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Mononuclear Nitrogen/Sulfur-Ligated Zinc Methoxide and Hydroxide Complexes: Investigating Ligand Effects on the Hydrolytic Stability of Zinc Alkoxide Species

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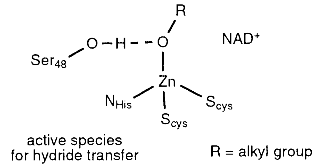
The synthesis and structural characterization of mononuclear nitrogen/sulfur-ligated zinc methoxide and hydroxide complexes are reported. Elucidation of thermodynamic parameters for equilibrium mixtures of these complexes provides insight into how ligand structural features influence the hydrolytic stability of zinc alkoxide species.

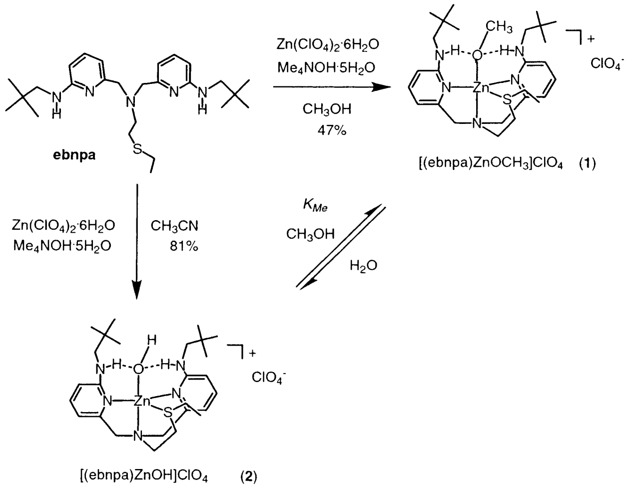
The formation of a mononuclear nitrogen/sulfur-ligated zinc alkoxide species, via displacement of a zinc-bound water molecule accompanied by an additional proton transfer, is a key proposed step in the mechanistic pathway of alcohol oxidation catalyzed by liver alcohol dehydrogenase (LADH).[1−3] Once formed, the zinc alkoxide species (Scheme 1) is suggested to be the active moiety for hydride transfer to NAD^+, a chemical reaction that yields the oxidized alcohol product (an aldehyde or ketone) and NADH. On the basis of the proposed pivotal role of a zinc alkoxide species in the catalytic pathway of LADH, elucidation of chemical factors that govern the formation and reactivity of Zn−OR species is clearly important toward fully understanding LADH-mediated alcohol oxidation.

One structural feature of the LADH active site that may influence the chemistry of a zinc-bound alkoxide is a serine residue (Ser_{48}) positioned near the active site zinc ion. On the basis of biochemical and computational studies,[2,3] Ser_{48} is proposed to interact with the zinc-bound alkoxide species via a strong hydrogen bond involving the alkoxide oxygen ($O(Ser_{48})\cdots O(R)$ 2.44 Å (calculated)).[3] To begin to examine how ligand effects, including hydrogen bonding, influence the chemistry of mononuclear zinc alkoxide species, we report herein studies of the zinc coordination chemistry of a mixed nitrogen/sulfur ligand possessing two internal hydrogen bond donors (ebnpa, *N*-2-(ethylthio)ethyl-*N*,*N*-bis(6-neopentylamino-2-pyridylmethyl)amine). Notably, while this ligand does not provide an exact mimic of the primary and secondary coordination environment found in LADH, the ebnpa ligand system has enabled, for the first time, isolation and structural characterization of a mononuclear nitrogen/sulfur-ligated zinc methoxide complex. In the presence of water, this methoxide derivative ($[(ebnpa)Zn-OCH_3]ClO_4$) forms an equilibrium mixture with the corresponding hydroxide complex and free alcohol that is notably shifted from that observed for $[Tp^{tBu,Me}ZnOCH_3]$.[4] The results presented herein thus suggest an important biologically relevant influence of ligand effects, including hydrogen bonding, on the stability of a zinc alkoxide moiety with respect to hydrolysis.

