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Thioester Hydrolysis Reactivity of an Fe(III)Zn(II) Complex

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Glyoxalase II enzymes catalyze the hydrolysis of a thioester substrate and have been found to coordinate a variety of dimetal combinations, including Fe(III)Zn(II), within the enzyme active site. Of relevance to these enzymes, the thioester hydrolysis reactivity of the Fe(III)Zn(II) compound [(BPBPMP)Fe(III)Zn(II)(μ-OAc)$_2$]ClO$_4$ (**1**) was evaluated in CH_3CN/H_2O (50:50; buffered) at 26.5 °C. Thioester hydrolysis in the absence and presence of **1** was monitored using ^{2}H NMR by following the loss of the thioester $-SCD_3$ signal. Two products are generated in the reaction involving the metal complex, D_3CSSCD_3 and CD_3SH. Kinetic studies of this reaction as a function of pH revealed maximum rate above the pK_a of a Zn$-$OH$_2$ moiety of [(BPBPMP)Fe(III)(OH)(μ-OH)Zn(II)(OH$_2$)]$^+$, which forms from **1** in CH_3CN/H_2O (50:50). UV$-$vis and electron paramagnetic resonance (EPR) studies of a single turnover thioester hydrolysis reaction in the presence of 1 equiv of **1** at pH = 9.0 suggest that the thioester does not initially interact with the Fe(III) center, but that changes occur at this site over the course of the reaction. The formation of a Fe(III)$-SCD_3$ moiety is proposed based on the observed D_3CSSCD_3 formation, which likely results from redox activity involving a iron(III) thiolate species. A mechanism for thioester hydrolysis is proposed involving initial coordination of the deprotonated α-hydroxy thioester to the zinc center followed by nucleophilic attack by a terminal Fe(III)$-$OH moiety and thiolate leaving group stabilization by the Fe(III) center. Overall, this study outlines a novel approach of using an aliphatic thioester substrate and ^{2}H NMR to provide mechanistic insight into thioester hydrolysis involving an Fe(III)Zn(II) complex of relevance to glyoxalase II.

Introduction

Metallohydrolases containing an active site heterobinuclear metal cluster are an area of intense current investigation.[1,2] Falling within this classification are some glyoxalase II (GlxII) enzymes, which have been identified as containing a bimetallic iron/zinc cluster that catalyzes thioester hydrolysis.[3] GlxII is a member of the metallo-β-lactamase superfamily of proteins[4] and is one of the two metalloenzymes found in the ubiquitous glyoxalase pathway.[5] The primary physiological substrate of the glyoxalase pathway (Figure 1) is believed to be methylglyoxal ($CH_3C(O)CHO$), a small molecule byproduct of lipid and glucose metabolism that is cytotoxic and mutagenic.[5−8] GlxII catalyzes the hydrolysis of a *S*-(2-hydroxyacyl)glutathione thioester (SLG) to produce the corresponding *S*-2-hydroxyacid and free glutathione (Figure 1). As SLG is also cytotoxic and can inhibit DNA synthesis,[5,9] the reaction catalyzed by glyoxalase II is important in cellular detoxification.[10] Elevated levels of GlxI and GlxII mRNA and protein have been reported in tumor cells, and inhibitors of the glyoxalase system have been shown to affect tumor growth in vitro.[11]

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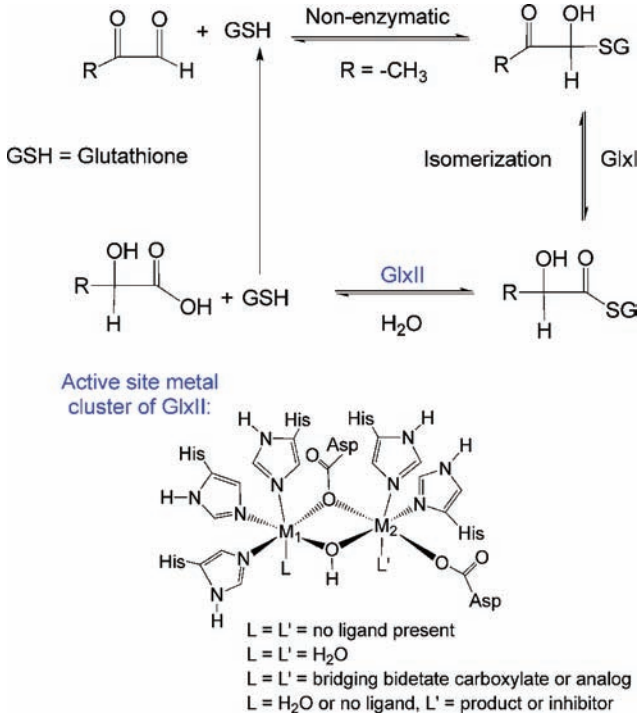

Figure 1. (Top) Glyoxalase pathway. (Bottom) Active site structural features in crystallographically characterized GlxII enzymes.

For this reason, several studies have been reported toward the design of antitumor agents that target the glyoxalase system.[10a,11,12]

Glyoxalase II enzymes from a variety of sources have been characterized by X-ray crystallography.[3b,c,13] In each structure the active site contains a binuclear metal cluster in which each metal center is coordinated by three terminal amino acid ligands, a bridging aspartate, and a bridging water/hydroxide moiety. In a subset of the X-ray structures, a bridging carboxylate or a terminal water molecule occupies the sixth coordination position on each metal center. The active site structural features of GlxII enzymes are similar to purple acid phosphatase in that one metal site has three terminal neutral donors and the second site contains a terminal anionic oxygen donor ligand.[2d]

A problem that has hampered comprehensive mechanistic studies of the GlxII-catalyzed thioester hydrolysis reaction is the variable metal ion content of GlxII enzymes which depends on the source of the enzyme and preparation conditions.[3,13,14] Specifically, different amounts of iron, zinc, and manganese ions are incorporated into the active site binuclear cluster depending on conditions. Metal ion contents that have been proposed on the basis of spectroscopic studies and metal analyses in GlxII enzyme samples include Fe(III)Zn(II), Fe(II)Zn(II), Fe(III)Fe(II), Zn(II)Zn(II), Fe(II)Fe(II), and Mn(II)Mn(II).[3,13,14] For the mitochondrial *Arabidopsis thaliana* GlxII produced recombinantly in *Escherichia coli* in rich media, the predominant iron-containing form of the enzyme (~70%) is proposed to have an Fe(III)Zn(II) metal ion content.[3c]

Neves and co-workers previously reported the Fe(III)Zn(II) complex $[(BPBPMP)Fe(III)Zn(II)(\mu\text{-}OAc)_2]ClO_4$ (**1**, Figure 2) which, when dissolved in CH_3CN/H_2O (50:50), exhibits three pK_a values.[2a] These have been assigned to the deprotonation of a bridging water molecule, a terminal $Fe(III)-OH_2$ and a terminal $Zn(II)-OH_2$, indicating that the bridging acetate ligands are displaced in favor of water ligands in aqueous solution. A structurally relevant species to the proposed $Fe(III)(H_2O)(\mu\text{-}OH)Zn(II)(H_2O)$ complex, $[(BPBPMP)Fe(III)(H_2O)(\mu\text{-}OH)Zn(II)](ClO_4)_2$ (**2**), has been crystallized from a methanol/H_2O solution and characterized by X-ray crystallography.[2e] In 1:1 CH_3CN/H_2O, this compound exhibits pK_a values of 2.93, 4.81, and 8.30. These have been assigned to similar ionizations to those depicted in Figure 2. X-ray absorption spectroscopic studies of **2** in CH_3CN/H_2O (70:30) provided evidence that both metal centers are six-coordinate in solution.

To our knowledge, no thioester hydrolysis reactivity studies involving an Fe(III)Zn(II) complex of relevance to GlxII have been reported. To address this deficiency, we report herein our initial studies of the thioester hydrolysis reactivity of the Fe(III)Zn(II) complex **1** in a CH_3CN/H_2O (buffered) solution. Our approach involves the use of ^2H NMR as a monitoring technique to follow the loss of a deuterium-labeled thioester. This method has allowed us to examine the reactivity of an aliphatic α-hydroxy thioester of structural relevance to the glutathione adduct of the glyoxalase pathway. We note that only one study of thioester hydrolysis promoted by a metal complex has been previously reported.[15] This investigation, which involved binuclear zinc compounds and reactivity studies in organic solvent, suggested the need for a terminal Zn−OH for thioester hydrolysis.

