# Structural, spectroscopic, and magnetic properties of a diphenolate-bridged Fe$^{III}$Ni$^{II}$ complex showing excellent phosphodiester cleavage activity

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A B S T R A C T

To mimic the phosphate ester hydrolysis behavior of purple acid phosphatases the heterobimetallic complex [(BNPP)Fe$^{III}$L(μ-BNPP)Ni$^{II}$(H$_2$O)](ClO$_4$) (**1**) has been synthesized from the precursor complexes [Fe$^{III}$(LH$_2$)(H$_2$O)$_2$](ClO$_4$)$_3$·3H$_2$O and [Fe$^{III}$(LH$_2$)(H$_2$O)Cl](ClO$_4$)$_2$·2H$_2$O. In these compounds, L$^{2-}$ is the anion of the tetraiminodiphenol macrocyclic ligand (H$_2$L), while LH$_2$ is the zwitterionic form in which the phenolic protons are shifted to the two metal-uncoordinated imine nitrogens, and BNPP is bis(4-nitrophenyl)phosphate. The X-ray crystal structure of compound **1** has been determined. The structure of **1** comprises of two edge-shared distorted octahedrons whose metal centers are bridged by two equatorial phenolate oxygens and two axially disposed oxygens of a BNPP ligand. The internuclear Fe···Ni distance is 3.083 Å. The high-spin iron(III) and nickel(II) in **1** are antiferromagnetically coupled ($J = -7.1$ cm$^{-1}$; $H = -2J\ S_1·S_2$) with $S = 3/2$ spin ground state. The phosphodiesterase activity of **1** has been studied in 70:30 H$_2$O–(CH$_3$)$_2$SO medium with NaBNPP as the substrate. The reaction rates have been measured by varying pH (3–10), temperature (25–50 °C), and with different concentrations of the substrate and complex at a fixed pH and temperature. Treatment of the rate data, obtained at pH 6.0 and at 35 °C, by the Michaelis–Menten approach have provided the following parameters: $K_M = 3.6 \times 10^{-4}$ M, $V_{max} = 1.83 \times 10^{-7}$ M s$^{-1}$, $k_{cat} = 9.15 \times 10^{-3}$ s$^{-1}$. As compared to the uncatalyzed hydrolysis rate of BNPP, the $k_{cat}$ value is $8.3 \times 10^8$ times higher, showing that **1** behaves as an excellent model for phosphate ester hydrolysis.

## 1. Introduction

The purple acid phosphatases (PAPs) represent a type of dinuclear metallohydrolases that contain the heterovalent Fe$^{III}$M$^{II}$ (M = Fe, Zn or Mn) active site and catalyze hydrolysis of phosphate esters at pH 4–7 [1–4]. The identity of divalent metal ion in the active site of PAPs depends upon the source (plant, animal or bacteria) from which the enzyme is isolated. For instance, the red kidney bean PAP contains the Fe$^{III}$Zn$^{II}$ active site, while the PAP isolated from another plant source sweet potato has the Fe$^{III}$Mn$^{II}$ site. On the other hand, mammalian PAPs isolated from bovine spleen, pig liver, procine uterus fluid (uteroferrin) etc. contain the Fe$^{III}$Fe$^{II}$ site. Despite the difference of the active site metal contents, the metal-coordinated protein residues of PAPs are conserved and their enzymatic activities also remain largely similar. Further, zinc(II)- substituted mammalian PAPs and iron(II)- substituted kidney bean PAP are kinetically indistinguishable [5,6]. The X-ray structures of the enzymatically active kidney bean PAP [7] and inactive PO$_4$/XO$_4$ (X = As, S, Mo) – ligated derivatives of PAPs [8–12] of different origins have been reported. The crystal structure of the kidney bean PAP has been resolved [7] by including three exogenous OH$^-$/H$_2$O ligands in the active site. The iron(III) and zinc(II) sites, which being separated by 3.2–3.3 Å, are bridged by a hydroxide ion as well as a carboxylate oxygen atom of Asp$_{164}$. A second hydroxide ligand is thought to be terminally bound to iron(III) center, while the zinc(II) is coordinated by a water molecule.

