Inorg. Chem. **2002**, *41*, 5641–5643

New $Fe^{III}Zn^{II}$ Complex Containing a Single Terminal $Fe-O_{phenolate}$ Bond as a Structural and Functional Model for the Active Site of Red Kidney Bean Purple Acid Phosphatase

Mauricio Lanznaster, Ademir Neves,* Adailton J. Bortoluzzi, Bruno Szpoganicz, and Erineu Schwingel

Departamento de Química—LABINC—Universidade Federal de Santa Catarina, Campus Trindade, BR-88040-900 Florianópolis—SC, Brasil

Received July 22, 2002

The new heterodinuclear complex $[Fe^{III}Zn^{II}(BPBPMP)(OAc)_2]ClO_4$ (**1**) with the unsymmetrical N_5O_2 donor ligand 2-bis[{(2-pyridylmethyl)-aminomethyl}-6-{(2-hydroxybenzyl)(2-pyridylmethyl)}-aminomethyl]-4-methylphenol (H_2BPBPMP) has been synthesized and characterized by X-ray crystallography, which reveals that the complex cation has an $Fe^{III}Zn^{II}$(μ-phenoxo)-bis(μ-carboxylato) core. Solution studies of **1** indicate that a pH-induced change of the bridging acetate occurs, and the formation of an active $[(OH)Fe^{III}$-$Zn^{II}(OH_2)]$ species as a highly efficient catalyst under weakly acidic conditions for phosphate diesters hydrolysis is proposed.

Purple acid phosphatases (PAPs) constitute a class of metalloenzymes that contain a dinuclear $Fe^{III}M^{II}$ (M = Fe, Mn or Zn) in their active sites which are able to catalyze the hydrolysis of a variety of phosphoric acid esters and anhydrides within a pH range 4−7.[1] The purple color of this class of acid phosphatases results from a tyrosinate-to-Fe^{III} charge-transfer at about 560 nm.[1b] These enzymes have been isolated from animals, plants, and fungi. Recently, the crystal structures of the kidney bean (kbPAP),[2a] the rat (TRAP),[2b] the rat bone,[2c] and uteroferrin PAPs[2d] have been determined, and their similarities suggest a common mechanistic behavior for phosphate hydrolysis. In the kbPAP (resolution 2.65 Å),[2a] the Fe^{III} ion is coordinated by a tyrosine, a histidine, and an aspartate, and the zinc is coordinated by two histidines and an asparagine. The $Fe^{III}Zn^{II}$ ions are bridged by a monodentate carboxylate group of an aspartate and by a modeled μ-hydroxo group. Terminal aqua and hydroxo ligands, which were also modeled, complete the coordination spheres of the Zn^{II} and Fe^{III} ions, respectively. It is important to emphasize that the two chemically distinct environments around the Fe^{III} and Zn^{II} centers are very likely essential for the catalytic properties of this enzyme. Thus, according to the proposed mechanism,[2a] during catalysis the phosphate group is bound to the zinc ion and oriented/activated by this center in such a way that the terminal Fe^{III}-bound hydroxide can selectively attack the phosphorus atom, and the product is formed.

From the preceding information, it seems clear that the use of dinucleating N,O-donor ligands that can induce unsymmetrical metal ligation environments and vacant or labile sites for reactivity is a convenient strategy in the search for structural and functional models for PAPs. Heterodinuclear $Fe^{III}Zn^{II}$ complexes have been described as models for the active site of kbPAP.[3] However, none of them contain the $Fe^{III}-O_{phenolate}$ motif which in PAPs is responsible for the visible chromophore[1b] and most probably for the stabilization of the Fe^{III} ion, which acts as a strong Lewis acid on a bound H_2O molecule, giving rise to the formation of a nucleophilic hydroxide ion under acidic conditions.

In this communication, we report the first $Fe^{III}Zn^{II}$ complex containing a terminal $Fe^{III}-O_{phenolate}$ bond with the unsymmetrical ligand H_2BPBPMP[4a] as a structural and functional model for kbPAP. The mixed-valence $Fe^{II}Fe^{III}$,[4b] $Mn^{II}Fe^{III}$,[4a] and $Mn^{II}Mn^{III}$ [4c] containing the $M^{II}(\mu\text{-}OAc)_2M^{III}$ moiety, with the H_2BPBPMP ligand, have been already described in the literature.

