A Model for the Interaction of Alcohol with the Zinc Thiolate Site of Alcohol Dehydrogenase

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Non-covalent interactions between cysteinate-ligated metal sites and protic substrates and/or the surrounding protein environment[1,2] can have an important influence on metalloprotein function and enzyme activity. For this reason, we are investigating these effects at a wide variety of synthetic thiolate-ligated metal sites.[3–5] For example, the active site of liver alcohol dehydrogenase (LADH) has been shown by protein crystallography[6–9] to consist of a Zn^{2+} ion tetrahedrally ligated by two cysteinate (SR^-) residues, a histidine N, and a water. It functions to oxidize alcohols to aldehydes or ketones in the human body.[10,11] The mechanism of this reaction has been the subject of numerous proposals;[12–18] however, synthetic model reactions to support these proposed mechanisms are scarce.[17] Of greatest interest has been the mechanism by which the proton is removed from the alcohol.[10,11,17,19,20] Synthetic modeling studies with cationic $[Zn^{II}N_4]^{2+}$ complexes demonstrate that the pK_a of an alcohol drops considerably when it binds to zinc so that deprotonation occurs spontaneously.[17] However, with thiolates (SR^-) in the coordination sphere one would expect the Zn^{2+} ion to be a much poorer Lewis acid. The influence of SR^- on the reactivity of zinc in LADH has not yet been investigated. Since S-alkylation of one of the Zn-bound S-cys has been reported to shut down LADH activity,[21] it is important that the role of SR^- receive attention. The few reported reactivity models for LADH [14,15,17,22] do not contain thiolates. Structural models containing SR^-/N-ligated Zn^{2+} have been reported;[23–25] however, reactivity studies were not included. Herein we report structural evidence which suggests that with thiolates in the coordination sphere a kinetically more favorable mechanism for LADH-promoted alcohol activation might involve initial partial proton transfer to a Zn-bound thiolate without prior coordination of the alcohol to the metal.

The Schiff base compound $ZnL_{S2(Me)N3(Pr)}$·MeOH ((1,2-dimethyl-3,7,1-triazatrideca-2,11-diene-1,13-dithiolato)zinc(II)–methanol (**1**)) was synthesized[26] and structurally characterized as described below.[27] Metrical features of **1** (Figure 1) are similar to those of the water-soluble Ni^{2+} complex $NiL_{S2(Me)N3(Pr)}$ previously reported by our group.[4] The most notable structural feature of **1** is the short S···O (3.213(4) Å) separation between the Zn–SR and a cocrystallized MeOH molecule, which is indicative of the presence of a weak intermolecular S···H hydrogen bond.[28–30] This is supported[31] by the presence of a red-shifted ($\Delta\nu_{OH} = 400$ cm^{-1}) ν_{O-H} stretch at 3242 cm^{-1} in the IR (solid state), which shifts to 2431 cm^{-1} upon incorporation of CD_3OD. Refinement of H(1O) places it 2.34(6) Å away from S(2) and 0.88(6) Å from O(1) (Figure 3).[32]

Sodium borohydride reduction converts imine/thiolate-ligated **1** to amine/thiolate-ligated $ZnL_{S2(Me)N3(Pr)H4}$·MeOH (**2**)[33] and, as can be seen in ORTEP diagrams[34] of Figures 2 and 3, causes the ligand to change its configuration so that a chelated MeOH molecule fits between one of the Zn–SR's and the in-plane Zn–NH. The methanolic O···Zn separation (3.831 Å) indicates that there is no interaction with the zinc ion. The observed configurational change is most likely induced by the removal of ligand constraints and consequent lengthening of the apical

