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Thioester Hydrolysis Promoted by a Mononuclear Zinc Complex

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The mononuclear zinc complex $[(bpta)Zn](ClO_4)_2 \cdot 0.5H_2O$ promotes the hydrolysis of the thioester $PhCH(OH)C(O)SCD_3$ when dissolved in CH_3CN:H_2O (50:50 buffered at pH 9.0). This reaction results in the formation of a mixture of CD_3SH and a zinc thiolate complex, the latter of which can be protonated to generate additional CD_3SH. Kinetic studies revealed an overall second-order reaction with an activation energy that is similar to that found for aqueous OH^- promoted thioester hydrolysis. These studies represent the first investigation of chemistry relevant to that occurring in the monozinc-containing form of human glyoxalase II.

The glyoxalase pathway is ubiquitous in biological systems and involves two metalloenzymes, glyoxalase I (GlxI) and glyoxalase II (GlxII), with glutathione as an essential cofactor.[1] GlxI catalyzes the isomerization of a hemithioacetal of methyl glyoxal to a thioester, and GlxII then catalyzes the hydrolysis of the thioester to produce nontoxic products. The glyoxalase pathway is involved in cellular detoxification and is important in preventing the formation of advanced glycation end products, which are linked to aging and various diseases.[2] Because of its critical role in cellular detoxification, the glyoxalase system is under investigation as a possible antitumor target.[3]

Crowder and co-workers recently reported that human glyoxalase II contains an Fe(II)Zn(II) center but is catalytically active as a mononuclear zinc enzyme.[4] This is similar to the reactivity found for some metallo-β-lactamases, which catalyze the hydrolytic ring-opening of β-lactam antibiotics.[5] In the monozinc form of both enzymes, a $(His)_3Zn$ site catalyzes the hydrolysis of the substrate. While the reactivity of a mononuclear Zn−OH complex with the β-lactam-containing nitrocefin has been investigated,[6] there are no reports in the literature of detailed studies of a thioester hydrolysis reaction involving a mononuclear zinc complex. Such studies would have relevance to human glyoxalase II. Additionally, these investigations have relevance to a recently reported Zn−OH-promoted hydrolysis of the thioester compound thiocoumarin within the active site of carbonic anhydrase (CA). This reaction results in the formation of a ring-opened product that is a nanomolar inhibitor of three CA isozymes.[7]

We have previously shown that use of an aliphatic, deuterium-labeled thioester ($PhCH(OH)C(O)SCD_3$), with ^2H NMR as the monitoring method, is a feasible approach for investigating thioester hydrolysis reactions promoted by dizinc and Fe(III)Zn(II) complexes.[8,9] These studies revealed that the presence of a terminal Zn−OH species enhanced thioester hydrolysis reactivity in both types of complexes. In the research outlined herein, we have studied thioester hydrolysis promoted by the mononuclear zinc complex $[(bpta)Zn](ClO_4)_2 \cdot 0.5H_2O$ (**1**). Nitrate and triflate analogs of this complex have been previously reported.[6] However, both were prepared in solution, and no structural or spectroscopic data was reported. We have fully characterized **1** using elemental analysis, ^1H and ^{13}C NMR, FTIR, mass spectrometry, and X-ray crystallography.[10] When crystallized from CH_3CN/Et_2O, the cationic portion exhibits facial coordination of the bpta ligand, with two acetonitrile donors and one water molecule completing the coordination sphere (Figure 1). The water ligand is positioned *trans* to a pyridyl nitrogen, with a Zn−O distance of 2.110(3) Å. The two coordinated acetonitrile ligands have Zn−N distances of 2.221(3) and 2.185(2) Å, respectively. Three distinct Zn−N distances are found involving the bpta ligand, with the shortest being Zn(1)−N(3) (2.055 Å), which is *trans* to the coordinated water molecule. Upon crushing and drying of the crystals, the acetonitrile ligands and half of the water is

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(10) **1**: $C_{20}H_{29}Cl_2N_5O_9Zn$, M = 619.75, orthorhombic, *P*bca, colorless plate, a = 10.8255(2) Å, b = 14.0872(2) Å, c = 32.7227(8) Å, V = 5245.30(18) Å^3, Z = 8, T = 150(1) K, 10038 total reflections, 5736 independent {R_{int} = 0.0295, R1 [$I > 2$sigma(I)] = 0.0443, wR2 (all data) = 0.1160}.

