interaction between the hydroxyl proton of the zinc-bound alcohol substrate and the hydroxyl oxygen of Ser_{48} ($O(Ser_{48})\cdots O$(alcohol) 2.6 Å).[3] This interaction forms a portion of a proton transfer pathway from the active site of LADH to the solvent. Following deprotonation of the zinc-bound alcohol in LADH, Ser_{48} is proposed to stabilize the reactive zinc alkoxide species via a strong hydrogen-bonding interaction with the zinc-bound alkoxide oxygen.[4] On the basis of recent theoretical studies, this hydrogen-bonding interaction is proposed to weaken upon hydride transfer and formation of the zinc-bound aldehyde or ketone product.[5] Notably, X-ray crystallographic analyses of inhibited forms of LADH, in which the alcohol-binding site is occupied by a neutral oxygen donor (e.g., a secondary or tertiary formamide[6] or sulfoxide[7] derivative), indicate the presence of a hydrogen-bonding interaction between Ser_{48} and the zinc-bound oxygen of the inhibitor.

In efforts toward addressing various aspects of the chemistry involved in the reaction pathway of LADH, several synthetic modeling studies have previously been undertaken.[8,9] However, despite these efforts, mononuclear nitrogen/sulfur-ligated zinc alcohol, alkoxide, and aldehyde complexes relevant to proposed reactive species in the catalytic cycle of LADH have yet to be reported. As an approach toward isolating such complexes, we report herein our initial studies of the zinc coordination chemistry of a novel ligand system (bmapa, *N*,*N*-bis-2-(methylthio)ethyl-*N*-(6-amino-2-pyridylmethyl)amine) that combines a mixed nitrogen/sulfur coordination environment with a single internal hydrogen bond donor. Significantly, utilizing this ligand we have isolated and structurally characterized the first examples of mononuclear nitrogen/sulfur-ligated zinc methanol and *N*,*N*-dimethylformamide complexes that exhibit secondary hydrogen-bonding interactions akin to those found between zinc-bound molecules and Ser_{48} in the active site of LADH.

Scheme 1



The bmapa ligand (Scheme 1) was initially isolated as the product of a zinc-mediated amide cleavage reaction.[10] However, because this reaction involved the use of a zinc perchlorate complex, an alternative synthetic procedure that employs a zinc

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(1) Eklund, H.; Brändén, C.-I. In *Zinc Enzymes*; Spiro, T. G., Ed.; Wiley: New York, 1983; pp 124−152.

(2) (a) Klinman, J. P. *Crit. Rev. Biochem.* **1981**, *10*, 39−78. (b) Pettersson, G. *Crit. Rev. Biochem. Mol. Biol.* **1987**, *21*, 349−389.

(3) Ramaswamy, S.; Eklund, H.; Plapp, B. V. *Biochemistry* **1994**, *33*, 5230−5237.

(4) Ramaswamy, S.; Park, D.-H.; Plapp, B. V. *Biochemistry* **1999**, *38*, 13951−13959.

(5) Agarwal, P. K.; Webb, S. P.; Hammes-Schiffer, S. *J. Am. Chem. Soc.* **2000**, *122*, 4803−4812.

(6) (a) Ramaswamy, S.; Scholze, M.; Plapp, B. V. *Biochemistry* **1997**, *36*, 3522−3527. (b) Schindler, J. F.; Berst, K. B.; Plapp, B. V. *J. Med. Chem.* **1998**, *41*, 1696−1701. (c) Deng, H.; Schindler, J. F.; Berst, K. B.; Plapp, B. V.; Callender, R. *Biochemistry* **1998**, *37*, 14267−14278.

(7) (a) Cho, H.; Ramaswamy, S.; Plapp, B. V. *Biochemistry* **1997**, *36*, 382−389. (b) Al-Karadaghi, S.; Cedergren-Zeppezauer, E.; Hövmoller, S.; Petratos, K.; Terry, H.; Wilson, K. S. *Acta Crystallogr.* **1994**, *D50*, 793−807.