Complex **1** was prepared by simultaneously adding methanolic solutions of $Zn(OAc)_2 \cdot 6H_2O$ (0.5 mmol) and $Fe(ClO_4)_3 \cdot 9H_2O$ (0.5 mmol) to a methanolic solution containing the ligand H_2BPBPMP (0.5 mmol) and NaOAc (1.0 mmol) with

* To whom correspondence should be addressed. E-mail: ademir@qmc.ufsc.br.

(1) (a)Vincent, B.; Olivier-Lilley, G. L.; Averill, B. A. *Chem. Rev.* **1990**, *90*, 1447. (b) Antanaitis, B. C.; Aisen, P. *Adv. Inorg. Biochem.* **1983**, *5*, 111. (c) Klabunde, T.; Krebs, B. *Struct. Bonding (Berlin)* **1997**, *89*, 177.

(2) (a) Klabunde, T.; Sträter, N.; Fröhlich, R.; Witzel, H.; Krebs, B. *J. Mol. Biol.* **1996**, *259*, 737. (b) Uppenberg, J.; Lindqvist, F.; Svensson, C.; Ek-Rylander, B.; Anderson, G. *J. Mol. Biol.* **1999**, *290*, 201. (c) Lindqvist, Y.; Johansson, E.; Kaija, H.; Vihko, P.; Schneider, G. *J. Mol. Biol.*, **1999**, *291*, 135. (d) Guddat, L. W.; McAlpine, A. S.; Hume, D.; Hamilton, S.; Jersey, J.; Martin, J. L. *Structure* **1999**, *7*, 757.

(3) (a) Schepers, K.; Bremer, B.; Krebs, B.; Henkel, G.; Althaus, E.; Mosel, B.; Müller-Warmuth, W. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 531. (b) Borovik, A. S.; Papaefthymiou, V.; Taylor, L. F.; Anderson, O. P.; Que, L., Jr. *J. Am. Chem. Soc.* **1989**, *111*, 6183. (c) Albedyhl, S.; Averbuch-Pouchot, M. T.; Belle, C.; Krebs, B.; Pierre, J. L.; Saint-Aman, E.; Torelli, S. *Eur. J. Inorg. Chem.* **2001**, 1457. (d) Albedyhl, S.; Schnieders, D.; Jancsó, A.; Gajda, T.; Krebs, B. *Eur. J. Inorg. Chem.* **2002**, 1400. (e) Machinaga, H.; Matsufuji, K.; Ohba, M.; Kodera, M.; Ökawa, H. *Chem. Lett.* **2002**, 716.

(4) (a) Karsten, P.; Neves, A.; Bortoluzzi, A.; Drago, V.; Lanznaster, M. *Inorg. Chem.* **2002**, *41*, 4624. (b) Neves, A.; de Brito, M. A.; Drago, V.; Griesar, K.; Haase, W. *Inorg. Chim Acta* **1995**, *237*, 131. (c) Karsten, P.; Neves, A.; Bortoluzzi, A. J.; Strähle, J.; Maichle-Mössmer, C. *Inorg. Chem. Commun.* **2002**, *5*, 434. (d) Neves, A.; Horn, A., Jr,; Lanznaster, M.; Vencato, I.; Bortoluzzi, A. J. Manuscript in preparation.

10.1021/ic025892d CCC: $22.00
Published on Web 10/02/2002

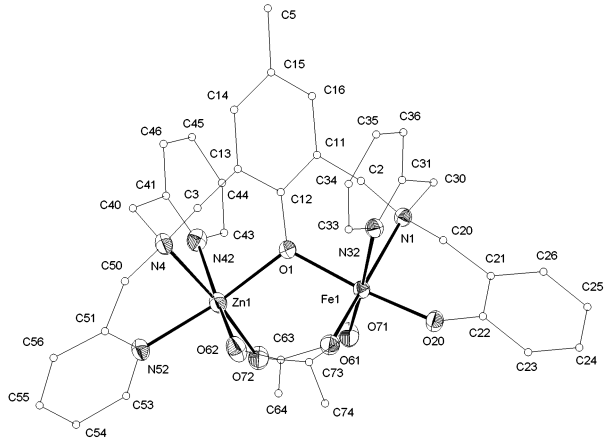

Figure 1. ORTEP plot of the cation of **1**. Selected bond lengths (Å) and angles (deg): Fe1−O1 2.006(3), Fe1−O20 1.890(3), Fe1−O61 1.967(3), Fe1−O71 2.034(3), Fe1−N1 2.214(3), Fe1−N32 2.182(3), Zn1−O1 2.105(3), Zn1−O62 2.137(3), Zn1−O72 2.030(3), Zn1−N4 2.185(4), Zn1−N42 2.190(4), Zn1−N52 2.142(3), Fe1···Zn1 3.490(9), Fe1−O1−Zn1 116.22(12).