Scheme 1



Scheme 2



Examples of structurally characterized mononuclear zinc alkoxide complexes are rare, with no reported examples possessing a mixed nitrogen/sulfur supporting coordination environment.[4−7] Of particular importance to LADH are complexes exhibiting structural and functional relevance to the proposed zinc alkoxide active form of the enzyme, which have been reported by Parkin,[4,5] Kimura,[6d−g] and Vahrenkamp.[6a,b] Generation of a portion of these complexes was achieved via reaction of a zinc hydroxide precursor with an alcohol.[4−6] However, the isolation of several zinc hydroxide complexes from methanol solution has demonstrated that, despite similar pK_a values for water (15.7)[8] and methanol (15.1),[8] zinc hydroxide species predominate in methanol solution for many ligand systems.[4,5,9] We have found that treatment of the ebnpa ligand with equimolar amounts of $Zn(ClO_4)_2{\cdot}6H_2O$ and $Me_4NOH{\cdot}5H_2O$ in methanol (Scheme 2) results in the isolation of a complex with spectroscopic properties and an analytical composition consistent with the formation of a methoxide derivative, $[(ebnpa)Zn-OCH_3]ClO_4$ (**1**). Notably, this complex is spectroscopically distinct from the hydroxide derivative $[(ebnpa)Zn-OH]ClO_4$ (**2**), which has been produced from the same reagents in CH_3CN solution. For example, important ^1H NMR spectroscopic differences between **1** and **2**

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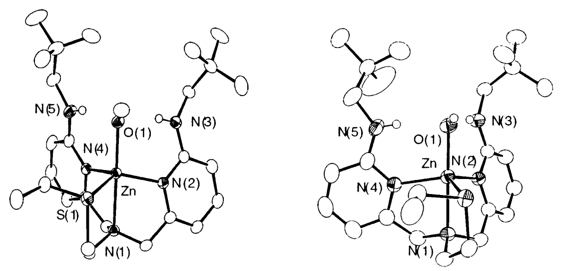

Figure 1. ORTEP diagrams of the cationic portion of **1** (left) and **2** (right). All ellipsoids are drawn at the 50% probability level (all hydrogens except the secondary amine and hydroxyl protons not shown for clarity). Only one of two chemically similar, but structurally unique, cations within the asymmetric unit of **1** is shown. Selected bond lengths: (**1**) Zn(1)−O(1) 1.953(8), Zn(1)−N(1) 2.212(10), Zn(1)−N(2) 2.095(8), Zn(1)−N(4) 2.076(9), Zn(1)−S(1) 2.490(3) Å; (**2**) Zn(1)−O(1) 1.925(2), Zn(1)−N(1) 2.193(2), Zn(1)−N(2) 2.077(2), Zn(1)−N(4) 2.109(2), Zn(1)−S(1) 2.533(1) Å. Selected hydrogen-bonding interactions: (**1**) N(3)···O(1) 2.77 Å, N(3)−H··O(1) 166°, N(5)···O(1) 2.77 Å, N(5)···O(1) 163°; (**2**) N(3)··O(1) 2.74 Å, N(3)−H···O(1) 171°; N(5)···O(1) 2.73 Å, N(5)−H···O(1) 169°.

include (a) a singlet resonance in CD_3CN spectra of **1** at 3.72 ppm which integrates to three hydrogens, and (b) an upfield shifted secondary amine N−*H* resonance in **1** (10.37 ppm) as compared to that found in [(ebnpa)Zn(OH)]ClO_4 (10.97 ppm).

X-ray crystallographic analysis of single crystals of **1** and **2** (Figure 1) revealed that, for each complex, the mononuclear nitrogen/sulfur-ligated zinc center binds a single anion (**1**, methoxide; **2**, hydroxide), resulting in a slightly distorted trigonal bipyramidal geometry (**1**, $\tau = 0.84$, 0.88; **2**, $\tau = 0.84$).[10] The metrical parameters of the zinc coordination environment in **1** and **2** are similar, with the most notable perturbation being in the Zn(1)−S(1) distance (**1**, Zn(1)−S(1) 2.490(3) Å; **2**, Zn(1)−S(1) 2.533(1) Å). The secondary environments in **1** and **2** both exhibit two hydrogen-bonding interactions with the oxygen atom of the zinc-bound anion (N(3/5)···O(anion)$_{avg}$ 2.73−2.77 Å).

