Amide Alcoholysis in Mononuclear Zinc and Cadmium Complexes Ligated by Thioether Sulfur and Nitrogen Donors

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Amide hydrolysis reactions mediated by metalloenzymes are of considerable current interest.[1] For example, elucidation of a detailed chemical understanding of the formamide hydrolysis reaction mediated by the metalloamidase peptide deformylase (PDF) is a significant present goal due to the involvement of this enzyme in bacterial protein biosynthesis and its potential as an antibiotic target.[2,3] In PDF, a mononuclear divalent iron center, tetrahedrally ligated by two histidine residues, a cysteine thiolate, and a water/hydroxo moiety, catalyzes the hydrolytic cleavage of a N-terminal formamide group in nascent polypeptides.[4] Notably, PDF is highly active when the native Fe^{2+} is replaced by Co^{2+} or Ni^{2+}, but exhibits low activity when Fe^{2+} is replaced by Zn^{2+}, the metal ion typically found in metalloamidases. From X-ray crystallographic studies, it appears that change in the active site metal ion in PDF results in only minor structural differences, suggesting that the loss in catalytic activity for the Zn^{2+}-containing enzyme is likely due to transition-state, rather than ground-state, effects.[4f] As an approach toward examining the fundamental chemical features of amide cleavage reactions mediated by mononuclear nitrogen/sulfur-ligated zinc species relevant to Zn^{2+}-containing PDF, we report herein our development of a novel family of nitrogen/sulfur-ligated Zn^{2+} and Cd^{2+} complexes that mediate the alcoholysis of an internal amide substrate.

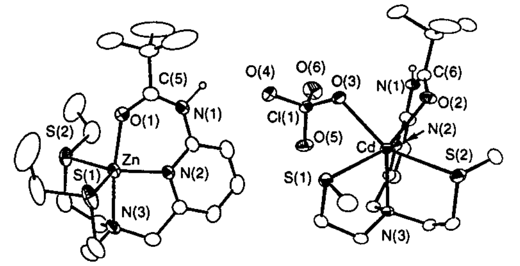

Figure 1. ORTEP representations of the cationic portions of $[(beppa)Zn](ClO_4)_2$ (**1**) and $[(bmppa)Cd(ClO_4)](ClO_4)\cdot 1.5MeOH$ (**3**). All ellipsoids are drawn at the 30% probability level (all hydrogen atoms except amide protons not shown for clarity). Selected bond lengths (Å) and angles (deg): Zn−O(1) 2.006(3), Zn−N(2) 2.046(4), Zn−N(3) 2.141(4), Zn−S(1) 2.366(1), Zn−S(2) 2.427(1), C(5)−O(1) 1.254(6), C(5)−N(1) 1.333(7), O(1)−Zn−N(2) 87.7(2), O(1)−Zn−N(3) 167.5(2), N(2)−Zn−N(3) 82.4(2), O(1)−Zn−S(1) 104.6(1), N(2)−Zn−S(1) 125.4(1), N(3)−Zn−S(1) 87.4(1), O(1)−Zn−S(2) 91.5(1), N(2)−Zn−S(2) 199.1(1), N(3)−Zn−S(2) 86.8(1), S(1)−Zn−S(2) 113.6(1), C(5)−O(1)−Zn 129.7(4), O(1)−C(5)−N(1) 124.0(5); Cd−O(2) 2.302(2), Cd−O(3) 2.468(2), Cd−N(2) 2.353(2), Cd−N(3) 2.428(2), Cd−S(1) 2.6135(7), Cd−S(2) 2.7069(8), C(6)−O(2) 1.227(3), C(6)−N(1) 1.344(4), O(2)−Cd−N(2) 75.8(2), O(2)−Cd−N(3) 133.99(8), N(2)−Cd−N(3) 73.4(7), O(2)−Cd−S(1) 145.6(6), O(2)−Cd−S(2) 79.7(6), N(2)−Cd−S(1) 131.7(6), N(3)−Cd−S(1) 79.4(5), N(2)−Cd−S(2) 108.4(6), N(3)−Cd−S(2) 78.6(6), S(1)−Cd−S(2) 104.2(3), C(6)−O(2)−Cd 126.7(2), O(2)−C(6)−N(1) 123.1(3).