Experimental Section

General and Physical Methods. All reagents and solvents were obtained from commercial sources and were used as received unless otherwise noted. When necessary for synthetic procedures, solvents were dried according to published procedures[16] and were distilled under N_2 prior to use. IR spectra were recorded on a Shimadzu FTIR-8400 as KBr pellets. ^1H NMR spectra for characterization purposes were recorded in CD_3CN at ambient temperature on a JEOL ECX-300 or Bruker ARX-400 NMR spectrometer. ^1H NMR parameters appropriate for paramagnetic complexes were used for obtaining spectra of **1**.[17] Chemical shifts (in ppm) are referenced to the residual solvent peak in CHD_2CN (^1H: 1.94 (quintet)). ^2H NMR spectra were collected as previously described and were referenced to an internal standard of C_6D_6 (7.16 ppm).[17] ESI/APCI mass spectra

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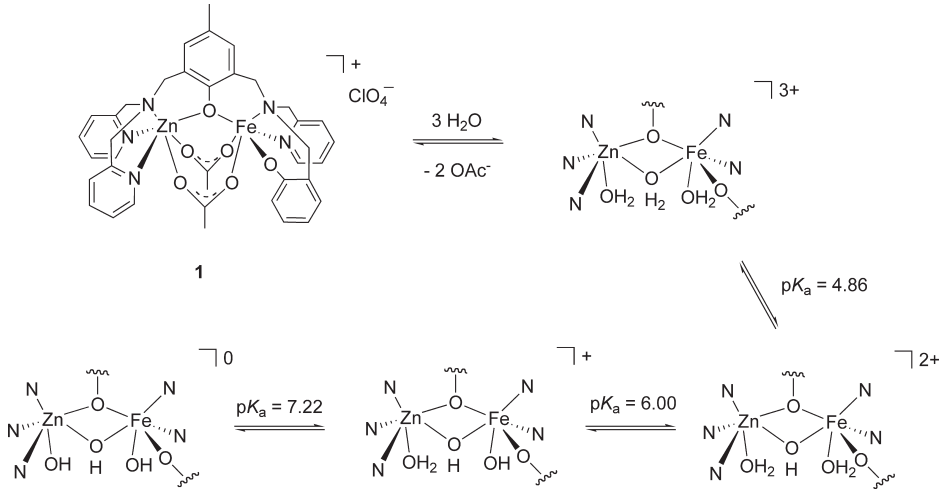

Figure 2. Solution properties of **1** in CH_3CN/H_2O (50:50).

were obtained at the University of California, Riverside. UV−vis spectra were recorded on a Hewlett-Packard HP8453 diode array spectrophotometer at ambient temperature. Electron paramagnetic resonance (EPR) spectra were collected at 5(1) K using a Bruker EMXPlus spectrometer system fitted with a continuous flow liquid helium cooled cyrostat. Elemental analyses were performed by Atlantic Microlabs of Norcross, GA.

Synthesis of Ligand Precursors. The organic precursors 2, 6-bis-(chloromethyl)-4-methylphenol,[18] bis-(2-pyridylmethyl)-amine,[19] and (2-hydroxybenzyl)(2-pyridylmethyl)amine[20] were synthesized according to literature procedures with minor modifications.

Synthesis of Hydroxyphenyl Thioacetic Acid *S*-methyl(d_3) Ester (PhCH(OH)C(O)SCD$_3$). This d_3-labeled thioester was prepared according to literature procedures starting from d_6-DMSO.[21] ^{1}H NMR (CD_3CN, 300 MHz): δ 7.41−7.35 (m, 5H), 5.22−5.20 (d, J = 5.2 Hz, 1H), 4.46−4.44 (d, J = 5.2 Hz, 1H, O−H) ppm; $^{13}C\{^1H\}$ NMR (CD_3CN, 100.6 MHz) δ 204.7, 140.4, 129.8, 129.7, 128.0, 80.3, 11.6 (7 signals expected and observed); ^{2}H NMR (CH_3CN, 61.4 MHz) δ 1.92 (s, pwhh ~3 Hz); ^{2}H NMR (CH_3CN/H_2O (50:50, buffered, pH = 9.0)) δ 1.92 (s, pwhh ~4 Hz); FTIR (KBr, cm^{-1}) 3441 (ν_{OH}), 1682 ($\nu_{C=O}$); Anal. calcd for $C_9H_7D_3SO_2$: C, 58.35; H+D, 5.39. Found: C, 58.48; H+D, 5.49.

Synthesis of 2-{[Bis(2-pyridylmethyl)amino]methyl}-6-[{[2-hydroxyphenyl)methyl]-(2-pyridylmethyl)amino}methyl]-4-methylphenol (L^1).[22] To a solution of 2,6-bis(chloromethyl)-4-methylphenol (14.4 g, 0.0702 mol) in dry CH_2Cl_2 (500 mL) was added a solution of (2-hydroxybenzyl)(2-pyridylmethyl)amine (9.14 g, 0.0427 mol) in dry CH_2Cl_2 (55 mL) and triethylamine (19.6 mL, 0.141 mol). The reaction mixture was stirred overnight (~12 h). At this point, bis-(2-pyridylmethyl)amine was added (22.4 g, 0.113 mol), and the solution was allowed to stir for an additional 24 h at room temperature. The solution was then transferred to a separation funnel, and 300 mL of brine solution was added. The mixed aqueous/organic solution was extracted with CH_2Cl_2 (3 × 100 mL). The combined organic fractions were then dried over sodium sulfate, and the solvent was removed under reduced pressure to produce a brown solid. Purification of this solid by column chromatography on silica gel using a 4:1:1 ratio of ethyl acetate/hexanes/triethylamine as the eluent yielded a white powder (R_f ~ 0.50; Yield: 9.21 g, 40%); ($CDCl_3$, 400 MHz) δ 8.57−8.56 (m, 3H), 7.61−6.81 (m, 15H), 3.90 (s, 8H), 3.80 (s, 2H), 3.75 (s, 2H), 2.27 (s, 3H) ppm. The ^{1}H NMR of L^1 matched that previously reported.[22]

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.*[23]

[(L^{1}Fe(III)Zn(II)(μ-O$_2$C$_2$H$_3$)$_2$]ClO$_4$ (1). A procedure for the preparation of **1** has been previously reported.[24] In our hands this procedure resulted in the formation of a significant amount of the Fe(III)Fe(II) complex as detected by ^{1}H NMR.[24] To a methanol solution of L^1 (50.0 mg, 0.0920 mmol) was added a methanol solution of $Me_4NOH \cdot 5H_2O$ (33.3 mg, 0.184 mmol). The reaction was stirred for ~5 min, at which time a methanol solution of $Zn(ClO_4)_2 \cdot 6H_2O$ (34.1 mg, 0.0920 mmol) was added, followed by a methanol solution of $Fe(ClO_4)_3 \cdot 6H_2O$ (42.4 mg, 0.0920 mmol). The resulting mixture became dark purple and was stirred for approximately 2 min, at which point a methanol solution of NaOAc (15.0 mg, 0.184 mmol) was added. This mixture was stirred for 1 h and then the solvent was removed under reduced pressure. The remaining solid was dissolved in CH_2Cl_2, and solution was passed through a Celite/glass wool plug. The filtrate was pumped to dryness, and the remaining solid was recrystallized 3−4 times from MeOH at room temperature. FTIR (KBr, cm^{-1}) 3441 (ν_{N-H}), 1597 (ν_{COO}), 1435 (ν_{COO}), 1096 (ν_{ClO4}), 764 (ν_{ClO4}). ESI/APCI-MS, m/z (relative intensity), 781.15 ([M − ClO$_4$]$^+$, 100%). Anal. calcd. for $C_{38}H_{39}N_5FeZnClO_{10} \cdot 1.5H_2O$: C, 50.27; H, 4.67; N, 7.72. Found: C, 50.15; H, 4.65; N, 7.63.

