Structure of a (μ-Oxo)(dihydroxo)diiron(III) Complex and Its Reactivity toward Phosphodiesters

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Functional models for nonheme diiron proteins have received much attention during the recent years. Some (μ-oxo)diiron(III) complexes were reported as catalysts for oxidations,[1] thus modeling the activity of the diiron centers of methane monooxygenase,[2] desaturase,[3] and ribonucleotide reductase.[4] No relevant functional models for purple acid phosphatase (PAP),[5] containing an $Fe^{III}Fe^{II}$ active center, have been reported so far. Recently, hydration of acetonitrile by diiron(III) complexes was performed.[6,7] The key role of a coordinated hydroxide, acting as a nucleophile, and the activation of acetonitrile by ligation to the metal center have been demonstrated. In the course of our effort to model such enzyme activities, we have undertaken a general structure/reactivity study of a series of (μ-oxo)diiron(III) species. Here, we report a study of the acid/base properties of $Fe_2O(Phen)_4(OH_2)_2(NO_3)_4$,[8] **1**. The characterization of the first (μ-oxo)(dihydroxo)diferric complex stable in aqueous medium allowed us to investigate the hydrolytic properties of iron-bound hydroxides.

Spectrophotometric titration of **1** by imidazole or NaOH in water showed the formation of a new stable species **2** upon addition of 2 equiv of base, the green solution turning red. Both charge transfer bands at 580 (ϵ = 0.16 mM·cm^{-1}) and 365 nm (10 mM·cm^{-1}), characteristic of complex **1**, shifted to 540 (shoulder, 0.10 mM·cm^{-1}) and 350 nm (8.0 mM·cm^{-1}) respectively, suggesting the presence of a stronger electron-donating ligand in the diferric unit.[9] Proton resonances between 0 and 30 ppm in the ^{1}H NMR spectrum and the absence of an EPR signal as well confirmed that the (μ-oxo)diiron(III) core was retained in solution. The transformation is reversible since **1** was quantitatively re-formed when perchloric acid or acetic acid was added to complex **2**. Complex **2**, $Fe_2O(Phen)_4(OH)_2(NO_3)_2$, could be isolated as red crystals by allowing the solution to stand for 1 day.[10]

The X ray structure of the cation of **2**·8H_2O[11] is shown in Figure 1. The iron atoms are each coordinated by four nitrogen atoms provided by two phenanthroline ligands and by the oxygen atom of the bridge [Fe−O1 = 1.788(1) Å]. The sixth coordination site is occupied by an oxygen atom from a hydroxo ligand. The Fe−OH bond lengths in **2** [1.896(5) Å] are shorter than the Fe−OH_2 bond lengths in **1** [2.027(9) and 2.021(5) Å],[8] reflecting the higher basicity of the hydroxo ligand. They are in the range of the Fe−OH bond lengths recently reported.[6,12] Each hydroxo ligand is hydrogen-bonded to a lattice water molecule.

*Thus, reaction of complex **1** with a base involves deprotonation of coordinated water molecules, exclusively, with no degradation of the dinuclear unit*. Furthermore, potentiometric titration of complex **1** demonstrated the presence of two successive titrable protons with pK_a values of 5.00(10) and 6.85(10), respectively (see Supporting Information). This showed that complex **1** behaved as a diacid in solution (Figure 2). Complex **3**, its base conjugate, is supposed to contain one water molecule on one iron and one hydroxo ligand on the adjacent iron. Spectroscopic studies of complex **1** at pH 6 confirmed that complex **3** retained the dinuclear structure and is structurally analogous to $Fe_2O(TPA)_2(H_3O_2)(ClO_4)_3$ (see Figure 2).[6]

Hydrolysis of 100 μM bis(2,4-dinitrophenyl) phosphate (BDNPP)[13] was promoted at 50 °C by 13 μM complex **1** in 50 mM buffered solution as shown by the formation of 2,4-dinitrophenolate. Initial rate constants k_{obs} were pH dependent, rising from low pH to reach a maximum at pH 6.0 and then

