Model Complexes of the Active Site in Peptide Deformylase: A New Family of Mononuclear N_2S−M(II) Complexes

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Mononuclear zinc enzymes comprise a large and varied class in bioinorganic chemistry and have received considerable attention from researchers with biochemical as well as synthetic interests.[1−3] They perform a number of diverse functions in biology with different residues (His, Cys, Asp, Glu) anchoring a tetrahedral zinc(II) ion to the active site. Recently, a large body of information has become available on peptide deformylase (PDF), a related enzyme with a relatively unusual [$His_2CysM^{II}(H_2O)$] site.[4] PDF is responsible for the hydrolysis of an N-terminal formyl group during bacterial protein synthesis and has been under investigation as a new target for antibiotics.[5] The presence of the cysteinyl sulfur ligand places PDF in a new subfamily of the superfamily of zinc metalloproteases that include astacin (His_3 donor set) and thermolysin (His_2Glu donor set). Remarkably, recent studies on PDF show that iron(II) is the likely metal ion in vivo,[6,7] and not zinc(II), which is the metal ion found in all other metallopeptidases.[1] Interestingly, the Zn^{II} form of the protein shows low activity, while the Fe^{II} form and the Ni^{II} and Co^{II} forms of PDF show high activity.[6,7] Our initial goal is to construct a series of $N_2S_{thiolate}M^{II}$ models in which we can vary the metal ion and that in particular bear an aliphatic thiolate as a close electronic mimic of the cysteine donor in PDF. In the long term we intend to illuminate the fundamental properties that may be responsible for such striking and unexpected differences in reactivity as well as to determine the effect of a thiolate ligand on hydrolytic function. Here we report our initial results on the preparation of a new family of [$N_2S_{thiolate}M^{II}L$] (M = Zn, Co) complexes as small-molecule analogues of peptide deformylase.

The synthesis of numerous tetrahedral [$N_3Zn^{II}L$] (L = H_2O/OH^-, substrate, etc.) model complexes has provided important information concerning the structure and mechanism of (His_3)-Zn^{II} proteins such as carbonic anhydrase.[8] However, the frustrating propensity of thiolate (RS^-) ligands to yield sulfur-bridged polymeric metal species[9] has stymied progress in the synthesis of discrete [$N_xS_yZn^{II}L$] (*S* = thiolate; L ≠ N, S) complexes, despite a great deal of effort toward their preparation.[10] Thus, the synthesis of such complexes not only would have important biological relevance but also would be of fundamental synthetic interest.

A new $N_2S_{thiolate}$ ligand, 2-methyl-1-[methyl-(2-pyridin-2-yl-ethyl)amino]propane-2-thiol (PATH, **1**), has been synthesized and used to prepare two novel zinc compounds [(PATH)Zn^{II}Br] (**2**) and [(PATH)Zn^{II}NCS] (**3**). These molecules are close analogues of the active site of PDF and the first crystallographically characterized [$N_2S_{thiolate}Zn^{II}L$] complexes in which S is a cysteinelike *aliphatic* thiolate. The analogous isostructural cobalt compounds [(PATH)Co^{II}Br] and [(PATH)Co^{II}NCS] are also synthetically accessible, and to our knowledge, there are no previous examples of mononuclear [$N_2SCo^{II}L$] (L ≠ N, S) complexes. We also provide evidence that these complexes have the potential to serve as functional models.

The new $N_2S_{thiolate}$ ligand **1** was synthesized according to Scheme 1. Addition of isobutylene sulfide to 2-(2-methylamino-ethyl)pyridine in CH_3CN at 60 °C gave **1** in good yield.[11] The one-step synthesis of the PATH ligand routinely gives multigram quantities of pure product. Similar pyridine−amine−thiol $N_2S_{thiolate}$ ligands, which lack the methyl substituents on the thiolate arm of PATH, invariably give polynuclear M^{II} complexes with bridging sulfur atoms.[9] The *gem*-dimethyl substituents in **1** were incorporated as steric blocking groups, and this simple yet critical feature of our ligand design predisposes the PATH ligand to give mononuclear complexes.[12−14]

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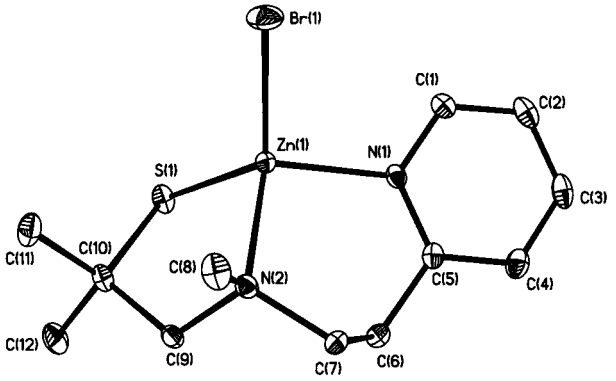
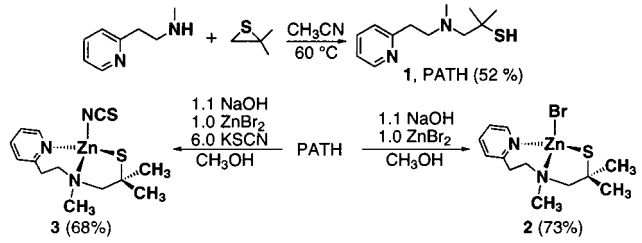


Figure 1. ORTEP diagram of **2** with hydrogen atoms omitted for clarity. Selected bond distances (Å): Zn−N(1) = 2.058(2); Zn−N(2) = 2.110(2); Zn−S(1) = 2.2548(7); Zn−Br = 2.3777(5).