Thioester Hydrolysis Monitored by ^{2}H NMR. Product Identification. A 0.75 mL solution composed of CH_3CN (0.30 mL), buffer solution (0.36 M CHES, 0.30 mL) containing $NaNO_3$ (I = 0.61 M), and doubly distilled H_2O (0.15 mL) was prepared. An internal chemical shift standard (C_6D_6, 1 μL) was added, and the pH of this solution was adjusted to pH = 9.0 using 0.1 M NaOH. To this solution was added a 0.15 mL solution of the hydroxyphenylthioacetic acid *S*-methyl(d_3) ester in CH_3CN (2.0 mg dissolved in 1.0 mL, 0.011 M). The resulting mixture was transferred to an NMR tube, and the tube was placed in a Bruker ARX NMR probe which had been previously optimized for ^{2}H data collection at 26.5°. Over the course of > 450 h, the intensity of the 1.92 resonance of the thioester −SCD$_3$ group

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decreased while a new resonance appeared at 2.12 ppm. This product was confirmed as CD_3SH by identification of its volatility (purging the solution with N_2 results in loss of the 2.12 ppm resonance) and by a positive Ellman's reagent test, which indicates the presence of a free thiol. Similar experiments were performed with appropriate buffers at pH = 7.0, 7.5, 7.9, and 8.6. In each case, the only product was CD_3SH.

For the reaction involving the metal complex, a similar reaction mixture was prepared, with **1** (variable concentration) dissolved in the CH_3CN portion. For these reactions, as the intensity of the 1.92 ppm signal decreases, two new resonances appear at 2.18 and 2.12 ppm, respectively. The resonance at 2.18 ppm was identified as being from the disulfide D_3CSSCD_3 by comparison of the chemical shift to that of CH_3SSCH_3 in 50% D_2O/CD_3CN (2.18 ppm). The resonance at 2.12 ppm was identified as belonging to a volatile species (e.g., CD_3SH) by purging the reaction mixture with N_2, which results in the loss of this resonance. Similar product mixtures were identified at all pH values (7.0, 7.5, 7.9, 8.6, 9.0).

Kinetic Measurements. General Procedure for Background Reactions Involving Only PhCH(OH)C(O)SCD$_3$. Reaction mixtures were prepared in an identical manner to that outlined for the qualitative NMR experiments described above. The pH of each solution was adjusted to the desired value using 0.1 M NaOH. To this solution was added a 0.15 mL solution of the hydroxyphenylthioacetic acid *S*-methyl(d_3) ester in CH_3CN (2.0 mg dissolved in 1.0 mL, 0.011 M). The resulting mixture was transferred to an NMR tube, and the tube was placed in a Bruker ARX NMR probe which had been previously optimized for ^{2}H data collection. ^{2}H NMR spectra were collected at 26.5 °C as a function of time and were referenced to the chemical shift of the C_6D_6 standard (δ = 7.16 ppm). The progress of the reaction was monitored by observing the loss of the $-SCD_3$ resonance for the starting thioester at 1.92 ppm. Data was collected until >80% of the thioester had undergone reaction. Data was collected for a minimum of two runs at pH 8.6 and 9.0. Background data at lower pH values was collected for only one run because of the slow nature of the hydrolysis reaction (k_{obs} $\sim 10^{-7}-10^{-8}$ s^{-1}). Background observed rate constants are as follows: pH = 7.0 (5.7×10^{-8} s^{-1}), pH = 7.5 (9.2×10^{-8} s^{-1}), pH = 7.9 (1.5×10^{-7} s^{-1}), pH = 8.6 ($1.2(1) \times 10^{-6}$), pH = 9.0 ($1.1(1) \times 10^{-6}$ s^{-1}).

Kinetic Measurements. General Procedure for Reactions Containing 1 and PhCH(OH)C(O)SCD$_3$. A 0.75 mL solution composed of **1** in CH_3CN (0.30 mL), buffer solution (0.30 mL) containing $NaNO_3$ (I = 0.61 M), and doubly distilled H_2O (0.15 mL) was prepared. An internal chemical shift standard (C_6D_6, 1 μL) was then added. The pH of this solution was adjusted to the desired value using 0.1 M NaOH. To this solution was added a 0.15 mL solution of the hydroxyphenylthioacetic acid *S*-methyl(d_3) ester in CH_3CN (2.0 mg dissolved in 1.0 mL, 0.011 M). The resulting mixture was transferred to an NMR tube, and the tube was placed in a Bruker ARX NMR probe which had been previously optimized for ^{2}H NMR data collection. Each ^{2}H NMR spectrum was referenced to the chemical shift of the C_6D_6 standard (δ = 7.16 ppm). Each reaction was monitored by observing the loss of the $-SCD_3$ resonance for the starting thioester at 1.92 ppm as a function of time until the reaction was >80% complete. As the intensity of the 1.92 ppm signal decreases, two new resonances appear at 2.18 and 2.12 ppm, respectively. The resonance at 2.18 ppm was identified as being from D_3CSSCD_3 by comparison of this chemical shift to that of CH_3SSCH_3 in 50% D_2O/CD_3CN (2.18 ppm). The resonance at 2.12 ppm was identified as belonging to a volatile species (e.g., CD_3SH) by purging the reaction mixture with N_2, which results in the loss of this resonance. The initial concentration of metal complex was varied from 9.0×10^{-4} M to 0.018 M. Data was collected for a minimum of two runs at all metal complex concentrations and pH values.

Deconvolution of ^{2}H NMR Data for the Reactions Involving PhCH(OH)C(O)SCD$_3$. Integrated intensities of the $-SCD_3$ resonances in the ^{2}H NMR kinetic data were determined using Lorentzian−Gaussian curve fitting program provided in the Delta NMR Software v 4.3.6 package (JEOL, Ltd.). For each spectrum acquired, the chemical shift of the resonance of C_6D_6 was set to 7.16 ppm. The integrated intensity of the HDO resonance at 3.95 ppm was set to one deuteron. The concentration of $-SCD_3$ labeled thioester was then determined via integration of the 1.92 ppm resonance versus the HDO integrated intensity. Pseudo first-order rate constants were determined from slopes of plots of ln[thioester] versus time. Typical correlation coefficients for these plots were ≥0.98.

UV−vis Experiments. (A) A solution of **1** (0.008 mmol) was prepared in CH_3CN (15 mL), buffer solution (10 mL) containing $NaNO_3$ (I = 0.61 M), and doubly distilled H_2O (5 mL). The absorption spectrum of this solution was then measured. The entire solution was then added to solid PhCH(OH)C(O)SCD$_3$ (0.008 mmol), and the absorption spectrum was again recorded. (B) An identical solution of **1** to that described in part (A) was prepared. This solution was then added to solid mandelic acid (0.008 mmol), and the absorption spectrum was again recorded. (C) An NMR sample of the thioester hydrolysis reaction mixture, judged complete by ^{2}H NMR, was diluted from 1.8×10^{-3} M to 2.67×10^{-4} in terms of **1**, and an absorption spectrum was obtained.

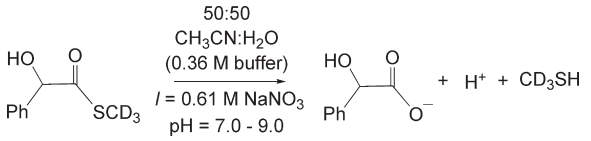
EPR Experiments. A single turnover thioester hydrolysis reaction in the presence of **1** (2.67×10^{-4} M) in 50:50 CH_3CN/H_2O (CHES buffer, pH = 9, I = 0.61 M ($NaNO_3$)) was monitored by removal of aliquots for EPR experiments. Each aliquot was immediately frozen at 77 K prior to EPR data acquisition at 5(1) K.