The steric environments of the active sites of PAPs have been addressed by various spectroscopic and magnetic measurements [13–15] to ascertain the exogenous ligands present. These studies have confirmed the presence of a μ-OH linkage and a water molecule coordinated to the divalent metal site of mammalian PAPs and the kidney bean PAP, although magnetization measurement of the sweet potato PAP seems to indicate the presence of a μ-oxo bridge between the iron(III) and manganese(II) [16]. Notwithstanding the evidences obtained from the X-ray structural studies [7,11], the ENDOR measurement carried out for a mammalian PAP has failed

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to reveal the presence of the terminal hydroxide ligand [17]. The hydrolysis of phosphate esters by PAPs is accompanied by inversion of stereochemistry at the phosphorous atom [18]. The nucleophile is presumably added to the phosphorous atom on the opposite side of the leaving group and thus forming a trigonal bipyramid intermediate. However, the mode of substrate binding and the identity of the attacking nucleophile are still debatable issue [1,3]. Two alternative reaction mechanisms have been proposed. (i) The substrate binds to the $M^{II}$ site in a monodentate form and the terminal hydroxide ligand of the $Fe^{III}$ site acts as the nucleophile [7,19,20]. (ii) The substrate gets attached to the $M^{II}Fe^{III}$ site as a μ-1,3 bridge and is attacked by the sterically-oriented metal-bridged hydroxide [12,16,17,21].

In recent years, a number of $Fe^{III}M^{II}$ complexes have been synthesized to replicate the structural, spectroscopic, magnetic, and redox features of PAPs and to get an insight of the mechanism of hydrolysis of phosphate ester [22–27]. We report here the structure and properties of the iron(III)-nickel(II) complex [(BNPP)$Fe^{III}$L(μ-BNPP)$Ni^{II}$(H$_2$O)](ClO$_4$), which in polar solvent dissociates to [(H$_2$O)$Fe^{III}$L(μ-BNPP)$Ni^{II}$(H$_2$O)]$^{2+}$ and catalyzes hydrolysis of bis(4-nitrophenyl)phosphate (BNPP$^-$) at pH 6.0.

## 2. Experimental

### 2.1. Materials

Reagent grade chemicals obtained from commercial sources were used as received. Solvents were purified and dried according to standard methods [28]. A Universal buffer system introduced by Ellis [29] was used for this study in the pH range 3–10. Buffers of appropriate pH values were prepared by mixing together a solution containing sodium carbonate, 2-amino-2-methyl propan-1,3-diol, sodium dihydrogen phosphate, and citric acid each of which is 0.4 M with 0.1 M HCl or NaOH in different volume ratio. The precursor complexes [$Fe^{III}$(LH$_2$)(H$_2$O)$_2$](ClO$_4$)$_3$·3H$_2$O and [$Fe^{III}$(LH$_2$)(H$_2$O)Cl](ClO$_4$)$_2$·2H$_2$O have been prepared by following the methods already described by us [25b].

### 2.2. Physical measurements

Elemental (C, H, and N) analyses were performed on a Perkin–Elmer 2400 II elemental analyzer. The electrospray ionization mass spectra (ESI-MS) were measured on a Micromass Qtof YA 263 mass spectrometer. IR spectra were recorded using KBr disks on a Shimadzu FTIR 8400S spectrophotometer. The electronic absorption spectra and kinetic measurements over the temperature range 25–50 °C were performed on a Perkin–Elmer 950 UV–Vis–NIR spectrophotometer equipped with thermostated cell and Peltier temperature controller assembly. The $^1$H NMR and $^{31}$P NMR spectra were recorded on a Bruker Avance III 500 MHz NMR spectrometer. Variable temperature (2–300 K) magnetic susceptibility measurements were carried out on a MPMS Quantum Design SQUID magnetometer. Diamagnetic corrections were made using Pascal's constants [30]. The cyclic voltammetric (CV) and square wave voltammetric (SWV) measurements were carried out using a BAS 100B electrochemical analyzer in the same way as reported earlier [31]. EPR spectrum for a frozen acetonitrile solution (77 K) of 1 ($5 \times 10^{-4}$ M) was recorded on a JEOL JES-FA200 ESR spectrometer.

### 2.3. Preparation of metal complex

*Caution:* Perchlorate salts used in this study are potentially explosive and therefore should be handled with care.

#### 2.3.1. [(BNPP)FeL(μ-BNPP)Ni(H$_2$O)](ClO$_4$) (1)

To an acetonitrile solution of [$Fe^{III}$(LH$_2$)(H$_2$O)$_2$](ClO$_4$)$_3$·3H$_2$O (0.421 g, 0.5 mmol) were added solid Ni(ClO$_4$)$_2$·6H$_2$O (0.18 g, 0.5 mmol), a methanol solution (10 mL) of bis(4-nitrophenyl)phosphate (HBNPP, 0.34 g, 1 mmol), and triethylamine (0.20 g, 2 mmol). On standing at room temperature for overnight period, bright red crystals of 1 were deposited. The product was filtered, washed with ethanol, and recrystallized from acetonitrile-methanol (1:1). Yield 0.54 g (82%).