Scheme 1



The PATH ligand was deprotonated with NaOH and added to $ZnBr_2$ in MeOH to give [(PATH)Zn^{II}Br] (**2**) as a white microcrystalline powder in good yield. An X-ray quality crystal of **2** was grown from MeOH/Et_2O, and the ORTEP diagram is shown in Figure 1. The mononuclear structure of **2** is as designed; the zinc(II) ion is bound in a pseudotetrahedral array by the tridentate PATH ligand and a fourth Br^- ligand. The Zn−S and Zn−N bond lengths closely match the $Zn-S_{Cys90}$ (2.1−2.3 Å), and $Zn-N_{His132}$/$Zn-N_{His136}$ (2.0−2.2 Å) distances that are found for native and inhibitor-bound structures of Zn^{II}−PDF.[4] Thus, complex **2** is a close structural analogue of the active site in PDF and in particular provides an aliphatic thiolate that duplicates the coordination of Cys90 in the active site.

Synthesis of the thiocyanate complex [(PATH)Zn^{II}NCS] (**3**) follows a similar procedure in which deprotonated **1** is combined with $ZnBr_2$ and excess KSCN to give **3** (Scheme 1). The mononuclear structure of **2** is retained in **3**, with SCN^- replacing Br^- (Figure S1 of Supporting Information). Both complexes **2** and **3** have particular biological significance in that anions such as Br^- and SCN^- have been well-studied as inhibitors of zinc enzymes and their cobalt-substituted analogues,[2] and recently, a detailed study of Cl^- binding to Co^{II}−PDF proved to be very fruitful in delineating the function of the active-site metal.[15] Although the SCN^-/Br^- forms of PDF have not been structurally characterized, the Zn−Br and Zn−NCS distances in **2** and **3** compare well with the corresponding distances for the related carbonic anhydrase inhibitor complexes (for human CAII: Zn−Br = 2.5 Å;[16] Zn−NCS = 1.9 Å[17]).

In order for complexes of the new PATH ligand to act as *functional* as well as structural models of PDF, they need to retain their mononuclear structures in solution. To our satisfaction the ^{1}H NMR spectra of **2** and **3** (see Supporting Information) are consistent with the X-ray structures. For example, from Figures 1 and S1 it is evident that the *gem*-dimethyl groups are diastereotopic, and this inequivalence is reflected in the two separate singlets at δ 1.28 (1.29) and 1.32 (1.46) ppm for **2** (**3**). These spectra are essentially unaffected by solvent (CD_3OD, CD_3CN, and $CDCl_3$) and show little change even after the samples are left standing for several weeks, indicating that the monomeric structures of **2** and **3** are stable in solution.

Moreover, the catalytically active species in PDF is a metal-bound OH^-/H_2O, and the proposed mechanism for PDF, like many other metallopeptidases, requires an enzyme−M^{II}−OH/OH_2 species to undergo substitution at the OH^-/H_2O site.[4,15] Importantly, we have established that complexes **2** and **3** can undergo such substitution reactions at the labile position. As evidenced by variable-temperature ^{1}H NMR spectroscopy (Figure S2), **2** and **3** can be interconverted through a reversible substitution of the Br^- and SCN^- ligands. An exchange-averaged peak for the pyridine proton closest to the labile site (H_α, ortho to the N atom) is observed at δ 8.64 ppm for an ∼1:1 mixture of **2** and **3** in CD_3CN at 23 °C. When the sample is cooled from 23 to −30 °C, this signal resolves into two peaks at δ 8.68 ppm (H_α for pure **2**) and δ 8.54 ppm (H_α for pure **3**) . There is no evidence at any temperature for the presence of any other species in significant concentration besides compounds **2** and **3**. Although the mechanism of exchange has not been determined, the variable-temperature NMR results show that **2** and **3** can undergo transformations at the "open" site while the PATH−Zn^{II} unit remains intact.[18]

To determine the fundamental properties of different metal ions bound by an $N_2S_{thiolate}$ environment, it is imperative for the PATH ligand to yield structurally analogous complexes with other M^{II} ions. Toward this goal the cobalt(II) complexes [(PATH)Co^{II}Br] and [(PATH)Co^{II}NCS] have also been synthesized and have structures that are the same as **2** and **3**, respectively.[19] The cobalt(II) ion has a well-known role as a spectroscopic probe (e.g., UV−vis, paramagnetic NMR) when substituted into Zn^{II} metalloproteins.[2] The spectroscopic assignments made for Co^{II}-substituted proteins are largely based on data that have been gathered from small-molecule model compounds. Thus, [(PATH)Co^{II}Br] and [(PATH)Co^{II}NCS] will fill important gaps in the current database of cobalt(II) model compounds.

In summary, we have synthesized a new $N_2S_{thiolate}$ ligand and the first models of PDF that contain an *alkylthiolate* cysteine mimic.[20] The zinc complexes are stable toward substitution chemistry at the open site, a prerequisite for using these complexes as functional models. Because of the previous lack of suitable model complexes, almost nothing is known about metal-mediated hydrolysis with sulfur in the coordination sphere. Efforts toward synthesizing Fe^{II} and Ni^{II} complexes, as well as determining the hydrolytic power of suitable ($N_2S_{thiolate}$)M^{II}−OH/OH_2 complexes, are underway.

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Supporting Information Available: Synthetic procedures for **1**−**3** and crystallographic data for **2** and **3** and variable-temperature NMR data. This material is available free of charge via the Internet at http://pubs.acs.org.

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