(8) (a) Kimblin, C.; Bridgewater, B. M.; Churchill, D. G.; Parkin, G. *Chem. Commun.* **1999**, 2301−2302. (b) Bergquist, C.; Parkin G. *Inorg. Chem.* **1999**, *38*, 422−423. (c) Kimblin, C.; Hascall, T.; Parkin, G. *Inorg. Chem.* **1997**, *36*, 5680−5681. (d) Shoner, S. C.; Humphreys, K. J.; Barnhart, D.; Kovacs, J.A. *Inorg. Chem.* **1995**, *34*, 5933−5934. (e) Kimura, E.; Shionoya, M.; Hoshino, A.; Ikeda, T.; Yamada, Y. *J. Am. Chem. Soc.* **1992**, *114*, 10134−10137. (f) Engbersen, J. F. J.; Koudijs, A.; van der Plas, H. C. *J. Org. Chem.* **1990**, *55*, 3647−3654. (g) Kellogg, R. M.; Hof, R. P. *J. Chem. Soc., Perkin Trans. 1* **1996**, 1651−1657. (h) Müller, B.; Schneider, A.; Tesmer, M.; Vahrenkamp, H. *Inorg. Chem.* **1999**, *38*, 1900−1907. (i) Walz, R.; Weis, K.; Ruf, M.; Vahrenkamp, H. *Chem. Ber.* **1997**, *130*, 975−980. (j) Ruf, M.; Schell, F. A.; Walz, R.; Vahrenkamp, H. *Chem. Ber.* **1997**, *130*, 101−104.

(9) We note that these efforts have led to the synthesis of several mixed nitrogen/sulfur ligands which produce thiolate bridged zinc complexes and are therefore unsuitable for modeling the active site chemistry of LADH: (a) Curtis, N. J.; Brown, R. S. *Can. J. Chem.* **1981**, *59*, 65−75. (b) Kaptein, B.; Wang-Griffin, L.; Barf, G.; Kellogg, R. M. *J. Chem. Soc., Chem. Commun.* **1987**, 1457−1459. (c) Kaptein, B.; Barf, G.; Kellogg, R. M.; Van Bolhuis, F. *J. Org. Chem.* **1990**, *55*, 1890−1901. (d) Bochmann, M.; Webb, K. J.; Hursthouse, M. B.; Mazid, M. *J. Chem. Soc., Chem. Commun.* **1991**, 1735−1737. (e) Brand, U.; Vahrenkamp, H. *Z. Anorg. Allg. Chem.* **1996**, *622*, 213-218. (f) Burth, R.; Stange, A. Schäfer, M.; Vahrenkamp, H. *Eur. J. Inorg. Chem.* **1998**, 1759−1764.

(10) Berreau, L. M.; Makowska-Grzyska, M. M.; Arif, A. M. *Inorg. Chem.* **2000**, *39*, 4390−4391.

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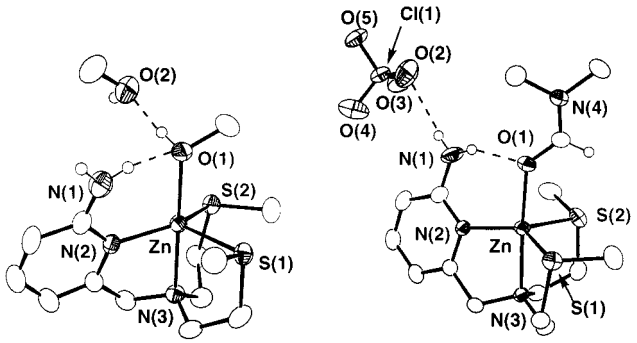

