Inorg. Chem. **1996,** *35,* 7472−7473

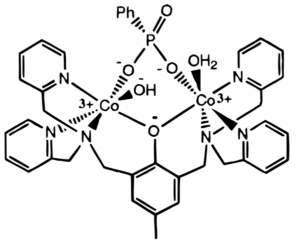
Structure and Reactivity of a Dinuclear Cobalt(III) Complex with a Bridging Phosphate Monoester

Jin Seog Seo, Nack-Do Sung,[†] Rosemary C. Hynes, and Jik Chin*

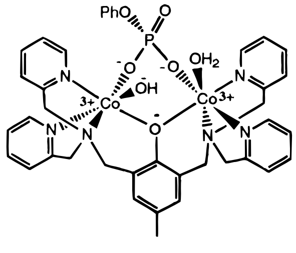
Department of Chemistry, McGill University, Montreal, Canada H3A 2K6

Received April 9, 1996

In nature there are many enzymes that hydrolyze phosphate monoesters that are activated by two or more metal ions. They include alkaline phosphatase,[1] purple acid phosphatase,[2] inositol monophosphatase,[3] and D-fructose 1,6-biphosphate 1-phosphatase.[4] The active sites of protein serine and threonine phosphatases may also consist of dinuclear metal centers.[5] Over the years there has been considerable interest in understanding the mechanism of hydrolysis of phosphate monoesters[6,7] and diesters[8,9] and the role of simple mononuclear[6,8] and dinuclear[7,9] metal complexes in the hydrolysis reaction.[10] Since hydrolyses of phosphate mono- and diesters often take place by different mechanisms,[11] it is important to study the mechanisms of both reactions. Here we report the structure of a phenylphosphonate bridging a dinuclear Co(III) complex (**1**) and the reactivity of a phenyl phosphate bridging the same dinuclear Co(III) complex (**2**).



1



2

The perchlorate salt of **1** was synthesized by bubbling air into a methanolic solution containing equimolar amounts of Co(ClO$_4$)$_2$, disodium phenylphosphonate, and 2,6-bis[(bis(2-pyridylmethyl)amino)methyl]-4-methylphenol (Hbpmp). The crude product, [Co$_2$(bpmp)(O$_3$P(Ph))(OH$_2$)(OH)](ClO$_4$)$_2$ (**1**), was recrystallized from a solution of acetonitrile and water, yielding purple cubic crystals. The structure of **1** (Figure 1) reveals that the two cobalt centers are different.[12] In one of the cobalt centers, the tertiary alkylamine is coordinated *trans* to the coordinated phosphonate oxygen while in the other cobalt center the tertiary alkylamine is coordinated *trans* to the coordinated water or hydroxide molecule. ^{31}P NMR spectrum of the dinuclear Co(III) complex shows a sharp singlet (**1** in D$_2$O (trimethyl phosphate) δ 33 ppm), indicating that there is only one diastereomer in the sample. Replacing phenylphosphonate in the above synthesis with phenyl phosphate gave [Co$_2$(bpmp)(O$_3$P(OPh)(OH$_2$)(OH)](ClO$_4$)$_2$ (**2**).[13] The ^{31}P NMR signal for **2** (δ 10 ppm relative to trimethyl phosphate) in D$_2$O is about 12 ppm downfield shifted relative to the corresponding signal for the unbound phosphate, which is characteristic of a phosphate bridging two Co(III) centers.[13]

Hydrolysis of the bridging phosphate monoester in **2** was monitored by ^{1}H and ^{31}P NMR (Varian XL 300). In an aqueous solution of **2** (1 mM) at 25 °C and pD 6.8 (10 mM 1,4-piperazinebis(ethanesulfonic acid) (PIPES)), the ^{31}P NMR signal due to **2** gradually decreases with a concomitant increase in the signal at 28 ppm (Supporting Information). Over the same time period, the ^{1}H NMR signal due to the methyl group in **2-H** is converted to that for **3**. Production of phenol from hydrolysis of the phosphate monoester in **2** (1 mM) at 25 °C and pH 6−13 was also monitored by HPLC (Supporting Information).[14] The pH−rate profile for the hydrolysis reaction

---

[†] Permanent address: Department of Agricultural Chemistry, Chung-Nam National University, Taejeon 305-764, Korea.

(1) Kim, E. E.; Wyckoff, H. W. *J. Mol. Biol.* **1991**, *218*, 449.

(2) Klabunde, K.; Sträter, N.; Tucker, P.; Witzel, H.; Krebs, B. *Science* **1995**, *268*, 1489.

(3) Pollack, S. J.; Atack, J. R.; Knowles, M. R.; McAllister, G.; Ragan, C. I.; Baker, R.; Fletcher, S. R.; Iversen, L. L.; Broughton, H. B. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 5766.