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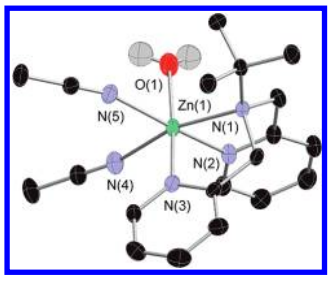


Figure 1. Cationic portion of $[(bpta)Zn(CH_3CN)_2(H_2O)](ClO_4)_2$ (**1**·**2CH$_3$CN·0.5H$_2$O**). Hydrogen atoms other than those of the coordinated water molecule have been omitted for clarity. Selected bond distances (Å) and angles (°): Zn(1)−N(3) 2.055(2), Zn(1)−O(1) 2.1103(3), Zn(1)−N(2) 2.130(2), Zn(1)−N(5) 2.185(2), Zn(1)−N(4) 2.221(3), Zn(1)−N(1) 2.254(2), N(1)−Zn(1)−N(2) 77.48(9), N(2)−Zn(1)−N(3) 100.02(9), and N(1)−Zn(1)−N(3) 80.66(9).

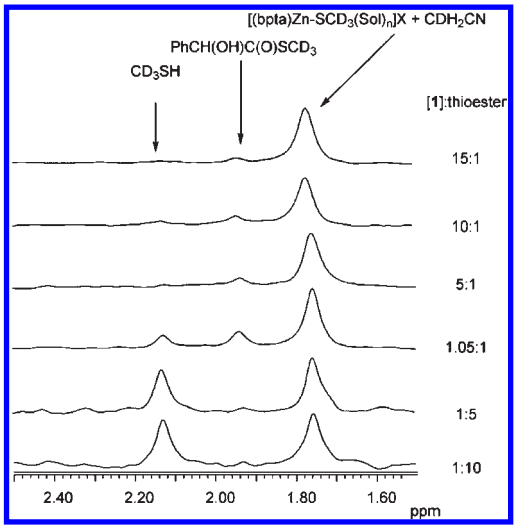


Figure 2. ^{2}H NMR spectra obtained following hydrolysis of $PhCH(OH)C(O)SCD_3$ promoted by **1** at pH 9.0. The species at 2.12 ppm has been identified as CD_3SH. The resonance at 1.77 ppm is proposed to be the signal for $[(bpta)Zn\text{-}SCD_3(Sol)_n]X$ (Sol = CH_3CN or H_2O; X = anion present in solution).

lost, leading to an analytical formulation for the dried solid as $[(bpta)Zn](ClO_4)_2\cdot 0.5H_2O$ (**1**).

Kinetic studies of nitrocefin hydrolysis promoted by $[(bpta)Zn](NO_3)_2\cdot nH_2O$ yielded a kinetic pK_a of 7.84(2) for the Zn−OH_2 moiety.[6] For the thioester hydrolysis studies reported herein, we have performed all reactions at pH 9.0 to ensure that a Zn−OH species is present. Admixture of 1.05 equivalents of **1** and one equivalent of $PhCH(OH)C(O)SCD_3$ in 50:50 CH_3CN:H_2O (buffered at pH 9.0), followed by heating of this solution at 329.5 K for ∼12 h, results in the formation of two −SCD_3-containing products as identified by ^{2}H NMR (Figure 2). The more abundant product has a ^{2}H NMR signal at 1.77 ppm,[11] and a smaller signal is found at 2.12 ppm. The latter signal corresponds to CD_3SH (pK_a = 10.4).[9] We note that in the absence of **1**, the hydrolysis of $PhCH(OH)C(O)SCD_3$ under identical conditions results in the formation of CD_3SH as the only sulfur-containing product.[9] We propose that the species at 1.77 ppm is a zinc thiolate complex, with an analytical formulation such as $[(bpta)Zn\text{-}SCD_3(Sol)_n]X$ (sol = H_2O or CH_3CN).[12] The relative amounts of CD_3SH and the zinc thiolate complex produced in the reaction mixture change as a function of the **1**:thioester ratio (Figure 2), with more of the zinc thiolate species being produced in solutions having a higher initial concentration of zinc complex. Using the 10:1 ([**1**]:thioester) reaction mixture, which following stoichiometric thioester hydrolysis contains only a trace amount of CD_3SH (Figure 2), lowering of the pH to ∼8.6 resulted in the formation of additional CD_3SH (2.12). Further lowering of the pH to 8.3 resulted in additional CD_3SH being generated. This data is consistent with the presence of a Zn−SCD_3 complex that can undergo protonation to release CD_3SH. Additional evidence that the 1.77 ppm species contains a Zn−SCD_3 moiety comes from treatment of the reaction mixture with excess CH_3I, which results in the formation of the sulfonium salt $[(CD_3)S(CH_3)_2]X$ (^{2}H NMR 2.59 ppm; Supporting Information).

Table 1. Rate Constants for the Hydrolysis of $PhCH(OH)C(O)SCD_3$ Promoted by **1**[a]

temperature (K)	k_2 (M^{-1} s^{-1})
299.5	$1.87(26)\times 10^{-3}$
309.5	$4.69(24)\times 10^{-3}$
319.5	$1.06(11)\times 10^{-2}$
329.5	$2.44(6)\times 10^{-2}$

[a] $[PhCH(OH)C(O)SCD_3]$ = 0.0018 M; [**1**] = 0.0019−0.027 M; 50:50 CH_3CN:H_2O [CHES buffer, 0.36 M, I = 0.61 M ($NaNO_3$)].