Figure 1. ORTEP representations of the cationic portions of [(bmapa)-Zn(CH_3OH)](ClO_4)$_2$·MeOH (**1**·MeOH) and [(bmapa)Zn(DMF)](ClO_4)$_2$ (**2**). All ellipsoids are drawn at the 50% probability level (all hydrogen atoms except the primary amine, methanol, and formamide protons not shown for clarity). Selected bond lengths (Å): (**1**) Zn−O(1) 2.077(1), Zn−N(2) 2.054(2), Zn−N(3) 2.163(2), Zn−S(1) 2.434(1), Zn−S(2) 2.452(1); (**2**) Zn−O(1) 2.049(2), Zn−N(2) 2.054(2), Zn−N(3) 2.189(2), Zn−S(1) 2.434(1), Zn−S(2) 2.441(1).

triflate precursor and is suitable for large-scale preparation of the bmapa ligand has been developed (Scheme S1, Supporting Information).[11] Treatment of the bmapa ligand with an equimolar amount of Zn(ClO_4)$_2$·6H_2O (Scheme 1) in methanol followed by crystallization via diethyl ether diffusion into a MeOH:*i*-PrOH (2:1) solution at ambient temperature yielded [(bmapa)Zn-(MeOH)](ClO_4)$_2$·MeOH (**1**·MeOH) as colorless crystalline blocks.[12] Addition of *N*,*N*-dimethylformamide to a reaction mixture identical to that utilized for the synthesis of **1**·MeOH yielded [(bmapa)-Zn(DMF)](ClO_4)$_2$ (**2**), which deposited from MeOH:*i*-PrOH:Et_2O as block-type crystals.

X-ray crystallographic analysis of **1**·MeOH revealed a mononuclear nitrogen/sulfur-ligated zinc alcohol complex (Figure 1, left) with all of the donor atoms of the bmapa ligand coordinated to the zinc center. The five-coordinate zinc ion exhibits a slightly distorted trigonal bipyramidal geometry ($\tau = 0.65$)[13] with the pyridyl nitrogen and the thioether sulfur donors in the equatorial plane. Complex **2** (Figure 1, right) exhibits a similar overall geometry ($\tau = 0.73$)[13] in the solid state with equatorial bond distances comparable to those found in **1**·MeOH. Due to the pentacoordinate nature of the zinc ion in **1**·MeOH, the Zn−O(MeOH) distance in this complex (2.077(1) Å) is longer than that found in the four-coordinate tetrahedral $C_6F_5CH_2OH$ adduct of LADH (2.0 Å) and in a recently reported S_3-ligated tetrahedral zinc complex possessing a single methanol ligand ({[Tm^{Mes}]-Zn(HOMe)}ClO_4·MeOH: 1.993(3) Å).[3,8a] The Zn−O(DMF) distance in **2** (2.049(2) Å) is noticeably shorter than that reported for the *N*-formylpiperidine adduct of LADH (2.3 Å),[6a] but is comparable to amide oxygen ligation in other synthetic zinc complexes.[10]

Complexes **1**·MeOH and **2** exhibit several hydrogen-bonding interactions in the solid state, one of which for each complex is derived from the strategic positioning of the primary amine hydrogen bond donor in the supporting ligand. Specifically, in each complex, the primary amine moiety of the bmapa ligand acts as a hydrogen bond donor to the oxygen atom of the zinc-bound exogenous molecule. In **2**, this interaction (N(1)···O(1) 2.86 Å, N(1)−H(1A)···O(1) 156°) mimics the hydrogen bonding observed between Ser_{48} and the oxygen atom of zinc-bound formamide derivatives in LADH (*N*-cyclohexylformamide and *N*-formylpiperidine, O(Ser_{48})···O(formamide) ∼ 2.6 Å), albeit the heteroatom separation in **2** is longer. In **1**·MeOH, a comparable hydrogen-bonding interaction is observed between a primary amine proton of the ligand and the oxygen atom of the zinc-bound methanol molecule (N(1)···O(1) 2.92 Å, N(1)−H(N1A)···O(1) 147°). Similar to a recently reported model complex ({[Tm^{Mes}]Zn(HOMe)}ClO_4·MeOH: O(MeOH)···O(Zn−HOMe) 2.58 Å),[8a] **1**·MeOH exhibits a hydrogen-bonding interaction between the hydroxyl proton of the zinc-bound methanol and an additional molecule of methanol (O(1)···O(2) 2.58 Å, O(1)−H(O1)···O(2) 169°). This short hydrogen-bonding interaction involving the zinc-bound methanol mimics the hydrogen bond network involving Ser_{48} in LADH (O(Ser_{48})···O(alcohol) 2.6 Å)[3] that is responsible for proton transfer from the active site of the enzyme to the solvent.