(4) Zhang, Y.; Liang, J.-Y.; Huang, S.; Ke, H.; Lipscomb, W. *Biochemistry* **1993**, *32*, 1844.

(5) Vincent, J. B.; Averill, B. A. *FEBS Lett.* **1990**, *263*, 265.

(6) (a) Fife, T. H.; Pujari, M. P. *J. Am. Chem. Soc.* **1988**, *110*, 7790. (b) Jones, D. R.; Lindoy, L. F.; Sargeson, A. M. *J. Am. Chem. Soc.* **1983**, *105*, 7327. (c) Harrowfield, J. M.; Jones, D. R.; Lindoy, L. F.; Sargeson, A. M. *J. Am. Chem. Soc.* **1980**, *102*, 7733.

(7) (a) Hendry, P.; Sargeson, A. M. *Prog. Inorg. Chem.* **1990**, *38*, 201. (b) Jones, D. R.; Lindoy, L. F.; Sargeson, A. M. *J. Am. Chem. Soc.* **1984**, *106*, 7807. (c) Vance, D. H.; Czarnik, A. W. *J. Am. Chem. Soc.* **1993**, *115*, 12165. (d) Chin, J.; Banaszczyk, M. *J. Am. Chem. Soc.* **1989**, *111*, 4103.

(8) (a) Kim, J. H.; Chin, J. *J. Am. Chem. Soc.* **1992**, *114*, 9792. (b) Linkletter, B.; Chin, J. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 472. (c) Hendry, P.; Sargeson, A. M. *Inorg. Chem.* **1990**, *29*, 92. (d) Magda, D.; Miller, R. A.; Sessler, J. L.; Iverson, B. *J. Am. Chem. Soc.* **1994**, *116*, 7439. (e) Hall, J.; Hüsken, D.; Pieles, U.; Moser, H. E.; Häner, R. *Chem. Biol.* **1994**, *1*, 185. (f) Bashkin, J. K.; Frolova, E. I.; Sampath, U. S. *J. Am. Chem. Soc.* **1994**, *116*, 5981. (g) Matsumura, K.; Endo, M.; Komiyama, M. *J. Chem. Soc., Chem. Commun.* **1994**, 2019. (h) Morrow, J. R.; Buttrey, L. A.; Shelton, V. M.; Berback, K. A. *J. Am. Chem. Soc.* **1992**, *114*, 1903. (i) Breslow, R.; Huang, D. L. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 4080. (j) Schneider, H.-J.; Rammo, J.; Hettich, R. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1716.

(9) (a) Wall, M.; Hynes, R. C.; Chin, J. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1633. (b) Young, M. J.; Chin, J. *J. Am. Chem. Soc.* **1995**, *117*, 10577. (c) Wahnon, D.; Lebuis, A.-M.; Chin, J. *Angew. Chem., Int. Ed. Engl.* **1995**, 2412. (d) Takasaki, B. K.; Chin, J. *J. Am. Chem. Soc.* **1995**, *117*, 8582. (e) Williams, N.; Chin, J. *J. Chem. Soc., Chem. Commun.* **1996**, 131. (f) Tsubouchi, A.; Bruice, T. C. *J. Am. Chem. Soc.* **1994**, *116*, 11614. (g) Breslow, R.; Zhang, B. *J. Am. Chem. Soc.* **1994**, *116*, 7893. (h) Yashiro, M.; Ishikubo, A.; Komiyama, M. *J. Chem. Soc., Chem. Commun.* **1995**, 1793.

(10) (a) Karlin, K. *Science* **1993**, *261*, 701. (b) Göbel, M. W. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1141.

(11) Guthrie, J. P. *J. Am. Chem. Soc.* **1977**, *99*, 3991.

(12) Crystal structural data for **1**(ClO$_4$)$_2$·3H$_2$O: Co$_2$C$_{39}$H$_{41}$N$_6$O$_{17}$PCl$_2$, monoclinic, space group $P2_1/c$, $a = 11.6403(15)$ Å, $b = 19.5420(18)$ Å, $c = 19.7515(18)$ Å, $V = 4474.5(8)$ Å^3, $Z = 4$; 4895 measured reflections, 2976 with $I > 2.5\sigma(I)$, 647 parameters, $R = 0.049$ and $R_w = 0.053$.

(13) (a) Connolly, J.; Kim, J. H.; Banaszczyk, M.; Drouin, M.; Chin, J. *Inorg. Chem.* **1995**, *34*, 1094. (b) Haight, G. P., Jr. *Coord. Chem. Rev.* **1987**, *79*, 293. Anal. Cald for C$_{39}$H$_{41}$N$_6$O$_{15}$PCl$_2$Co$_2$ (**2**(ClO$_4$)$_2$): C, 44.46; H, 3.92; N, 7.98; Cl, 6.73; Co, 11.19. Found: C, 44.22; H, 3.92; N, 7.90; Cl, 6.54; Co, 11.50.