Two amide-appended N_2S_2 ligands *N*-bis-2-(ethylthio)ethyl-*N*-(6-pivaloylamido-2-pyridylmethyl)amine (beppa) and *N*-bis-2-(methylthio)ethyl-*N*-(6-pivaloylamido-2-pyridylmethyl)amine (bmppa), were prepared as outlined in Scheme S1 (Supporting Information).[5] Divalent zinc complexes of beppa and bmppa and a Cd^{2+} complex of bmppa were generated ($[(beppa)Zn](ClO_4)_2$ (**1**), $[(bmppa)Zn](ClO_4)_2$ (**2**), and $[(bmppa)Cd(ClO_4)](ClO_4)\cdot MeOH$ (**3**)), and the solid-state structures of **1** and **3** have been determined by X-ray crystallography (Figure 1).[6,7] The Zn^{2+} ion of **1** adopts a slightly distorted trigonal bipyramidal geometry ($\tau = 0.70$)[8] with the pyridyl nitrogen and the thioether sulfur atoms (Zn−S(1), 2.366(1) Å; Zn−S(2) 2.427(1) Å) comprising the equatorial plane. The observed canting of the ethyl groups in **1** sterically shields the metal center on one N_2OS face of the trigonal bipyramidal Zn^{2+} center, while leaving a coordination site notably accessible on the other N_2OS face (Figure S1,

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(6) **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.

(7) Characterization data for all new ligands and complexes is provided in the Supporting Information.

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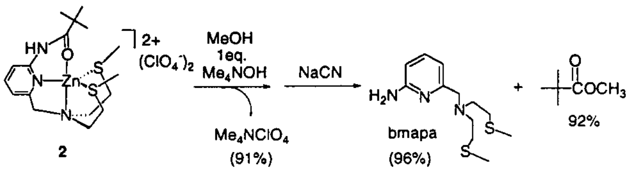
10.1021/ic000547e CCC: $19.00

Scheme 1. Amide Methanolysis



Supporting Information). In **3** (Figure 1), this available coordination position is occupied by a ClO_4^- anion, yielding a Cd^{2+} center with an overall distorted trigonal prismatic geometry, with the amide oxygen and the oxygen atom of a ClO_4^- anion occupying adjacent coordination positions (O(3)···C(6), 3.075 Å; Cd−O(ClO_4), 2.468(2) Å).[9] Notably, this arrangement of anion and amide is structurally akin to proposals put forth for nucleophile/substrate-bound (or double Lewis activated) forms of mononuclear metal centers poised for substrate attack in hydrolytic systems.[1] The binding of the anion results in a distortion of the planarity of the amide chelate ring (torsion angle O(2)−Cd−N(2)−C(7), −22°), presumably to minimize strain energy associated with the O(3)−Cd−O(2) angle (76.9(8)°), although a strong Cd−O(amide) interaction is maintained (Cd−O(2), 2.303(2) Å).

In d_4-MeOH at ambient temperature, complex **1** exhibits a single set of −S*Et* resonances, inconsistent with its observed solid-state structure in which two different −S*Et* environments are found. Examination of the ^1H NMR spectral properties of **1** and **2** in d_4-MeOH as a function of temperature (∼+60 to −83 °C) has revealed a coalescence point of ∼−65 °C for both complexes below which, consistent with the solid-state structure of **1**, signals for two distinct −S*R* substituents are observed. These results are suggestive of a rapid inversion process at ambient temperature wherein mirror images of **1** and **2**, which differ only in their respective direction of −S*R* canting (clockwise vs counterclockwise), are rapidly interconverted.[10]

In methanol solution, in the presence of 1 equiv of $Me_4NOH \cdot 5H_2O$, over the course of ∼2 h at 50(1) °C, complex **2** undergoes cleavage of the *tert*-butyl amide (Scheme 1), yielding quantitatively the products of methanolysis, specifically 1 equiv of methyltrimethylacetate and a single zinc-containing complex.[11,12] Specifically, FAB-MS analysis of the reaction mixture following stoichiometric formation of methyltrimethylacetate yielded a parent isotope cluster at *m*/*z* 436, a mass consistent with the formulation $\{[(bmapa)Zn](ClO_4)\}^+$ (bmapa = *N*-bis-2-(methylthio)ethyl-*N*-(6-amino-2-pyridylmethyl)amine) and with the loss in the mass spectral experiment of a second anion. While the nature of this anion is as yet undetermined, it is likely that it is derived from either solvent or water. To provide conclusive evidence for the primary amine ligand product in this complex, we have removed the Zn^{2+} ion using excess NaCN and isolated (96% yield) and characterized (^1H, ^{13}C, FTIR, HREIMS) the bmapa ligand.[13] We note that ^1H NMR, LRFAB-MS, and ligand recovery studies confirm that complexes **1** and **3** undergo an identical reaction with $Me_4NOH \cdot 5H_2O$, yielding methyltrimethylacetate and a single Zn^{2+} or Cd^{2+} complex of the bmapa or beapa (beapa = *N*-bis-2-(ethylthio)ethyl-*N*-(6-amino-2-pyridylmethyl)amine) ligands.