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(26) Complex **1** was prepared (yield: 30%) in a manner similar to that reported for its Ni analogue in ref 4. Anal. Calcd for $C_{13}H_{27}N_3S_2OZn$: C, 42.10; H, 7.34; N, 11.33. Found: C, 42.47; H, 7.41; N, 11.04. ^{1}H NMR (CD_3OD): δ 3.68 (m), 3.53 (m), 3.05 (t), 2.60 (t), 2.14 (m), 1.97 (s, CH_3), 1.76 (m). IR: 3242 cm^{-1} (ν_{O-H}), 1670 cm^{-1} ($\nu_{C=N}$).
(27) Crystallographic data with Mo Kα ($\lambda = 0.710\,73$ Å) radiation, Enraf-Nonius CAD4 diffractometer, at 183 K for **1** are as follows: $C_{13}H_{27}N_3S_2OZn$, monoclinic, space group $P2_1/n$, $a = 7.804(2)$ Å, $b = 12.039(2)$ Å, $c = 18.229(4)$ Å, $\beta = 100.93(3)°$, $V = 1681.6(6)$ Å^3, $Z = 4$, 2167 observed reflections ($F > 4.0\sigma(F)$), $R = 0.0436$, $R_w = 0.0462$. The structure was solved by direct methods using Siemens SHELXTL PLUS (PC version).
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(32) NH and OH H atoms were located in a difference map at intermediate stages of the refinement and then included in subsequent cycles of refinement with fixed temperature factors.
(33) To a solution containing 12 mmol (4.45 g) of **1** in 50 mL of MeOH was added fresh $NaBH_4$ (48 mmol, 1.82 g) over the course of 5 min. This solution was allowed to stir overnight at ambient temperature. The solution was then filtered and the volume reduced to ~30 mL. Cooling the reaction overnight at −35 °C afforded 1.57 g (35% yield) of colorless crystalline product **2**, (1,2-dimethyl-3,7,1-triazatridecane-1,13-dithiolato)zinc(II)–methanol. Anal. Calcd for $C_{13}H_{31}N_3S_2OZn$: C, 41.65; H, 8.33; N, 11.21. Found: C, 41.02; H, 8.24; N, 11.10. ^{1}H NMR (CD_3OD): δ 2.90, 2.71 (broad), 2.30 (m), 1.72 (m), 1.19 (m), 1.11 (d, CH_3). IR: 3200 cm^{-1} (ν_{O-H}), 1633 cm^{-1} (δ_{NH}).

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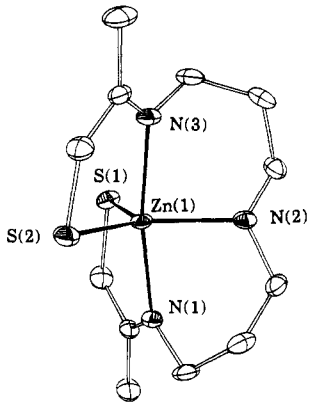

Figure 1. ORTEP diagram of $ZnL_{S2(Me)N3(Pr)}$ (**1**). Selected distances (Å) and angles (deg): Zn−S(1), 2.337(1); Zn−S(2), 2.329(1); Zn−N(1), 2.167(3); Zn−N(2), 2.142(4); Zn−N(3), 2.179(3); S(1)−Zn−S(2), 124.1(1); S(1)−Zn−N(2), 118.4(1); S(2)−Zn−N(2), 117.2(1); N(1)−Zn−N(3), 170.0(1). H atoms have been omitted for clarity.

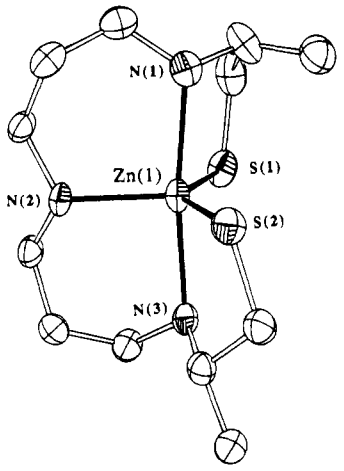

Figure 2. ORTEP diagram of $ZnL_{S2(Me)N3(Pr)H4}$ (**2**). Selected distances (Å) and angles (deg): Zn−S(1), 2.308(1); Zn−S(2), 2.340(1); Zn−N(1), 2.259(4); Zn−N(2), 2.133(3); Zn−N(3), 2.275(4); S(1)−Zn−S(2), 136.1(1); S(1)−Zn−N(2), 116.6(1); S(2)−Zn−N(2), 107.3(1); N(1)−Zn−N(3), 172.2(1). H atoms and a disordered methyl carbon have been omitted for clarity.