Addition of methanol to analytically pure **2** in dry CD_3CN leads to the formation of an equilibrium mixture involving **1** and **2** (eq 1). Measurement of the equilibrium constant of this reaction at 304(1) K via ^{1}H NMR yielded $K_{Me} = 0.30(8)$.

$$[(\text{ebnpa})\text{ZnOH}]\text{ClO}_4 + \text{CH}_3\text{OH} \xrightleftharpoons{K_{Me}} [(\text{ebnpa})\text{ZnOCH}_3]\text{ClO}_4 + \text{H}_2\text{O} \quad (1)$$

$$[\text{Tp}^{t\text{Bu,Me}}]\text{ZnOH} + \text{CH}_3\text{OH} \xrightleftharpoons{K_{Me}} [\text{Tp}^{t\text{Bu,Me}}]\text{ZnOCH}_3 + \text{H}_2\text{O} \quad (2)$$

Confirmation of the presence of an equilibrium in this system was achieved (1) via treatment of a dry CD_3CN solution of **1** with water, which yielded an identical equilibrium mixture, and (2) through variable temperature ^{1}H NMR studies. Examination of the temperature dependence of this equilibrium yielded thermodynamic parameters $\Delta H_{Me} = -0.9(1)$ kcal/mol and $\Delta S_{Me} = -5(1)$ eu that are interestingly different from those found for zinc methoxide/hydroxide equilibria involving the [Tp$^{t\text{Bu,Me}}$Zn−OH]/[Tp$^{t\text{Bu,Me}}$Zn−OCH_3] complexes (eq 2, K_{Me} (300 K) $1.4(2) \times 10^{-3}$, $\Delta H_{Me} = 1.2(1)$ kcal/mol, and $\Delta S_{Me} = -9(1)$ eu).[4,5] The negative ΔH_{Me} value for eq 1 indicates that spontaneous alkoxide formation from a zinc hydroxide precursor occurs in the ebnpa-ligated system at low temperature. This contrasts with the situation found for eq 2, where the positive ΔH_{Me} indicates that alkoxide formation from the Zn−OH precursor will be thermodynamically unfavorable at all temperatures.

Following a strategy employed by Parkin,[4,5] we used the experimentally determined ΔH_{Me} value for the equilibrium depicted in eq 1 to calculate the relative difference in Zn−O homolytic bond dissociation energies between the ebnpa-ligated Zn−OH and Zn−OCH_3 complexes. This difference, favoring the hydroxide by ∼14 kcal/mol, is ∼2 kcal/mol lower than that reported for the [Tp$^{t\text{Bu,Me}}$-ZnOH]/[Tp$^{t\text{Bu,Me}}$ZnOCH_3] (∼16 kcal/mol) system (eq 2).

The hydrogen-bonding interactions in **1** and **2** may be considered nearly equivalent, on the basis of structural and spectroscopic data, and may be expected to stabilize anion binding in both complexes. On the basis of a preliminary calculation of the solvent accessibility of the Zn−OCH_3 and Zn−OH units in **1** and **2**, the hydroxo moiety is exposed to solvent and thus may interact with solvent (including methanol) via hydrogen bonding (Figure S5, Supporting Information). Once the reaction takes place to form the methoxide derivative, the methyl carbon atom, in addition to the hydrogen-bonding interactions, limits water access to the alkoxide oxygen in **1**. This results in increased stabilization of the zinc alkoxide complex with respect to the hydroxide derivative. In regard to LADH, these results indicate that active site structural features, including hydrogen-bonding interactions involving Ser_{48}, likely enhance the hydrolytic stability of a zinc alkoxide moiety.

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Supporting Information Available: Synthetic details and characterization data for the ebnpa ligand, **1** and **2**; representative ^{1}H NMR spectra of **1**, **2** and an equilibrium mixture of **1** and **2**; van't Hoff plot; and calculated solvent accessibility figure (PDF). Two X-ray crystallographic files (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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