Results

Investigating Thioester Hydrolysis Promoted by an Fe(III)Zn(II) Complex. Rationale for the Use of a Deuterium-Labeled Thioester and ^{2}H NMR. We have initiated our studies using an Fe(III)Zn(II) complex (**1**, Figure 2) that has previously been shown to catalyze the hydrolysis of the activated phosphate diester 2,4-bis(dinitrophenyl)-phosphate (BDNPP) in mixed organic/aqueous solution.[2f] This complex contains pyridyl and phenolate ligation to the Fe(III) center, resulting in intense ligand based absorption features below 350 nm and a broad phenolate→Fe(III) ligand-to-metal charge transfer (LMCT) centered at ~464 nm (pH = 9.0, $\varepsilon \sim 2500$ M^{-1} cm^{-1}). Typically, hydrolysis reactions promoted by metal complexes (e.g., Zn−OH complexes) are investigated using an activated *p*-nitrolabeled substrate and UV−vis spectroscopy as the monitoring method.[25] This approach is not feasible for investigating thioester hydrolysis reactions promoted by **1** for several reasons. First, the absorption maximum of the *p*-nitrothiophenolate leaving group of $NO_2-C_6H_4-SC(O)CH_3$ (λ_{max} = 410 nm) overlaps to some extent with the LMCT of **1**. A more important issue is the low pK_a of the *p*-nitrothiophenolate leaving group (pK_a = 4.5),[26] which means that thioester bond scission in buffered aqueous solution at pH ≥ 7 at ambient temperature is fast relative to that of the oxygen analogue (*p*-nitrophenolate, pK_a = 7.15)[27] in the absence

(25) Berreau, L. M. *Adv. Phys. Org. Chem.* **2006**, *41*, 79–181.
(26) 25 °C, H_2O; Cadogan, J. I. G.; Buckingham, J.; MacDonald, F. J.; Rhodes, P. H. *Dictionary of Organic Compounds*, 6th ed.; CRC Press: Boca Raton, FL, 1996.
(27) Serjeant, E. P.; Dempsey, B. *Ionization Constants of Organic Acids in Aqueous Solution*; Pergamon: Oxford, U.K., 1979.

Scheme 1



of any metal complex. The presence of a methylene unit in the thioester $NO_2-C_6H_4-CH_2SC(O)CH_3$[28] gives a leaving group with a higher pK_a value (8.87), which therefore should produce a slower thioester hydrolysis reaction. However, the absorption maximum for the *p*-nitrobenzylthiolate leaving group is at 360 nm, which is within a region that also contains intense absorption features for the Fe(III)Zn(II) complex **1**. Additional methods that have previously been employed in studying thioester hydrolysis reactions in enzymatic systems also are not feasible for reactions involving **1**. For example, for kinetic studies involving glyoxalase II enzymes, thioester hydrolysis is monitored using the substrate S-D-lactoylglutathione, which is converted to reduced glutathione and D-lactic acid. The rate of hydrolysis of this substrate is followed by measuring the loss in absorbance of the substrate at 240 nm ($\varepsilon = 3100\ M^{-1}\ cm^{-1}$).[13b] For thioesterase enzymes, reactions are typically monitored by continuous assays based on the use of thiol derivatizing agents (e.g., Ellman's reagent, 5,5′-dithio-bis(2-nitrobenzoic acid) DTNB) that yield colored products ($\lambda_{max} = 412$ nm).[29] In considering thioester hydrolysis reactions involving **1**, both of these approaches used in enzymatic systems would be problematic because of overlap of the product absorption feature with absorption bands of the metal complex.

In the experiments outlined herein we have used a deuterium-labeled thioester $PhCH(OH)C(O)SCD_3$ and ^2H NMR to examine thioester hydrolysis in the absence and presence of **1** (Scheme 1). The use of this particular thioester is especially relevant to the chemistry of glyoxalase II enzymes as $PhCH(OH)C(O)SCD_3$ contains an α-hydroxy group and alkyl thiol leaving group (CD_3SH), both of which are found in glyoxalase II substrates (Figure 1). The hydrolysis products of this reaction in the pH range examined (7.0−9.0) should be mandelate anion (p$K_a = 3.85$)[27] and CD_3S^-/CD_3SH (CH_3SH, p$K_a = 9.7$).[30] As outlined below, in addition to the production of CD_3SH we have identified the formation of the disulfide D_3CSSCD_3 in reaction mixtures involving the Fe(III)Zn(II) complex **1**.

Product Identification in Thioester Hydrolysis Reactions Involving $PhCH(OH)C(O)SCD_3$. Mild heating of a solution of $PhCH(OH)C(O)SCD_3$ in 50:50 CH_3CN/H_2O (CHES buffer, $I = 0.61$ M) at pH = 9.0 at 26.5 °C results in the gradual disappearance of the $-SCD_3$ resonance of the starting thioester at 1.92 ppm and the appearance of a new resonance at 2.12 ppm (Figure 3). The new species produced is volatile, as purging of the reaction mixture with N_2 at room temperature results in loss of this

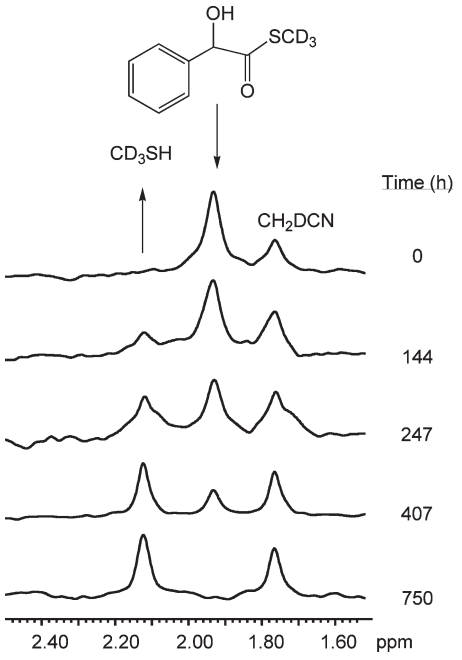


Figure 3. ^2H NMR spectra of a background thioester hydrolysis reaction collected as a function of time. Conditions: 50:50 CH_3CN/H_2O, pH = 9.0 (0.36 M CHES buffer), $I = 0.61$ M ($NaNO_3$), [$PhCH(OH)C(O)SCD_3$] = 1.8×10^{-3} M, 26.5 °C.

2.12 ppm resonance. On the basis of the volatility of this product and a positive Ellman's reagent test for free thiol at the conclusion of the reaction, we formulate this product as CD_3SH. As can be seen in Figure 3, the thioester hydrolysis reaction involving $PhCH(OH)C(O)SCD_3$ requires > 400 h to reach greater than > 80% completion. The formation of mandelate as the other product in the thioester hydrolysis reaction mixture is proposed based on stoichiometry and our previous study of thioester hydrolysis involving zinc complexes using the same substrate wherein the formation of mandelate was identified using spectroscopic and analytical approaches.[15]

Performing the thioester hydrolysis reaction in the presence of **1** (2.5 equiv relative to thioester) under identical conditions gives ^2H NMR spectra containing two product resonances (2.18 and 2.12 ppm; Figure 4). The species at 2.18 ppm was identified as the disulfide D_3CSSCD_3 via comparison of the chemical shift of this resonance with that of H_3CSSCH_3 in 50:50 CD_3CN/D_2O. This disulfide product should be generated from CD_3S^-, as alkylthiolates are known to undergo catalytic oxidation in the presence of O_2 and Fe(III).[31] For example, Fe(III)-EDTA in the presence of O_2 has been shown to catalyze the oxidative coupling of *n*-butyl thiol to give the disulfide in 93% yield in aqueous methanol (pH = 9.5). Overall, the thioester hydrolysis reaction in the presence of 2.5 equiv of **1** is considerably faster than the background reaction, requiring < 2 h to go to > 80% completion. It is important to note that in the presence of 0.5 equiv of **1** relative to the thioester, the hydrolysis reaction was found to proceed to completion, indicating catalytic turnover occurs under the reaction conditions.

(28) Aoyama, T.; Takido, T.; Kodomari, M. *Synth. Commun.* **2003**, *33*, 3817–3824.

(29) See for example: Zhao, H.; Martin, B. M.; Bisoffi, M.; Dunaway-Mariano, D. *Biochemistry* **2009**, *48*, 5507–5509.

(30) 20°C, H_2O; Lide, D. R. *Handbook of Chemistry and Physics*, 74th ed.; CRC Press: Boca Raton, FL, 1993.