magnetic stirring at 40 °C for 30 min to yield a dark purple solution. After leaving the solution to stand for a few days at room temperature, a crystalline solid was isolated. Yield: 300 mg, 68%. Anal. Calcd for $C_{38}H_{39.8}ClFeN_5O_{10.4}Zn$, fw = 889.62: C, 51.30; H, 4.51; N, 7.87. Found: C, 51.25; H, 4.41; N, 7.82. *CAUTION! Perchlorate salts of metal complexes are potentially explosive and therefore should be prepared in small quantities.* Recrystallization of this sample from MeOH afforded suitable crystals for X-ray structure analysis.[5] The structure and atomic numbering scheme of **1** are illustrated in Figure 1. The molecular structure of **1** shows that in the dinuclear $[Fe^{III}Zn^{II}(BPBPMP)(\mu\text{-}OAc)_2]^+$ unit the Fe^{III} and Zn^{II} ions are bridged by the phenolate oxygen O1 of $BPBPMP^{2-}$ and by two carboxylate groups of the acetate ligands. The two nitrogen atoms N1 and N32, from the tertiary amine and the pyridine group, and the O20 oxygen of the terminal phenolate complete the N_2O_4 coordination sphere of Fe1. The N_3O_3 octahedral coordination sphere of Zn1 is complemented by the three nitrogen atoms, N4, N42, and N52, of the adjacent bis(2-pyridylmethyl)amine pendant arm of the $BPBPMP^{2-}$ ligand. The average bond lengths around the two metal ions are significantly different as expected for a heterodinuclear mixed-valence species: those around Fe1 average 2.048 Å while those around Zn1 average 2.131 Å. The most obvious differences are found in the distances from the two metal ions to the bridging phenolate oxygen: Fe1−O1 2.006(3) versus Zn1−O1 2.105(3) Å.

The Fe1···Zn1 distance (3.490(9) Å) in **1** is similar to that observed in $[Fe^{III}Zn^{II}(BPMP)(\mu\text{-}OAc)_2]^{2+}$ (3.428 Å)[3b] and also compares favorably to the value of 3.33 Å measured in kbPAP in the presence of phosphate.[2a] The terminal $Fe^{III}-O_{phenolate}$ bond length (1.890(3) Å) in **1** is also in the same range of those found in the corresponding dinuclear $[Mn^{II}Mn^{III}]$,[4c] $[Mn^{II}Fe^{III}]$,[4a] and $[Fe^{II}Fe^{III}]$[4d] complexes but is significantly smaller compared to $Fe^{III}-O_{tyr}$ (2.05 Å) in the native kbPAP enzyme.[2a]

(5) $C_{38}H_{39.8}ClFeN_5O_{10.4}Zn$, fw = 889.62, monoclinic, $P2_1/c$, a = 19.612(4) Å, b = 10.795(2) Å, c = 20.807(4) Å, β = 116.67(3)°, V = 3936.4(13) Å^3, Z = 4. $F(000)$ = 1836, μ = 1.110 mm^{-1}, wavelength = 0.71073 Å, T = 293(2) K, data/restraints/params 6970/100/514. GOF = 1.036, final R indices, R1 ($I > 2\sigma I$) = 0.0439, wR2 = 0.1272. Largest diff peak and hole, 0.842 and −1.069 e·Å^{-3}.

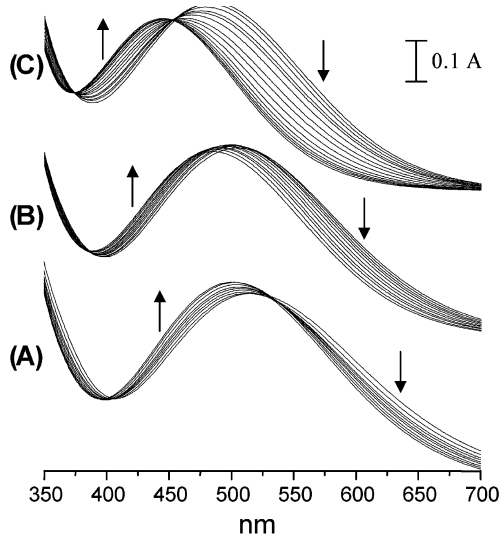

Figure 2. UV−vis spectra as a function of pH value: **(A)** 4.3−5.6, **(B)** 5.6−7.2, **(C)** 7.2−10. Addition of 2.0 mol L^{-1} NaOH in an ethanol/water 70:30 solution of **1** (2.0×10^{-4} mol L^{-1}) with I = 0.1 mol L^{-1} (KCl).