At pH 9.0, the thioester hydrolysis reaction promoted by **1** is catalytic, with 10 turnovers requiring ∼12 days to go to completion at 329.5 K. Monitoring the loss of thioester in single turnover reactions as a function of time at specific concentrations of **1** yielded plots from which pseudo first-order rate constants (k_{obs}) were determined.[13] Varying of the concentration of **1** from 1.9 to 27 mM, with $[PhCH(OH)C(O)SCD_3]$ = 1.8 mM, produced linear plots of k_{obs} versus [**1**] from which second-order rate constants (k_2) were determined (Figure S1 of the Supporting Information; Table 1). Variable temperature studies in the range of 299.5−329.5 K, and construction of an Eyring plot (Figure S2 of the Supporting Information), yielded $\Delta H^{\ddagger}$ = 13.6(6) kcal/mol, $\Delta S^{\ddagger}$ = −25.5(2.0) cal/mol K, and E_a = 14.1(6) kcal/mol.

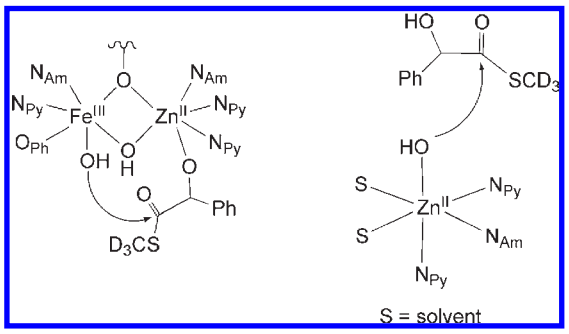
The kinetic data for the hydrolysis of $PhCH(OH)C(O)SCD_3$ promoted by **1** revealed that the reaction is second-order overall with an associative type mechanism that likely involves nucleophilic attack of the Zn−OH moiety on the thioester, with no formation of a precursor complex (Scheme 1). This differentiates thioester hydrolysis promoted by the mononuclear zinc complex from that found for a

(11) The CDH_2CN signal is positioned under the 1.77 ppm resonance.

(12) Mononuclear zinc alkyl thiolate complexes of N_3 donor ligands have been previously isolated and characterized. However, the properties of such complexes in aqueous solution have not been reported. (a) Brombacher, H.; Vahrenkamp, H. *Inorg. Chem.* **2004**, *43*, 6050. (b) Notni, J.; Gorls, H.; Anders, E. *Eur. J. Inorg. Chem.* **2006**, 1444. (c) Puerta, D. T.; Cohen, S. M. *Inorg. Chem.* **2002**, *41*, 5075. (d) Tekeste, T.; Vahrenkamp, H. *Inorg. Chem.* **2006**, *45*, 10799. (e) Boerzel, H.; Koeckert, M; Bu, W.; Spingler, B.; Lippard, S. J. *Inorg. Chem.* **2003**, *42*, 1604. (f) Brand, U.; Rombach, M.; Seebacher, J.; Vahrenkamp, H. *Inorg. Chem.* **2001**, *40*, 6151. (g) Matsunaga, Y.; Fujisawa, K.; Amir, N.; Miyashita, Y.; Okamoto, K. *Trans. Met. Chem.* **2006**, *31*, 897. (h) Ruf, M.; Burth, R.; Weis, K.; Vahrenkamp, H. *Chem. Ber.* **1996**, *129*, 1251. (i) Walz, R.; Weis, K; Ruf, M.; Vahrenkamp, H. *Chem. Ber.* **1997**, *130*, 975. (j) Calvo, J. A. M.; Vahrenkamp, H. *Inorg. Chim. Acta* **2006**, *359*, 4079. (k) Alsfasser, R.; Powell, A. K.; Vahrenkamp, H.; Trofimenko, S. *Chem. Ber.* **1993**, *126*, 685.

(13) Rates for the buffer promoted thioester hydrolysis reaction were determined at temperatures of 299.5−329.5 K. These values were subtracted from those obtained for the reactions involving **1** as described in the literature. diTargiani, R. C.; Chang, S.; Salter, M. H., Jr.; Hancock, R. D.; Goldberg, D. P. *Inorg. Chem.* **2003**, *42*, 5825.