(14) At appropriate time intervals, reactions were quenched by pipetting aliquots of the reaction mixture into equal volumes of 1 M HClO$_4$ prior to HPLC analysis. The production of phenol was monitored by HP1090 HPLC using a 100 × 2.1 mM C-18 column running 0.5 mL/min of 0.2 M NH$_4$H$_2$PO$_4$ buffer (pH 5.5) for the first 5 min followed by a linear gradient to 50% of a 60/40 methanol/water solution over the next 10 min. Buffers used for the pH−rate profile were MES (4-morpholineethanesulfonic acid), HEPES (*N*-(2-hydroxyethyl)piperazine-*N'*-ethanesulfonic acid), EPPS (4-(2-hydroxyethyl)-1-piperazinepropanesulfonic acid), CHES (2-(cyclohexylamino)ethanesulfonic acid), and CAPS (3-(cyclohexylamino)-1-propanesulfonic acid). No buffer catalysis was observed (at 10−50 mM). The rate constants were reproducible to within 3% error. At pH >10, production of phenoxide can also be monitored spectrophotometrically at 238 nm.

S0020-1669(96)00392-8 CCC: $12.00

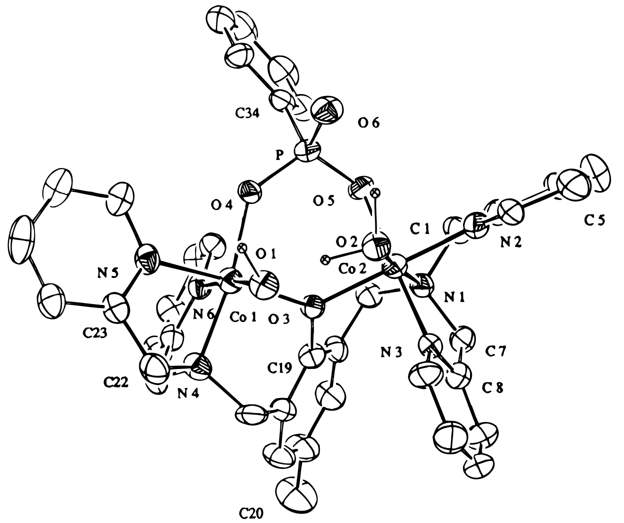
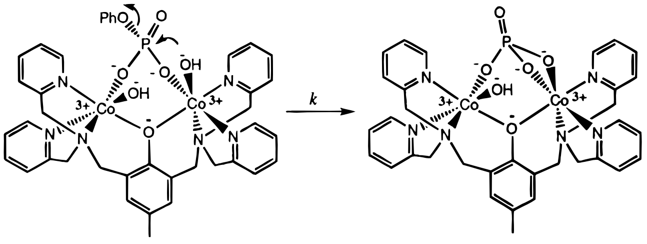


Figure 1. X-ray structure of **1** (ORTEP representation; ellipsoids at the 30% probability level). Selected distances (Å) and angles (deg): Co1−Co2 3.529(2), Co1−N4 1.966(6), Co1−N5 1.913(6), Co1−N6 1.947(6), Co1−O1 1.903(5), Co1−O3 1.959(5), Co1−O4 1.890(5), Co2−N1 1.984(6), Co2−N2 1.916(7), Co2−N3 1.926(6), Co2−O2 1.885(5), Co2−O3 1.968(5), Co2−O5 1.904(5), O4−P−O5 112.2(3), Co1−O4−P 138.8(3), Co2−O5−P 123.4(3), O1−Co1−O4 93.69(23), N4−Co1−N6 85.4(3), O2−Co2−O5 94.49(23), N1−Co2−N3 85.7(3).

Scheme 1



2-H **3**

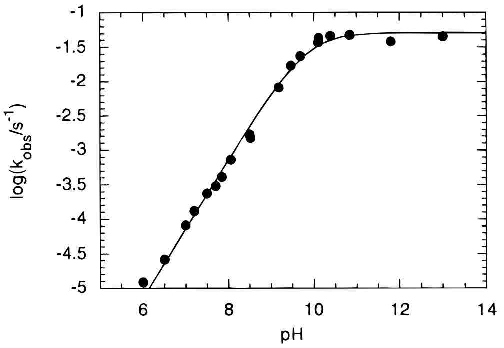
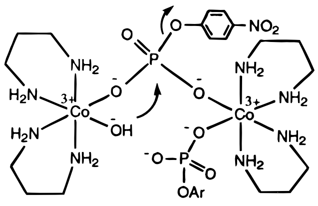


Figure 2. pH−rate profile for conversion of **2** to **3** at 25 °C.

is shown in Figure 2. The rate of the hydrolysis reaction increases with increase in pH but levels off above pH 10.