We have conducted preliminary chemical and spectroscopic studies toward elucidating the mechanism by which the amide cleavage reaction takes place in **2**. First, we have observed that the introduction of $Me_4NOH \cdot 5H_2O$ to a MeOH solution of $[(bmppa)Zn](ClO_4)_2$ (**2**) immediately induces precipitation of a white solid that we have identified as Me_4NClO_4 (91% yield) on the basis of comparison of spectroscopic data (FTIR) to an authentic sample. Following the reaction by ^1H NMR at 50(1) °C in d_4-MeOH (Scheme S2, Supporting Information), we have observed that, immediately after precipitation of Me_4NClO_4, the solution contains a mixture of two species (designated **A** and **B**; *tert*-butyl resonances 1.35 and 1.30 ppm, respectively). After ∼15 min, a third *tert*-butyl resonance (1.17 ppm) for the methyltrimethylacetate product appears. All three *tert*-butyl resonances (**A**, **B**, and methyltrimethylacetate) are then observed until the reaction reaches completion, wherein only the *tert*-butyl resonance associated with the methyltrimethylacetate product remains. Studies directed at conclusively identifying the chemical compositions of **A** and **B**, which are potentially zinc hydroxo species,[14] and determining the relationship of these intermediates to one another are currently in progress.

In summary, we have designed a novel family of mononuclear nitrogen/sulfur-ligated Zn^{2+} and Cd^{2+} complexes that exhibit amide cleavage reactivity. While these systems are not structural models for the active site in Zn^{2+}-containing PDF, they represent an excellent starting point for detailed studies of amide cleavage reactions mediated by a mononuclear nitrogen/sulfur-ligated metal center relevant to the enzyme active site. A particularly notable feature in this regard is that, with the isolation of the cleaved ligands (beapa and bmapa), a unique opportunity exists to introduce a variety of amide substrates, including formamides, for reactivity studies relevant to enzyme catalysis.

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Supporting Information Available: Synthetic details and characterization data for new ligands and complexes and two X-ray crystallographic files (CIF). Figures/schemes displaying (1) ligand and complex synthetic pathways (Scheme S1), (2) a space-filling model of $[(beppa)Zn](ClO_4)_2$ (Figure S1), and (3) ^1H NMR spectra at various times during the amide cleavage reaction of $[(bmppa)Zn](ClO_4)_2$ (Scheme S2). This material is available free of charge via the Internet at http://pubs.acs.org.

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(9) A weakly coordinated MeOH molecule (Cd−O(1), 2.54 Å) that is present in 50% occupancy has been omitted for clarity.

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(11) To confirm the metal dependence of the ligand alcoholysis reaction, a control reaction was performed in which a d_4-MeOH solution of bmppa (34.7 mg, 0.097 mmol) was treated with $Me_4NOH \cdot 5H_2O$ (17.7 mg, 0.097 mmol) under identical experimental conditions necessary for amide cleavage to occur in the $[(bmppa)Zn](ClO_4)_2$ system (40 °C, 140 h). ^1H NMR analysis of the reaction mixture after this time showed only the presence of unreacted bmppa.

(12) Preliminary experiments have revealed that addition of 1 equiv of NaOMe to a wet MeOH solution of $[(bmppa)Zn](ClO_4)_2$ (**1**) also initiates amide alcoholysis.

(13) To confirm that amide alcoholysis did not occur under the conditions necessary for removal of the ligand from the Zn^{2+} ion, a control reaction was performed in which a methanol solution of $[(bmppa)Zn](ClO_4)_2$ (14.6 mg, 0.024 mmol) was treated with excess NaCN (13.9 mg, 12 equiv). Analysis of the organic fraction obtained by ^1H NMR revealed only the presence of unaltered bmppa (98% yield).

(14) Preliminary electrospray ionization mass spectral analysis of the **A**/**B** mixture generated upon addition of $Me_4NOH \cdot 5H_2O$ to $[(bmppa)Zn](ClO_4)_2$ (**2**) in methanol followed by removal of the solvent under reduced pressure and redissolving of the resulting solids in CH_3CN (conditions under which no further amide cleavage takes place) yielded a molecular ion at *m*/*z* 454 (25% intensity). The isotopic pattern of this ion is consistent with the formulation $\{[(bmppa)Zn(OH)(H_2O)]\}^+$. No isotopic envelopes of greater than 3% intensity were observed in the region above *m*/*z* 500.