(31) (a) Rao, T. V.; Sain, B.; Murthy, P. S.; Rao, T. S. R. P.; Joshi, G. C.; Jain, A. K. *J. Chem. Res.* (*S*) **1997**, 300–301. (b) Plietker, B. *Iron Catalysis in Organic Chemistry*; Wiley-VCH: Weinheim, Germany, 2008.

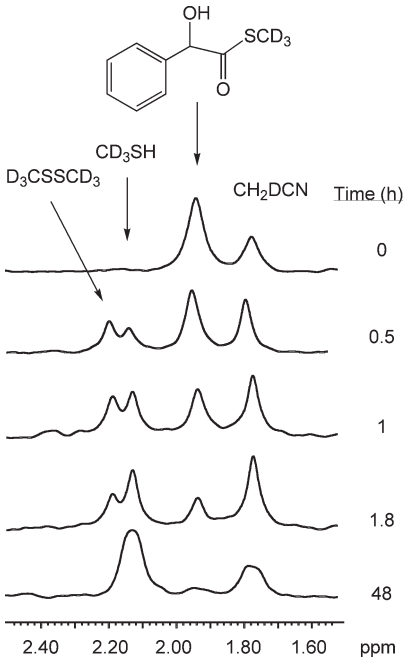


Figure 4. ^{2}H NMR spectra of a thioester hydrolysis reaction in the presence of **1** collected as a function of time. Conditions: 50:50 CH_3CN/H_2O, pH = 9.0 (0.36 M CHES buffer), I = 0.61 M ($NaNO_3$), [PhCHC(O)SCD_3] = 1.8×10^{-3} M, [**1**] = 4.5×10^{-3} M, 26.5 °C.

Thus, pseudo first-order conditions are present even at low concentrations of metal complex.

Kinetic Studies and Effect of pH. The thioester hydrolysis reaction was monitored as a function of the concentration of the metal complex (0−10 equiv relative to thioester) at pH = 7.0−9.0. The disappearance of the 1.92 ppm resonance of the thioester was monitored as a function of time. As can be seen in Figures 3 and 4, the ^{2}H NMR spectra involve peak widths for the $-SCD_3$ resonances of the starting thioester and resulting products (pwhh ~4 Hz) that are broad relative to standard ^{1}H NMR resonances. These broad peak widths required the use of curve-fitting to deconvolute peak overlap and determine accurate integration values for the 1.92 ppm thioester resonance. Fitting of the loss of the thioester versus time to a single exponential equation yielded pseudo first-order rate constants for each reaction. In the absence of **1**, the pseudo first-order rate constant for the thioester hydrolysis reaction at pH = 9.0 is $1.1(1) \times 10^{-6}$ s^{-1}. In the presence of 2.5 equiv of **1**, the pseudo first-order rate constant is $2.3(2) \times 10^{-4}$ s^{-1}, indicating a >200-fold rate enhancement. Rate constants for other pH values in the presence of 2.5 equiv of **1** are given in Table 1. For all pH values, a rate enhancement of at least >140-fold was identified relative to the background hydrolysis reaction. As shown in Figure 5, at all pH values plotting of the first-order rate constant versus [**1**] yielded a saturation curve wherein for low concentrations of **1** the rate of the reaction initially increased linearly. However, at higher concentrations of metal complex, the rate becomes independent of [**1**] indicating a rate-determining step that is more complicated than a single step. This kinetic behavior is consistent with a mechanism wherein an equilibrium step (K_1) precedes an irreversible step (k_2). Fitting of the pH = 9.0 data to eq 1 yielded

Table 1. Observed Rate Constants for Thioester Hydrolysis Promoted by **1**[a]

pH	k_{obs} (s^{-1})
7.0	$1.10(2) \times 10^{-5}$
7.5	$1.69(6) \times 10^{-5}$
7.9	$2.49(26) \times 10^{-5}$
8.6	$1.63(9) \times 10^{-4}$
9.0	$2.31(20) \times 10^{-4}$

[a] Conditions: 50:50 CH_3CN/H_2O, 0.36 M MOPS or CHES buffer, I = 0.61 M ($NaNO_3$), [PhCHC(O)SCD_3] = 1.8×10^{-3} M, [**1**] = 4.5×10^{-3} M, 26.5 °C.

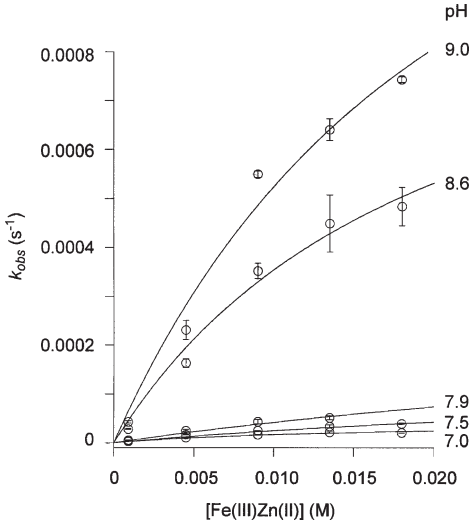


Figure 5. Plot of [**1**] versus k_{obs} for the thioester hydrolysis reaction. Conditions: 50:50 CH_3CN/H_2O, pH = 7.0−9.0 (0.36 M MOPS or CHES buffer), I = 0.61 M ($NaNO_3$), [PhCHC(O)SCD_3] = 1.8×10^{-3} M, 26.5 °C.

K_1 = 42(24) and $k_2 = 1.8(6) \times 10^{-3}$ s^{-1}. The K_1 value indicates a weak interaction of the thioester with the metal complex.

$$k_{obs} = \frac{k_2 K_1[\mathbf{1}]}{1+K_1[\mathbf{1}]} \quad (1)$$

The pH dependence on the rate of thioester hydrolysis for reactions containing 2.5 equiv of **1** is shown in Figure 6. The data is fit to eq 4 derived for a monoprotic system (Figure 7) and yields a kinetic pK_a value of 8.8. This value is similar to the thermodynamic pK_a (8.3) previously report by Neves et al. for the Zn−OH_2 moiety in the solution form of [(BPBPMP)Fe(III)(H_2O)(μ-OH)Zn(II)](ClO_4)$_2$ (**2**),[2e] indicating that the maximum rate is obtained upon formation of a Zn−OH unit. We note that data cannot be collected at higher pH values using the ^{2}H NMR approach as the reaction proceeds too rapidly at 26.5 °C.

Spectroscopic Investigations of the Thioester Hydrolysis Reaction. To gain further insight into the reaction pathway of thioester hydrolysis promoted by **1**, a single turnover reaction was monitored by UV−vis and EPR.

UV−vis Experiments. A solution of **1** (1.8×10^{-3} M) identical to that used in the kinetic studies at pH = 9.0 and containing 1 equiv of thioester was prepared and immediately diluted using CH_3CN/H_2O (0.36 M CHES buffer, I = 0.61 M $NaNO_3$) to [**1**] = 2.67×10^{-4} M for UV−vis spectral analysis. The features of the spectrum in the

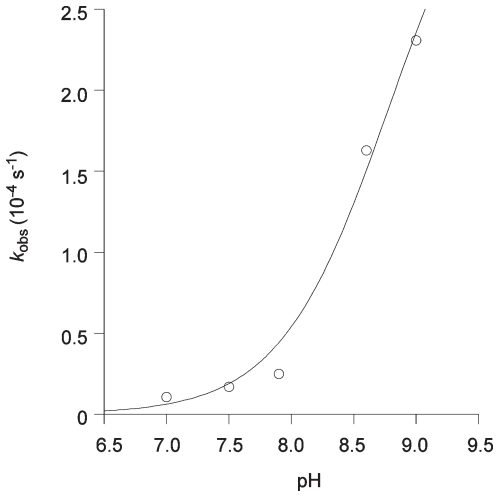


Figure 6. Dependence of k_{obs} on the pH of the reaction mixture. Conditions: 50:50 CH_3CN/H_2O, [buffer] = (0.36 M MOPS or CHES), I = 0.61 M ($NaNO_3$), [$PhCHC(O)SCD_3$] = 1.8×10^{-3} M, [**1**] = 4.5×10^{-3} M, 26.5 °C.

$$[(BPBPMP)Fe(III)(OH)(\mu\text{-}OH)Zn(II)(H_2O)]^+ \overset{K_a}{\rightleftharpoons} [(BPBPMP)Fe(III)(OH)(\mu\text{-}OH)Zn(II)(OH)] + H^+ \quad (2)$$

$$[(BPBPMP)Fe(III)(OH)(\mu\text{-}OH)Zn(II)(OH)] + PhCH(OH)C(O)SCD_3 \xrightarrow{k_2^{max}} \text{thioester hydrolysis products} \quad (3)$$

$$k_{obs} = \frac{k_2^{max} K_a}{K_a + [H^+]} \quad (4)$$

Figure 7. Equations describing the pH dependence of the thioester hydrolysis reaction involving **1**.

region of 300−850 nm are identical to that found for **1** in the absence of thioester under identical conditions (Figure 8). Of particular importance, no change is seen in the position or intensity of the terminal phenolate-to-Fe(III) LMCT at ~464 nm, which indicates that the thioester does not interact with the Fe(III) center.