The simplest reaction mechanism (Scheme 1) that fits the pH−rate profile (Figure 2) involves deprotonation of the metal-bound water molecule in **2** to form **2-H** followed by intramolecular nucleophilic attack of the metal hydroxide on the bridging phosphate monoester to form **3** and phenol. The pH−rate profile was fit according to $k_{\mathrm{obs}} = K_a k/(K_a + [\mathrm{H}])$ where K_a is the acid dissociation constant for the metal-bound water molecule in **2** and k is the rate constant in Scheme 1. The best fit gave $K_a = (1.4 \pm 0.2) \times 10^{-10}$ M and $k = (5.1 \pm 0.6) \times 10^{-2}\ \mathrm{s}^{-1}$. The p$K_a$ value of the metal-bound water molecule in **2** (9.8) obtained from the pH−rate profile is comparable to that for **1** (9.6) determined by potentiometric titration.[15] It is interesting that the reaction is so clean and simple with one ^{31}P NMR signal for the starting material going to one ^{31}P NMR signal for the product. The ^{31}P NMR signal for **3** is about 28 ppm downfield shifted relative to the corresponding signal of the unbound phosphate, which is characteristic of a phosphate with three of its oxygens coordinated to cobalt(III).[13] The X-ray structure of **1** shows that one of the coordinated water/hydroxide molecules is much closer to the phosphorus center (3.06 Å) than the other coordinated water/hydroxide molecule (3.67 Å).[16] We propose that only one product is formed because the coordinated water/hydroxide molecule closer to the phosphorus center is the nucleophile.

Sargeson et al. proposed a mechanism similar to that shown in Scheme 1 for the hydrolysis of *p*-nitrophenyl phosphate bridging two Co(III) centers.[7a,b] However, their system is highly complex due to competition between hydrolysis of the phosphate monoester and dissociation of the phosphate monoester from the cobalt complex. Through careful kinetic analysis, Sargeson et al. have been able to separate the rate of hydrolysis of the phosphate monoester from the dissociation reactions. They estimated that the rate constant for hydrolysis of the monoester in **4** is about $4 \times 10^{-1}\ \mathrm{s}^{-1}$ at 25 °C. The water rate for cleavage of the uncomplexed dianionic *p*-nitrophenyl phosphate is approximately $2 \times 10^{-9}\ \mathrm{s}^{-1}$ at 25 °C.[17] Hence the dinuclear complex **4** provides about 8 orders of magnitude rate acceleration for the hydrolysis reaction.



4

The water rate for hydrolysis of uncoordinated dianionic phenyl phosphate is about $5 \times 10^{-13}\ \mathrm{s}^{-1}$ at 25 °C.[17] The bridging phosphate monoester in **2-H** is cleanly hydrolyzed to completion with a first-order rate constant of about 5×10^{-2} s^{-1}. Therefore the dinuclear cobalt complex provides about 11 orders of magnitude rate acceleration for hydrolysis of phenyl phosphate. When phenyl phosphate is replaced with *p*-nitrophenyl phosphate in the synthesis of **2**, *p*-nitrophenol is rapidly released during the synthesis.[18] The greater rate acceleration in the hydrolysis of **2-H** may be due in part to the more rigid structure in **2-H** compared to that in **4**.

Acknowledgment. We thank the NSERC, Pioneer Hi-Bred International Inc., and the U.S. Army Research Office for support of this work.

Supporting Information Available: X-ray structural information for **1**, including a crystal packing diagram and listings of crystallographic data, refinement details, positional parameters, and interatomic distances and angles, typical time plots for production of phenoxide from **2** at pH 8 and 13 as determined by HPLC, and ^{31}P NMR spectra for conversion of **2-H** to **3** (11 pages). See any current masthead page for ordering information.

IC9603924

---

(15) Cobalt bound water molecules can have pK_a values greater than 9. For example, the pK_a value of $[(\mathrm{bamp})\mathrm{Co}(\mathrm{OH})_2(\mathrm{OH}_2)]^+$ is 9.4: Schwarzenbach, G.; Boesch, J.; Egli, H. *J. Inorg. Nucl. Chem.* **1971**, *33*, 2141.

(16) The sum of the van der Waals radii of oxygen and phosphorus is 3.3 Å.

(17) Kirby, A. J.; Jencks, W. P. *J. Am. Chem. Soc.* **1965**, *87*, 3209.

(18) If *p*-nitrophenyl phosphate in **4** is replaced with phenyl phosphate, the phosphate monoester is expected to dissociate from the cobalt complex without significant hydrolysis.[7a,b]