The cyclic voltammogram (Figure S1) of **1** in MeCN/0.1 mol L^{-1} n-But_4NPF_6 with scan rate of 150 mV s^{-1} reveals one reversible redox process at −910 mV versus Fc/Fc^+ which can be attributed to the one-electron transfer $Fe^{III}Zn^{II}/Fe^{II}Zn^{II}$. This value is in close agreement with the $Fe^{III}M^{II}/Fe^{II}M^{II}$ redox couple observed for the Fe_2[4b] (−890 mV) and FeMn[4a] (−870 mV) homologues, respectively.

The electronic spectrum of **1** in MeCN solution consists of a broad band centered at 540 nm (ϵ = 3700 L mol^{-1} cm^{-1}), which can be attributed to the terminal phenolate-to-Fe^{III} charge-transfer transition.[6] The PAP in red kidney bean shows a tyrosine-to-Fe^{III} charge-transfer transition at 544 nm (ϵ = 3200 L mol^{-1} cm^{-1}).[1]

Solution studies of **1** (water/ethanol, 30:70) demonstrate that the λ_{max} position of the LMCT process is strongly dependent on the pH of the solution. In the pH ranges 4.3−5.6 (Figure 2A), 5.6−7.2 (Figure 2B), and 7.2−10 (Figure 2C), strict isosbestic points can be observed indicating the presence of only two species in equilibrium, during each individual spectral change. From the distribution curves obtained by fitting the aborbance values versus pH (Figure 3), we propose the following equilibria:

The dissociation of the bridging carboxylate groups of **1** in aqueous medium is quite in line with its well documented

$$[(OH_2)Fe(OH_2)Zn(OH_2)] \leftrightarrows [(OH)Fe(OH_2)Zn(OH_2)] + H^+ \quad (\mathbf{2})$$

$$pK_1 = 4.86$$

$$[(OH)Fe(OH_2)Zn(OH_2)] \leftrightarrows [(OH)Fe(\mu\text{-}OH)Zn(OH_2)] + H^+ \quad (\mathbf{3})$$

$$pK_2 = 6.00$$

$$[(OH)Fe(\mu\text{-}OH)Zn(OH_2)] \leftrightarrows [(OH)Fe(\mu\text{-}OH)Zn(OH)] + H^+ \quad (\mathbf{4})$$

$$pK_3 = 7.22$$

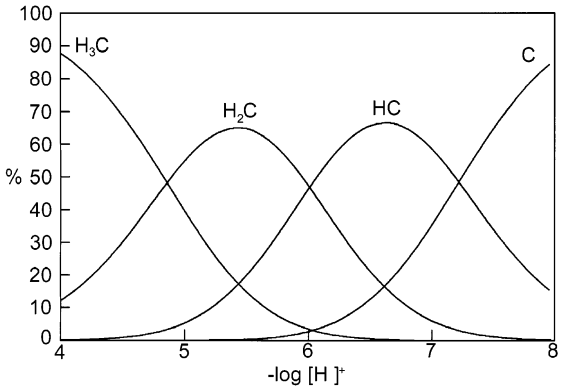

Figure 3. Species distribution curves of **1** as a function of pH. H_3C is the completely protonated species, C is the completely deprotonated species, and H_2C and HC are the intermediate species.

lability,[6] and the **2**/**3** species are most probably the active species toward the cleavage of phosphate diesters under the same pH conditions (vide infra).

Kinetic experiments to examine the phosphatase-like activity have been carried out with the activated substrate 2,4-bis(dinitrophenyl)phosphate (BDNPP)[7] by following spectrophotometrically the absorbance increase of the liberated 2,4-dinitrophenolate anion (λ_{max} = 400 nm with ϵ = 19100 L mol^{-1} cm^{-1}), under conditions of excess substrate. To take into account the spontaneous hydrolysis of the substrate, correction was accomplished by direct difference using a reference cell under identical conditions without adding the catalyst.