The absorption spectrum obtained at the conclusion of the single turnover thioester hydrolysis reaction differs from the starting reaction mixture, with an increase in intensity at ~400 nm and the phenolate-to-Fe(III) LMCT being less distinct. To evaluate whether the mandelate product interacts with the metal complex, a solution of **1** (2.67×10^{-4} M in CHES buffer (pH = 9), I = 0.61 M ($NaNO_3$)) was prepared containing 1 equiv of mandelic acid. This produces an absorption maximum at ~440 nm, which is somewhat similar to that found for the solution of the reaction products. As disulfide is generated in the thioester hydrolysis reaction mixture, which suggests that some reduction of Fe(III) to Fe(II) could be occurring, we also examined the absorption spectra of analytically pure **1** in the presence of equimolar amounts of sodium dithionite and mandelic acid. This produced a shift in the absorption maximum similar to the solution containing mandelic acid, but with a slightly lower intensity. Overall, the UV−vis experiments suggest that changes are occurring at the Fe(III) center over the course

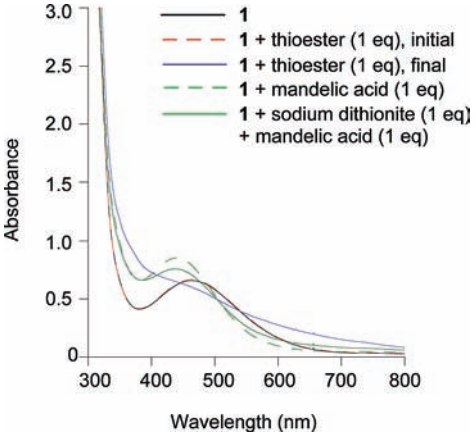


Figure 8. UV−vis absorption spectra. Conditions: 50:50 CH_3CN/H_2O, [buffer]=0.36 M CHES, I=0.61 M ($NaNO_3$), [**1**]=2.67×10^{-4} M, 26.5 °C.

of the reaction, with coordination of mandelate being a possibility.

The other product of the thioester hydrolysis reaction, CD_3S^-, may also coordinate to the metal complex. For this reason, we introduced 1 equiv of $NaSCH_3$ into a solution of **1** in CH_3CN/H_2O (2.67×10^{-4} M in CHES buffer (pH = 9.0), I = 0.61 M ($NaNO_3$)). This produced no shift in the absorption maximum of the complex. Addition of another 6 equiv of $NaSCH_3$ also produced no change in the absorption spectrum. ^2H NMR analysis of a solution of **1** and 1 equiv of $NaSCD_3$ in CH_3CN:CHES buffer at pH = 9.0 (I = 0.61 M) revealed the formation of CD_3SH. Thus, free thiolate is rapidly converted, at least in part, to thiol in the reaction mixture.

EPR Experiments. A reaction mixture was prepared as outlined for the ^2H NMR kinetic studies. This mixture was then immediately diluted to [**1**] = 2.67×10^{-4} M using CH_3CN/H_2O (0.36 M CHES buffer, I = 0.61 M $NaNO_3$). Using UV−vis to evaluate reaction progress (at this concentration the single turnover reaction takes ~120 h to go to completion), aliquots were removed at specified intervals and frozen at 77 K. EPR spectra were then collected at 5(1) K. Selected spectra are shown in Figure 9. Upon introduction of the thioester (90 s) a spectral change is identifiable wherein the intensity of the g = 9 and g = 4.3 features increased, whereas a decrease in intensity is found for the feature at g = 6.6. We note that it has been previously reported that the addition of 2,4-BDNPP to [(BPBPMP)-Fe(III)(H_2O)(μ-OH)Zn(II)](ClO_4)$_2$ (**2**) in acetonitrile/HEPES buffer at pH = 7.0 produces an increase in the g = 9 and g = 4.3 features similar to that identified here.[2f] The data for 2,4-BDNPP was interpreted as indicating that the phosphate diester monoanion did not directly interact with the Fe(III) center, and instead coordinates in a monodentate fashion to the Zn(II) center. One possible explanation for the initial EPR features of both systems is that in the absence of a substrate molecule there is a portion of the compound that exists as a dimer ([Fe(III)Zn(II)]$_2$ with $J > h\nu$ for Fe(III)···Fe(III) coupling) and is EPR silent and a portion that is a monomer ([Fe(III)Zn(II)] which gives an EPR signal. Upon reaction with the thioester or phosphate diester the proportion of the monomeric species is enhanced. Alternatively, the changes in the spectra upon addition of thioester could be due to changes in the zero field splitting parameters (i.e., D) for the S = 5/2 Fe(III)

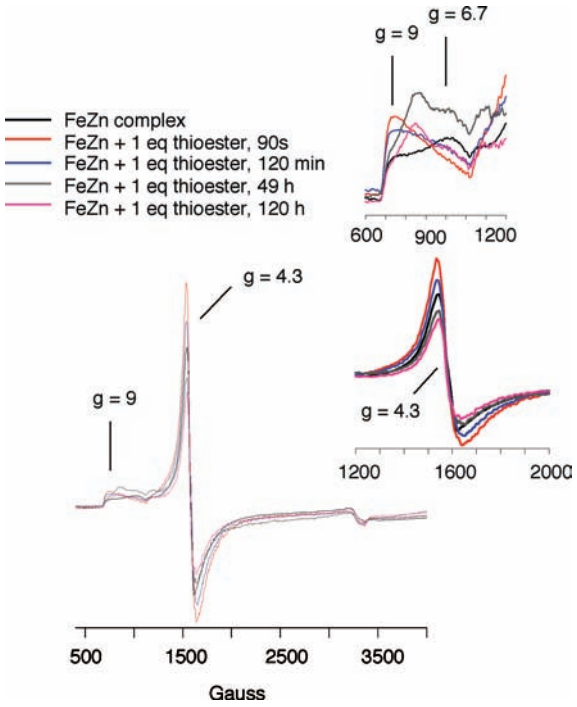


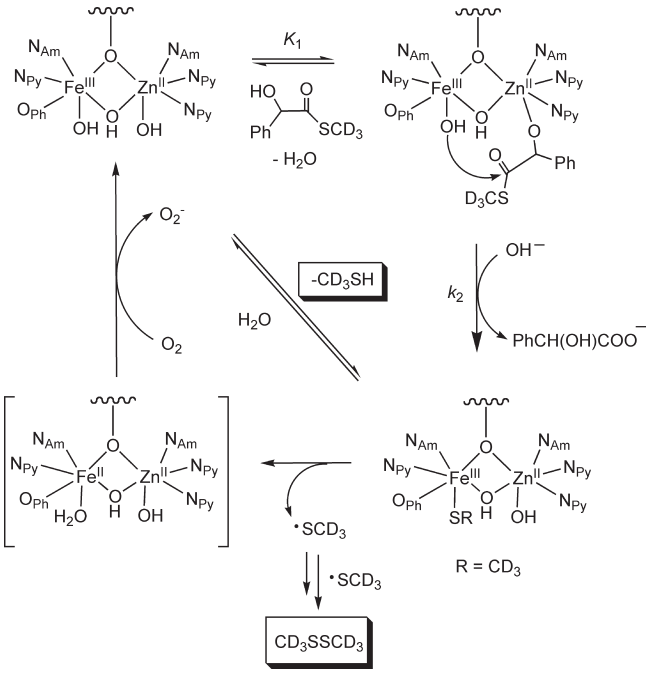
Figure 9. EPR spectra obtained at various intervals in the single turnover thioester hydrolysis reaction promoted by **1**. All spectra were recorded at 5(1) K using 1.0 mW microwave power, 100 kHz modulation frequency, 0.814 mT field modulation amplitude, and a 20.3 mT/s sweep rate.

center.[32] Overall, the similarity of the EPR spectral changes for the thioester and phosphate diester reactions suggests similar interactions with the metal complex.