Alternatively, compound 1 can be obtained by slowly adding to a stirred acetonitrile solution (30 mL) of [$Fe^{III}$(LH$_2$)(H$_2$O)Cl](ClO$_4$)$_2$·2H$_2$O (0.37 g, 0.5 mmol) and Ni(ClO$_4$)$_2$·6H$_2$O (0.18 g, 0.5 mmol), an aqueous solution (15 mL) of NaBNPP (0.90 g, 2.5 mmol). After 1 h, the product that precipitated was filtered, washed with water and ethanol, and recrystallized as stated above. Yield 0.59 g (90%). *Anal.* Calc. for C$_{48}$H$_{44}$ClFeN$_8$NiO$_{23}$P$_2$: C, 43.87; H, 3.35; N, 8.53. Found: C, 43.76; H, 3.39; N, 8.58%. ESI-MS (positive) in acetonitrile: m/z 1193.84 [FeLNi(BNPP)$_2$]$^+$ (10%); 871.91 [FeNiL(BNPP)(OH)]$^+$ (100%); 428.46 [FeLNi(BNPP)]$^{2+}$ (50%). FTIR (KBr, $v$/cm$^{-1}$): 3458(w, br), 1641(m), 1612(w), 1589(m), 1564(m), 1519(s), 1489(w), 1439(w), 1413(w), 1346(s), 1317(w), 1242(w), 1211(m), 1108(s), 1080(s), 918(m), 862(w), 815(w), 764(w), 625(m), 523(w), 430(sh). $^1$H NMR (500 MHz, (CD$_3$)$_2$SO): δ 8.15 (s, 4H, Ar–H); 7.34 (s, 4H, Ar–H). $^{31}$P NMR (202 MHz, (CD$_3$)$_2$SO): δ 27.0 (coordinated BNPP); −5.35 (PPh$_3$); −11.9 (free BNPP). UV–Vis–NIR ($\lambda_{max}$/nm ($\varepsilon$/M$^{-1}$ cm$^{-1}$)) in acetonitrile: 1020 (8), 535 (2350), 430 (sh), 350 (12750).

### 2.4. X-ray structure determinations

Crystal suitable for structure determinations of [(BNPP)FeL(μ-BNPP)Ni(H$_2$O)](ClO$_4$) (1) was mounted on glass fibers and coated with perfluoropolyether oil. Intensity data were collected at 150 K on a Bruker-AXS SMART APEX II diffractometer equipped with a CCD detector with graphite-monochromated MoKα radiation ($\lambda$ = 0.71073 Å). The data were processed with SAINT [32], and absorption corrections were made with SADABS [32]. The structure was solved by Direct and Fourier methods and refined by full-matrix least-squares based on $F^2$ using WINGX software of SHELXTL [33] and SHELX-97 [34]. The non-hydrogen atoms were refined anisotropically, while the hydrogen atoms were placed at geometrically calculated position with fixed isotropic thermal parameters. Wherever the positions of the hydrogen atoms could be detected equivocally, they were referred to as 'seen' in the text. The crystallographic data of compound 1 are given in Table 1.

### 2.5. Kinetic measurements

The hydrolytic cleavage of BNPP catalyzed by complex 1 was studied in H$_2$O–(CH$_3$)$_2$SO (70:30 v/v) solvent mixture. The reaction rate was followed by monitoring the increase in absorbance of the hydrolyzed product 4-nitrophenolate at 400 nm ($\varepsilon$ = 17000 M$^{-1}$ cm$^{-1}$). The p$K_a$ value of 4-nitrophenol (6.61) [35] was used to calculate the concentration of the liberated phenolate at the appropriate pH. Rate constants were obtained by the initial rate method and measurements were made over the pH range 3–10 and the temperature ranging from 25 to 50 °C. The pH of the solutions were adjusted with a fixed volume of the Ellis buffer of desired pH and after each kinetic run the pH of the solution was measured by a pH meter. Typically, the solution used for kinetic run was $2 \times 10^{-5}$ M in complex, $2 \times 10^{-4}$ M in NaBNPP, and $5 \times 10^{-2}$ M in buffer, and the ionic strength was maintained at 0.1 M with NaClO$_4$. To compensate the effect of spontaneous hydrolysis of BNPP, a reference cell identical to the sample cell except that it did not contain 1 was used. Reactions were monitored to about 5% cleavage of BNPP.