The FTIR spectroscopic properties of **1**[14] and **2** in the solid state are consistent with their X-ray crystallographically determined structures. For example, FTIR spectra of complexes **1** and **2** (collected as dilute KBr pellets) contain multiple broad bands in the 3250−3500 cm^{-1} region. These spectral features are consistent with the presence of the NH_2 moiety (symmetric and asymmetric vibrations are found in this region) in both complexes and the presence of a MeOH molecule in **1**, all of which are involved in hydrogen-bonding interactions as determined by X-ray crystallography. Spectroscopic features of the *N*,*N*-dimethylformamide moiety of **2** are indicative of binding of the formamide oxygen to the metal center. For example, the C−O stretching vibration of **2** is found at 1663 cm^{-1}, whereas the corresponding vibration in free DMF (neat sample) is found at 1677 cm^{-1}. While we are currently exploring various aspects of the solution properties of **1** and **2**, initial ^{1}H NMR spectra in CD_3CN (0.0193 M, 22(1) °C) have revealed notable downfield shifts (**1**, ∼5.8 ppm; **2**, ∼6.1 ppm) of the $-NH_2$ resonance compared to that observed for the free ligand (4.75 ppm) under identical conditions. This deshielding of the $-NH_2$ resonance in **1** and **2** suggests that some degree of hydrogen bonding involving the primary amine moiety is likely occurring in solution.

In summary, we have utilized a novel nitrogen/sulfur ligand possessing a single internal hydrogen bond donor to generate mononuclear zinc methanol and *N*,*N*-dimethylformamide complexes that are relevant to substrate- and inhibitor-bound forms of LADH. Importantly, while the bmapa ligand does not structurally mimic the coordination environment of the mononuclear zinc center in LADH in terms of overall coordination number (N_2S_2 vs NS_2 in LADH), we have demonstrated that it does enable the isolation of biomimetic complexes with a predictable hydrogen bond donor akin to Ser_{48} in the active site of LADH. With that in mind, we are currently examining further aspects of the zinc coordination chemistry of this ligand including its ability to stabilize mononuclear nitrogen/sulfur-ligated zinc alkoxide and aldehyde complexes relevant to the active and product-bound forms of LADH.

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Supporting Information Available: Synthetic details and characterization data for **1**·MeOH and **2** and two X-ray crystallographic files (CIF). A scheme outlining the large-scale synthetic procedure of the bmapa ligand (Scheme S1). This material is available free of charge via the Internet at http://pubs.acs.org.

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(11) A complete description of the new synthetic pathway for the bmapa ligand and characterization data for the new zinc complexes is provided in the Supporting Information.

(12) **CAUTION:** Perchlorate salts of metal complexes with organic ligands are potentially explosive. Only small amounts of material should be prepared, and these should be handled with great care. Wolsey, W. C. *J. Chem. Educ.* **1973**, *50*, A335−A337.

(13) Addison, A. W.; Rao, T. N.; Reedijk, J.; van Rijn, J.; Verschoor, G. C. *J. Chem. Soc., Dalton Trans.* **1984**, 1349−1356.

(14) Approximately one molecule of methanol is lost upon drying, as indicated by elemental analysis of a bulk dried sample. (See Supporting Information.) This bulk sample is referred to herein as **1**.