In the thioester hydrolysis reaction a second intermediate maximizes in intensity at 49 h (Figure 9). This species is characterized by a feature at ~850 G ($g \sim 8$), with a residual feature still present at $g \sim 9$. Overall, the EPR spectrum did not cleanly convert back to exactly that of the starting complex after 120 h. The $g = 4.3$ signal in the final spectrum is less intense and broadened compared to that of the starting material, which may be due to the reduction of a portion of the Fe(III) to Fe(II) over the course of the reaction.

An EPR spectrum of analytically pure **1** in the presence of 1 equiv of mandelic acid ($[\mathbf{1}] = 2.67 \times 10^{-4}$ M using 50:50 CH_3CN/H_2O (0.36 M CHES buffer, $I = 0.61$ M $NaNO_3$)) revealed a spectrum with $g \sim 6.4$, 4.3, and 2.0 (Supporting Information, Figure S1). The distinctly different nature of this spectrum versus that of **1** under identical conditions (Figure 1) indicates that the mandelate anion interacts with the Fe(III)Zn(II) complex in the absence of thioester or its hydrolysis products. However, for the single turnover thioester hydrolysis reaction mixture, a spectrum containing a significant $g \sim 6.4$ feature is not produced at any time interval (Figure 9). Importantly, this indicates that following thioester hydrolysis, the coordination properties (e.g., available coordination sites) of the Fe(III)Zn(II) complex have been altered, and mandelate does not interact with the metal complex to a significant degree. We hypothesize that this is due to the coordination of CD_3S^-, or chemical changes resulting from a Fe(III)-SCD_3 interaction. When produced near the Fe(III) center, the thiolate anion can be stabilized via metal coordination, and the formation of such a Fe(III)-SCD_3 adduct is a precursor to the observed

Scheme 2. Proposed Reaction Pathway for Thioester Hydrolysis Promoted by **1**



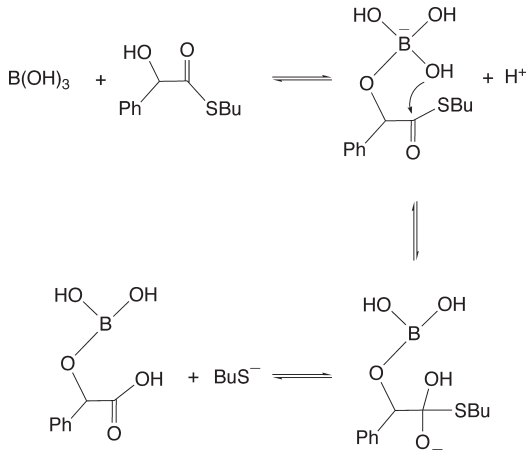
formation of the D_3CSSCD_3. Overall, the EPR studies suggest that while changes occur at the Fe(III) center over the course of the reaction, mandelate coordination is not significantly involved. This in turn indicates that the observed changes in the absorption spectrum over the course of the single turnover reaction are not due to carboxylate coordination.

Proposed Mechanism. On the basis of literature precedent[2e,f] and the kinetic and mechanistic data presented above, we propose a mechanism for thioester hydrolysis promoted by **1** as outlined in Scheme 2. Saturation kinetic behavior is found for the thioester hydrolysis reaction at pH = 7.0−9.0, with maximum rate being produced above the pK_a of the Zn−OH_2 moiety (pH > 8.3).[2e] Saturation kinetic behavior was also found for the phosphate diester hydrolysis reaction promoted by **1**, which occurs with a pH optimum of 6.5. The similarity of UV−vis and EPR spectral features found upon the introduction of thioester to **1** at pH = 9.0 to the data reported for the introduction of phosphate diester to **1** at pH = 7.0[2e] suggests that for both types of substrates (thioester or phosphate diester monoanion) similar initial chemistry is occurring. On this basis, we propose that at pH = 9.0 ligand exchange occurs at the zinc center via deprotonation of the α-hydroxy group of the thioester to give an equilibrium amount of a zinc alkoxide species.[33] Formation of such an alkoxide adduct is proposed based in part on the fact that at

(32) Copik, A. J.; Waterson, S.; Swierczek, S. I.; Bennett, B; Holz, R. C. *Inorg. Chem.* **2005**, *44*, 1160–1162.

(33) While monodentate coordination of the deprotonated α-hydroxythioester is proposed based on the six-coordinate ligand environment of the Zn(II) center, formation of a chelate structure via interaction with the Zn(II) center and loss of another ligand (e.g., bridging hydroxide) cannot be ruled out. Chelating structures involving Zn(II)-coordination of a deprotonated α-hydroxyester have been reported.(a) Chamberlain, B. M.; Cheng, M.; Moore, D. R.; Ovitt, T. M.; Lobkovsky, E. B.; Coates, G. W. *J. Am. Chem. Soc.* **2001**, *123*, 3229–3238. (b) Rieth, L. R.; Moore, D. R.; Lobkovsky, E. B.; Coates, G. W. *J. Am. Chem. Soc.* **2002**, *124*, 15239–15248.

Scheme 3. Proposed Mechanism for Borate-Catalyzed α-Hydroxythioester Hydrolysis[34]



pH = 9.0 no coordination position is available on the Zn(II) center in the absence of reaction with the Zn(II)−OH moiety. Importantly, an alkoxide type structure is also proposed to form in the borate-catalyzed hydrolysis of an α-hydroxy thioester (Scheme 3), wherein a borate-substrate complex is formed followed by intramolecular attack of a boron-coordinated OH^- to form a tetrahedral transition state.[34] For the Fe(III)−Zn(II) complex, attack of the terminal Fe(III)−OH moiety on the thioester carbonyl could lead to the formation of a tetrahedral transition state from which the CD_3S^- leaving group could be stabilized via coordination to the Fe(III) center. The formation of a Fe(III)-SCD_3 unit can serve as the precursor for the observed formation of the disulfide D_3CSSCD_3. Specifically, the proposed mechanistic pathway for disulfide formation catalyzed by Fe(III) complexes suggests Fe(III)-SCD_3 ligation prior to a redox process that results in the formation of Fe(II) and $CD_3S\cdot$.[31] Coupling of two $CD_3S\cdot$ species gives the disulfide product, and oxidation of the Fe(II) to Fe(III) via reaction with O_2 could regenerate **1**. Alternatively, the Fe(III)-SCD_3 species may undergo protonation and ligand displacement to give CD_3SH ($pK_a \sim 9.7$) and **1**. On the basis of independent EPR experiments, the mandelate anion does not interact to a significant degree with the Fe(III)Zn(II) complex following thioester hydrolysis. As this is different from what is observed for **1** and mandelate anion in the absence of thioester hydrolysis products, we hypothesize that the coordination properties of the Fe(III)Zn(II) complex are affected by the formation of the Fe(III)-SCD_3 entity. However, the observed turnover, as reaction mixtures containing 0.5 equiv of **1** promote thioester cleavage in >80% yield, is consistent with regeneration of at least a portion of the Fe(III)−OH moiety either by protonation and release of CD_3SH, or oxidation of an Fe(II)Zn(II) complex by O_2.