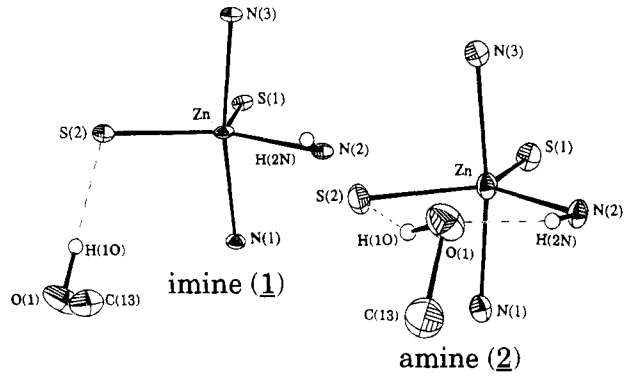

Figure 3. Comparison of the intermolecular S···H interactions between the imine complex $ZnL_{S2(Me)N3(Pr)}$ (**1**) (left) and the amine complex $ZnL_{S2(Me)N3(Pr)H4}$ (**2**) (right) and their cocrystallized MeOH molecules. Distances (Å) are as follows. For **1**: S(2)···O(1), 3.213(4); S(2)···H(1O), 2.34(6); O(1)−H(1O), 0.88(6). For **2**: S(2)···O(1), 3.151(4); S(2)···H(1O), 2.24(6); O(1)−H(1O), 0.98(6); O(1)···H(2N), 2.21(6).

Zn−N bonds (mean Zn−N = 2.27 Å in **2** vs 2.17 Å in **1**). The in-plane angles change substantially as a result, with the S−Zn−S angle widening by 12° and one of the S−Zn−N angles narrowing by 10°. Similar angle changes take place (Δ(S−Zn−S) = 14° and Δ(S−Zn−O) = −15°) in LADH upon conversion of the "open form" to the "closed form".[10] The resultant pinching of S(2) and N(2) together in **2** creates a cavity which binds the cocrystallized MeOH molecule via two hydrogen bonds (Figure 3). By locking the alcohol into position, the S···O interaction increases (S···O = 3.151(4) Å in **2** vs 3.213(4) Å in **1**),[35] and the alcoholic O−H bond becomes slightly more activated. In hydrogen-bonded systems, S···O separations are usually greater than or equal to 3.2 Å.[28] The sum of S and O van der Waals radii, ignoring the H (which has a radius of 1.20 Å), is 3.5 Å. Refinement of H(1O)[32] places it 2.24(6) Å away from S(2) and 0.98(6) Å away from O(1) (Figure 3). An increased S···H interaction is further supported by red-shifting of the ν_{O-H} stretch to 3200 cm^{-1} in the solid state IR, relative to free, matrix-isolated, MeOH (3642 cm^{-1})[31] and **1** (3242 cm^{-1}). The shift of 442 cm^{-1} for **2** is comparable to that observed (396 cm^{-1}) when MeOH is H-bonded to NEt_3.[36] Assignment of the ν_{O-H} stretch for **2** was verified by inserting CD_3OD (ν_{O-D} = 2366 cm^{-1}) in place of protio MeOH. Thus, in going from structure **1** to **2**, the proton moves away from the alcoholic oxygen toward the zinc-bound thiolate. Although **2** is not an exact representation of the LADH zinc site, it does provide us with the first opportunity to investigate the role of thiolates in the interaction of substrate with a site approximating that of LADH. There are very few H-bonding data available for thiolates[37] interacting with O−H protons[38,39] which correlates ν_{O-H} with strength of interaction,[36] so we plan to continue investigating this.

In conclusion, we have structural evidence which suggests that a metal-coordinated thiolate could participate in the activation of an O−H bond without requiring prior coordination to the metal ion. This would lower the kinetic barrier to H^+ transfer, since it could take place earlier along the reaction pathway, *i.e.*, as it approached the outer coordination sphere of the metal site. Since the local coordination number and geometry are sure to influence the pK_a of the thiolate and therefore the extent of S···HO interaction in thiolate-ligated metalloenzyme models, we are expanding this investigation to include both 4- and 5-coordinate Zn thiolates, both containing or lacking a labile binding site.

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Supporting Information Available: Text describing the structure determinations and listings of Crystallographic data and details of the data collection, atomic positional and isotropic thermal parameters, anisotropic thermal parameters, bond distances and angles, and hydrogen positional and isotropic thermal parameters for **1** and **2** (21 pages). Ordering information is given on any current masthead page.

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(34) Crystallographic data with Mo Kα (λ = 0.710 73 Å) radiation, Enraf-Nonius CAD4 diffractometer, at 183 K for **2** are as follows: $C_{13}H_{31}N_3S_2OZn$, tetragonal, space group $I4_1/a$, a = 22.240(3) Å, c = 14.411(3) Å, V = 7128(4) Å^3, Z = 16, 2426 observed reflections ($F > 4.0\sigma(F)$), R = 0.0450, R_w = 0.0707. The structure was solved using a Patterson map with Siemens SHELXTL PLUS (PC version).
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