The pH dependence of the catalytic activity between 3.63 and 10.00 shows a bell-shaped profile (Figure 4**)** with an optimum at about pH 6.5. Sigmoidal fits of the curve reveal p*K* values at 4.80 and 7.50, which are in good agreement with the values (4.86 and 7.22) obtained from the UV−vis titration experiments. These results strongly suggest that the monohydroxo form of the complex is the active species for cleaving BDNPP, because, as proposed previously, it provides a labile Zn−OH_2 bond for phosphodiester binding and an Fe^{III} bound hydroxide group for nucleophilic attack on the phosphorus atom. The decrease in reactivity at pH > 6.5 most probably arises because of the presence of the fully deprotonated form **4** in which the leaving tendency of the OH^- ion from the Zn−OH group becomes lower, even though a more concentrated OH^- solution can increase the spontaneous hydrolysis.

The determination of the initial rates at pH 6.1 as a function of substrate concentration reveals saturation kinetics

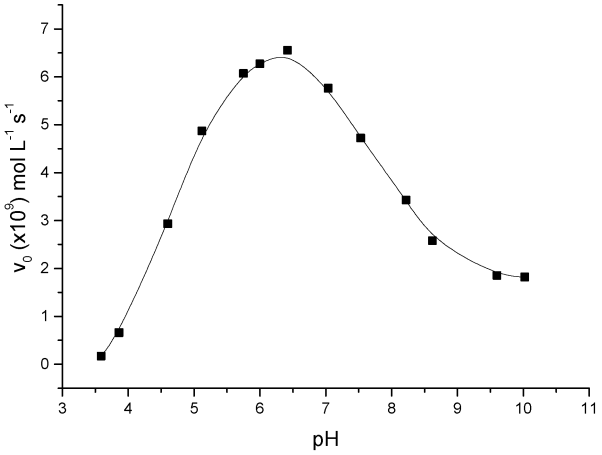

Figure 4. Dependence of the reaction rate on the pH, under the following conditions: water/acetonitrile 50% solution, [**1**] = 4.0 × 10^{-5} mol L^{-1}, [BDNPP] = 2.0 × 10^{-3} mol L^{-1}, [buffer] = 5.0 × 10^{-2} mol L^{-1} (buffer = MES, HEPES, and CHES), I = 0.1 mol L^{-1} ($LiClO_4$).

with Michaelis−Menten-like behavior (Figure S2). A linearization after Lineweaver−Burke (Figure S3) gives the following values: K_m = 8.10 × 10^{-3} mol L^{-1}, V_{max} = 2.92 × 10^{-8} mol L^{-1} s^{-1}, and the catalytic constant $k_{cat} = V_{max}/[\mathbf{1}]$ = 7.31 × 10^{-4} s^{-1}. Under these conditions, **1** shows a 4060 times acceleration rate compared to the uncatalyzed hydrolysis (k = 1.8 × 10^{-7} s^{-1}). To the best of our knowledge, this represents the best model complex so far which hydrolyzes BDNPP.[8]

In summary, we have described the first $Fe^{III}Zn^{II}$ complex which contains only one terminal $Fe^{III}-O_{phenolate}$ bond as a structural model for the active site of kbPAP. Furthermore, it was demonstrated that in aqueous solution the [(OH)FeZn-(OH_2)] species is a highly efficient catalyst in the hydrolysis of phosphate diester, probably through a mechanism similar to that proposed for the enzyme kbPAP. Further studies involving the syntheses of homo- and heterodinuclear $M^{III}M^{II}$ complexes with the unsymmetric H_2BPBPMP ligand and their interactions with phosphate diester bonds in model substrates and DNA are in progress and will be the subject of future reports.

Acknowledgment. Financial support was received from CNPq and PRONEX.

Supporting Information Available: X-ray crystallographic details for complex **1** in CIF format. Figures S1, S2, and S3 showing the cyclic voltammogram and kinetics details, respectively, in PDF format. This material is available free of charge via the Internet at http://pubs.acs.org.

IC025892D

(6) Lambert, E.; Chabut, B.; Chardon-Noblat, S.; Deronzier, A.; Chottard, G.; Bousseksou, A.; Tuchagues, J. P.; Laugier, J.; Bardet, M.; Latour, J. M. *J. Am. Chem. Soc*. **1997**, *119*, 9424.
(7) Bunton, C. A.; Farber, S. J. *J. Org. Chem.* **1969**, *34*, 767.
(8) Duboc-Toia, C.; Ménage, S.; Vincent, J. M.; Averbuch-Pouchot, M. T.; Fontecave, M. *Inorg. Chem.* **1997**, *36*, 6148.