Scheme 1. Comparison of the Thioester Hydrolysis Reaction Pathways Involving Binuclear Fe(III)Zn(II) and Mononuclear Zn(II) Complexes



Fe(III)Zn(II) complex, which mimics a common binuclear core found in glyoxalase II enzymes.[9] In reactions involving the Fe(III)Zn(II) complex, saturation kinetic behavior was interpreted as indicating equilibrium coordination of the deprotonated α-hydroxy group of the thioester to the Zn(II) prior to nucleophilic attack by an Fe(III)−OH moiety (Scheme 1). This coordination was proposed to occur via reaction of a Zn−OH moiety with the α-hydroxy group of the thioester.[9] With this in mind, we propose that the lack of thioester coordination to **1**, and the observation of a simple second-order reaction, is due to differences in the basicity of the Zn−OH moieties of **1** and the Fe(III)Zn(II) complex, with the latter being a better base, thus enabling deprotonation of the thioester α-hydroxyl group. The thioester hydrolysis reaction promoted by the Fe(III)Zn(II) complex is > 17-fold faster than the reaction performed under identical conditions using **1**.[9] With 10 equiv of Fe(III)Zn(II) complex or **1** present, the rate of thioester hydrolysis is enhanced over the background reaction by approximately 670- or 38-fold, respectively. Thus, the equilibrium coordination of the deprotonated thioester to the Zn(II) center of the Fe(III)Zn(II) complex facilitates the hydrolysis reaction by positioning the thioester carbonyl for nucleophilic attack by the Fe(III)−OH moiety. In terms of leaving group stabilization, for the reaction involving the Fe(III)Zn(II) complex, thiolate coordination was proposed to occur at the Fe(III) center on the basis of the formation of a disulfide side product that likely results from redox chemistry involving an Fe−SCD_3 moiety. For the thioester hydrolysis reaction promoted by **1**, the thiolate leaving group is stabilized via zinc coordination. For both systems, catalytic turnover presumably occurs via protonation of the M−SCD_3 species and generation of the reactive metal−hydroxide species. The proposed pathway for the thioester hydrolysis reaction promoted by **1** is shown in Scheme 2.

We note that the activation energy for the thioester hydrolysis reaction promoted by **1** is similar to that found for the alkaline hydrolysis of *n*-butylthioacetate.[14] This

Scheme 2. Thioester Hydrolysis Promoted by **1**

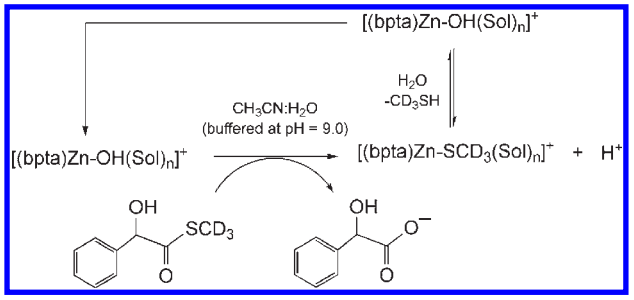


Table 2. Activation Energies for OH^- and Zn−OH-Promoted Thioester and Ester Hydrolysis Reactions

nucleophile	thioester or ester	E_a (kcal/mol)
OH^{-a}	*n*-BTA[b]	13.9[c]
1[d]	TE[e]	14.1(6)
OH^{-h}	4-NPA[f]	10.3(1)
[([12]aneN$_3$)Zn−OH]ClO_4[g]	4-NPA	11.7(1)
[([12]aneN$_4$)Zn−OH]ClO_4[h]	4-NPA	10.8(1)

[a] Ref 14. [b] *n*-BTA = *n*-butylthioacetate. [c] Temperature = 298 K. [d] This work. [e] TE = PhCH(OH)C(O)SCD_3. [f] 4-NPA = 4-nitrophenylacetate. [g] Ref 15. [h] Ref 16.

suggests that both reactions proceed via a rate-determining nucleophilic attack of hydroxide (either free or zinc-bound) on the thioester carbonyl carbon. Similar results have been reported for the hydrolysis of 4-nitrophenylacetate promoted by OH^- or mononuclear zinc complexes (Table 2).[15,16]

In summary, we report studies of thioester hydrolysis promoted by a mononuclear zinc complex in a mixed organic:aqueous environment. This reaction is relevant to the chemistry of the monozinc-containing form of human GlxII. In comparing the results of this study with those derived from an investigation involving a Fe(III)Zn(II) complex,[9] we found that the binuclear metal complex more effectively promotes thioester hydrolysis. In a broader context, our results provide evidence to support the notion that Zn−OH promoted ester and thioester hydrolysis reactions can proceed via similar reaction pathways. This is relevant to the hydrolysis of coumarin and thiocoumarin derivatives within the active site of CA enzymes.[7]

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Supporting Information Available: Synthetic and characterization details for **1**, experimental details for thioester hydrolysis reactions, plot of k_{obs} versus [**1**] at 299.5−329.5 K, and Eyring plot. This material is available free of charge via the Internet at http://pubs.acs.org.

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