Discussion

The glyoxalase pathway, by mediating the chemical modification of methylglyoxal, is directly involved in preventing the biosynthesis of advanced glycation end-products (AGEs), which are associated with aging and disease mechanisms.[35] Both enzymes of the glyoxalase pathway (Figure 1) are metalloenzymes, with glyoxalase I containing either Zn(II) or Ni(II) depending on the source,[10b] and glyoxalase II containing a variety of bimetallic combinations as described herein. Despite the health relevance of this pathway, very few studies involving synthetic complexes of relevance to the glyoxalase pathway have been reported.[15,21b,36]

The active site of the Fe(III)Zn(II) form of glyoxalase II has similarity to the active site of purple acid phosphatase (PAP).[2d,37] Specifically, they have in common a divalent metal binding site composed of three neutral donors, and a second metal binding site with one terminal carboxylate ligand. In both glyoxalase II and PAP the metal centers are bridged by a carboxylate and a hydroxide ligand. Because of this structural similarity, [(BPBPMP)Fe(III)Zn(II)(μ-$OAc)_2$]ClO_4 (**1**),[2f] which has been used in model studies of PAP enzymes, is an appropriate starting point for an investigation of thioester hydrolysis reactivity.

At pH values below the pK_a of the Zn−OH_2 in **1**, the observed saturation kinetic behavior (pH = 7.0, 7.5, 7.9; Figure 5) is consistent with a reaction pathway that involves coordination of the thioester carbonyl moiety to the Zn(II) center via displacement of water (Supporting Information, Scheme S1). Attack of the Fe(III)−OH on the thioester could then lead to a tetrahedral transition state and subsequent product formation. This reaction pathway is very similar to that proposed by Zang et al. for the Fe(III)Zn(II) containing form of the cytoplasmic glyoxalase II from *Arabidopsis*.[3f] In this mechanism, Zang and co-workers suggested that the $(His)_3$-ligated metal site (M1, Figure 1) activates the metal carbonyl, with the M2 site (Figure 1) being the source of a terminal hydroxide nucleophile and also assists in stabilizing the transition state and thiolate leaving group. Our results support the notion that the Fe(III) site (M2 in the Fe(III)Zn(II) form) could provide the nucleophilic hydroxide and stabilize the thiolate leaving group in the thioester hydrolysis reaction. Reaction mechanisms proposed for the dizinc form of human glyoxalase II based on the X-ray structure[13a] and computational studies[38] suggest that a bridging hydroxide can serve as the nucleophile to attack the thioester, with the M2 site serving to stabilize the transition state and the thiolate leaving group.[38]

We propose that the increased rate found above the pK_a of the Zn−OH_2 moiety in reactions involving **1** is associated with anionic coordination of the α-hydroxy thioester to the Zn(II) center. The reactivity of Zn−OH species with alcohols has been previously investigated.[39] Using mononuclear zinc complexes supported by a hydrophobic hydrotris(pyrazolyl)borate ligand, Parkin and co-workers found that more acidic alcohol hydroxyl groups produce equilibria that lie

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(37) We note that an Fe(III)Zn(II) form of metallo-β-lactamase L1 has been recently reported. (a) Hu, Z.; Gunasekera, T. S.; Spadafora, L.; Bennett, B.; Crowder, M. W. *Biochemistry* **2008**, *47*, 7947–7953. (b) Hu, Z.; Periyannan, G.; Bennett, B.; Crowder, M. W. *J. Am. Chem. Soc.* **2008**, *130*, 14207–14216.

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further toward the Zn-OR species. For example, *p*-methoxyphenol ($pK_a = 10.2$)[40] produces an equilbrium that lies toward the Zn-OR product ($K = 4.2(9)$ at 300 K), whereas aliphatic alcohols ($pK_a > 15$) produce equilibria that lie significantly toward the reactants ($K = 10^{-3}-10^{-5}$ at 300 K). The hydroxyl group of $PhCH(OH)C(O)SCD_3$ should have a lower pK_a than benzyl alcohol ($pK_a = 15$) because of the presence of the electron withdrawing thioester functionality. The formation of a Zn-OR moiety involving the deprotonated α-hydroxythioester may also be enhanced by the ability of this molecule to chelate via the ester carbonyl, which would further activate the thioester for nucleophilic attack. The K_1 values determined from fitting of the saturation curves at pH = 7.0−9.0 (Figure 5) have large errors (e.g., $K_1 = 42(24)$ at pH = 9.0) which precludes a detailed interpretation. However, the similarity of this proposed pathway to that outlined for borate-promoted thioester hydrolysis lends additional support to the proposed equilibrium Zn-OR formation.

With regard to possible Fe(III)-SCD_3 interactions, for all of the pH values examined for the thioester hydrolysis reaction promoted by **1**, the disulfide D_3CSSCD_3 is produced in the reaction mixture. This suggests the formation of a Fe(III)-SCD_3 species that can undergo redox reactivity. A decrease in the intensity of the $g = 4.3$ signal observed at pH = 9.0 (Figure 9) suggests the possible reduction of a portion of the Fe(III) to Fe(II) during the course of the single turnover reaction,[41] albeit this change in intensity may also indicate a perturbation in the electronic structure of the iron center, as the intensity of the $g = 4.3$ line is sensitive to the precise value of the axial ZFS parameter (D). We propose that the formation of the Fe(III)-SCD_3 species provides a rationale for why mandelate anion does not coordinate to the Fe(III)Zn(II) complex following thioester hydrolysis. Our control experiments indicate that free CD_3S^- anion in the reaction mixture at pH = 9.0 is quickly prontonated to produce CD_3SH. However, during the thioester hydrolysis reaction, we propose that CD_3S^- is generated in close proximity to the Fe(III) center thus enabling the formation of Fe(III)-SCD_3, which subsequently undergoes either redox to produce D_3CSSCD_3, or protonation to release CD_3SH.

Conclusions and Prospects

We have demonstrated that the Fe(III)Zn(II) complex **1** promotes thioester hydrolysis in a pH dependent reaction that achieves maximal rate above the pK_a of the $Zn-OH_2$ moiety. Equilibrium formation of a zinc alkoxide species via deprotonation of the α-hydroxythioester is proposed at pH values wherein the maximum rate of thioester hydrolysis is obtained. Products generated in the reaction include both a thiol and a disulfide, the latter of which indicates the involvement of redox activity that is likely derived from a Fe(III)-SCD_3 species.

The studies outlined herein are novel and have particular relevance to glyoxalase II enzymatic reactions, as the thioester substrate employed contains a α-hydroxy group and an aliphatic leaving group ($-SCD_3$) akin to the aliphatic glutathione-containing substrate hydrolyzed by glyoxalase II enzymes. In this regard, this model study is rare in terms of the use of a substrate that is not an activated nitro-substituted compound.[25]

This initial study sets the stage for follow up investigations using a similar approach ($-SCD_3$ labeled thioester and ^2H NMR as a monitoring tool) to examine the thioester hydrolysis reactivity of complexes having other binuclear metal combinations, or mononuclear zinc complexes. With regard to the latter, Crowder et al. recently reported that human glyoxalase II contains an Fe(II)Zn(II) center but that it is active as a mononuclear Zn(II) enzyme.[3a] The monozinc form would have $(His)_3$ ligation for the Zn(II) center (occupying the M1 site) and thus may have multiple solvent-occupied coordination positions for possible nucleophile and/or thioester substrate coordination. While activated ester hydrolysis reactivity involving mononuclear zinc complexes has been extensively investigated,[25] thioester reactions have been only minimally investigated to date.[15] Overall, our novel approach of using a deuterium-labeled aliphatic thioester and ^2H NMR to monitor hydrolysis reactions opens a window of opportunity into studies of metal complex-promoted thioester hydrolysis reactions of relevance to glyoxalase II enzymes. Understanding the mechanism of catalysis of glyoxalase II enzymes is an important goal as the glyoxalase pathway is being actively investigated with regard to its role in the clinical complications of diabetes, Alzheimer's disease, oxidative stress, and cancer.[42]

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Supporting Information Available: EPR spectrum of **1** in the presence of 1 equiv of mandelic acid; proposed mechanism for thioester hydrolysis promoted by **1** at pH values below the pK_a of the $Zn-OH_2$ moiety. This material is available free of charge via the Internet at http://pubs.acs.org.

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