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(10) Crystallization was optimal from a 20 mM solution of complex **1** in the presence of 2 equiv of imidazole. Elemental analysis satisfied the following formula: $Fe_2O(OH)_2(C_{12}H_8N_2)_4(NO_3)_2$·8$H_2O$. Anal. Calc for $C_{48}H_{50}Fe_2N_{10}O_{17}$: C, 50.12; H, 4.34; N, 12.20; Fe, 9.70. Found: C, 50.14; H, 4.12; N, 12.74; Fe, 8.54. ^{1}H NMR in D_2O (δ, ppm): 17.1, 15.0 (H_β); 8.4, 7.4 (H_γ); 11.4,10.2 (H_δ). This spectrum is different from that of complex **1**.

(11) Crystal data: $[Fe_2(\mu\text{-}O)(C_{12}H_8N_2)_4(OH)_2](NO_3)_2$·8$H_2O$, elongated red prisms, 0.32 × 0.32 × 0.64 mm, monoclinic, space group *C*2/*c* (No. 15), a = 12.874(5) Å, b = 24.584(8) Å, c = 18.411(8) Å, β = 92.62(4)°, V = 5821(4) Å^3, Z = 4, μ(Mo Kα) = 0.5895 mm^{-1}. For 2955 unique, observed reflections collected at 294 K with $I > 2\sigma(I)$ and 453 refined parameters, the current discrepancy indices are R = 0.063, R_w = 0.07 (direct methods with TEXSAN software).

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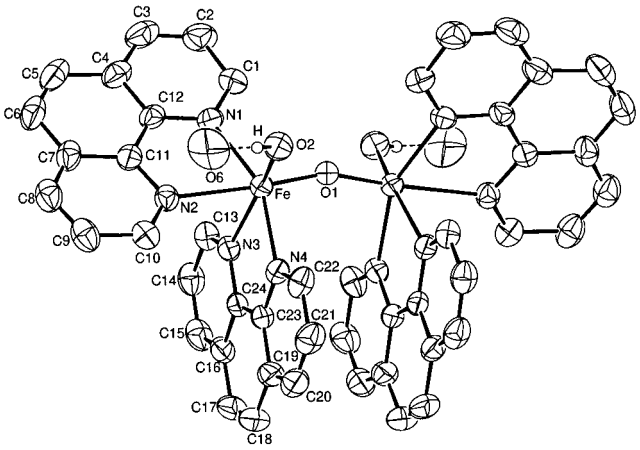

Figure 1. Core structure of complex **2** showing numbering scheme. Selected distances (Å) and angles (deg): Fe−O1, 1.788(1); Fe−O2, 1.896(5); Fe−N1, 2.157(1); Fe−N2, 2.305; Fe−N3, 2.248; Fe−N4, 2.164; O1−Fe−O2, 101.7(2); O1−Fe−N1, 95.2(2); O1−Fe−N2, 166.6(1); O1−Fe−N3, 91.0(2); O1−Fe−N4, 101.7(2); O2−Fe−N1, 98.4(2); O2−Fe−N2, 88.3(2); O2−Fe−N3, 98.4(2); O2−Fe−N4, 94.9(2); N2−Fe−N4, 86.2(2); N1−Fe−N2, 73.5(2); N1−Fe−N3, 88.3(2); N1−Fe−N4, 155.3(2); N2−Fe−N3, 80.6(2); N3−Fe−N4, 74.3(2); Fe···Fe, 3.530(2); Fe−O−Fe, 161.7. O6 corresponds to the lattice water molecule involved in the hydrogen bond with the hydroxo ligand. All hydrogen atoms and the nitrate anions, except the one bound to the hydroxo oxygen atom O2, are omitted for clarity.

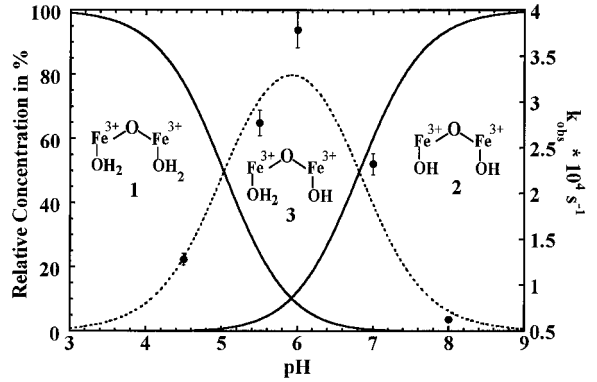

Figure 2. Relative concentrations of complexes **1** (—), **2** (—), and **3** (···) as a function of pH (calculated from potentiometric titration). k_{obs} values for 2,4-dinitrophenolate (ϵ = 19 100 cm^{-1}. M^{-1}) release during reaction of complex **1** with BDNPP as a function of pH (black circles) were determined spectrophotometrically (Supporting Information). Experimental conditions: T = 50 °C, [BDNPP] = 100 μM, [complex] = 13 μM, 50 mM buffered solution [acetate (pH 4.5), MES (pH range 5−6), and HEPES (pH range 7−8)].

falling off. The resulting bell curve remarkably matched the pH-dependent relative concentration curve of complex **3**, demonstrating that complex **3** was the active species during the hydrolytic reaction (Figure 2). At the optimal pH, the k_{obs} value was linearly correlated with the concentration of complex **1** between 17 μM (k_{obs} = 1 × 10^{-4} s^{-1}) and 80 μM (5 × 10^{-4} s^{-1}), consistent with the active species retaining its dinuclear structure during the reaction. These k_{obs} values correspond to a 100-fold enhancement of the hydrolytic reaction rate compared to that of the uncatalyzed reaction. A similar enhancement was obtained with Fe^{3+}/EDDA for hydrolysis of 4-nitrophenyl phosphate.[14] ^{31}P NMR of the final solution has revealed interactions between the complex and the reaction product,

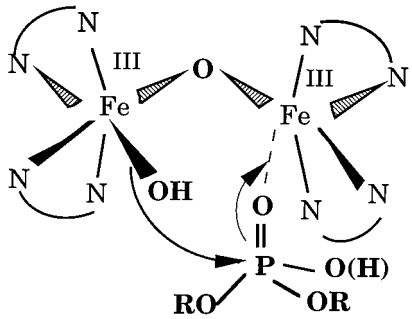

Figure 3. Proposed intermediate for hydrolysis of phosphodiesters catalyzed by complex **3**.

suggesting that the rate-limiting step is likely to be the release of the reaction product. Interestingly, the reaction was catalytic with the total conversion of 200 μM BDNPP in the presence of 65 μM complex **1** at pH 6 in 3 h whereas the uncatalyzed reaction afforded only less than 10% conversion.[15]

We propose that complex **3** is the active species as it provides both an exchangeable site for phosphodiester binding and a nucleophilic hydroxo group on the adjacent iron site. Substrate binding is supported by ^{31}P NMR and UV−visible spectroscopies and by kinetic experiments showing a saturation behavior for BDNPP (K_m = 90 μM).[16] The hydroxo ligand, then close to the bound substrate, is the best candidate for an intramolecular reaction with the phosphorus atom and the nucleophilic displacement of the phenolate anion (Figure 3). Complexes **2** and **1** are much less reactive because they lack the water-exchangeable ligand and the OH nucleophilic group, respectively.

Here we have described the first (μ-oxo)diferric unit containing two stable hydroxo ligands in water. Its acid conjugate was found to be catalytically active during hydrolysis of phosphodiesters, even though the reaction rate remains to be optimized. It thus mimicks the activity of PAP, which is optimally active under weakly acidic conditions.[5] Our results suggest that the enzyme has a similar mechanism and that PAP has selected nonheme dinuclear iron centers for their specific ability to (i) deprotonate water due to Lewis acidity of iron(III) and (ii) to provide a nucleophilic OH group adjacent to a coordinated phosphoester in a weakly acidic medium.[4,17]

Supporting Information Available: For complex **2**, tables of crystal data, structure refinement details, positional parameters, anisotropic displacement parameters, and bond lengths and angles, and a unit cell diagram, a potentiometric titration curve for **1**, text giving the experimental conditions for the potentiometric titration, and a table listing initial rates and K_{obs} values as a function of pH (10 pages). Ordering information is given on any current masthead page.

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