Modeling Carboxylate-Bridged Dinuclear Active Sites in Metalloenzymes Using a Novel Naphthyridine-Based Dinucleating Ligand

Chuan He and Stephen J. Lippard*

Department of Chemistry
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139

Received August 30, 1999

Nature uses carboxylate groups to bridge two metal ions in the catalytic centers of many dinuclear metalloenzymes. Besides providing an environment where two metal ions can bind with a flexible metal−metal separation, the geometry of the bridging carboxylate unit promotes the formation of additional bridging hydroxide (or water) groups and substrate molecules. When linking two metal ions, the pK_a value of water can be lowered to afford a hydroxide nucleophile at neutral pH, a strategy adopted in dinuclear metallohydrolases.[1−3] Substrates such as phosphate esters can also be activated upon binding to two metal ions. The bridging coordination mode of the carboxylate group also facilitates multielectron-transfer reactions that occur at dinuclear metal centers in biological systems.[4]

Despite their general utility in nature, multidentate ligands having a bridging carboxylate motif are challenging to prepare in model systems. The *syn, syn* coordination mode of the bridging carboxylate renders difficult the design and synthesis of such ligands with pendant groups that can form the desired dinuclear complexes. One approach to this problem is to employ naphthyridine, a nitrogen analogue of carboxylate, as the bridging unit.[5−8] Substitution at positions adjacent to the nitrogen heteroatoms facilitates the introduction of the desired functionalities. In the present work we describe the successful application of this strategy through the synthesis and utilization of a new dinucleating ligand BPAN, 2,7-bis[2-(2-pyridylethyl)aminomethyl]-1,8-naphthyridine. BPAN readily forms unprecedented biomimetic bridged dinuclear complexes, the 1,8-naphthyridine moiety providing a masked carboxylate group.

BPAN was prepared in 70% yield by reacting 2-(2-aminoethyl)-pyridine with 1,8-naphthyridine-2,7-dicarboxaldehyde[9] in methanol. It is an effective dinucleating ligand in both aqueous and organic media. Dinuclear copper(II), zinc(II), nickel(II), and tetranuclear cobalt(II) complexes were readily assembled, as illustrated in Scheme 1. In all cases, the naphthyridine portion of BPAN coordinates to two metal ions in a bridging mode, just like a carboxylate unit. Blue crystals of $[Cu_2(\mu\text{-OH})(BPAN)](ClO_4)_3$ (**1**) formed by allowing BPAN to react with 2 equiv of $Cu(OTf)_2$ in MeOH followed by addition of saturated aqueous $NaClO_4$ solution. Reacting **1** with 1 equiv of diphenyl phosphate afforded the blue derivative $[Cu_2(\mu\text{-OH})\{\mu\text{-}(PhO)_2PO_2\}(BPAN)](ClO_4)_2$ (**2**), the structure of which is depicted in Figure 1. The coordination geometry of each copper ion is square pyramidal with a bridging hydroxide and a bridging diphenyl phosphate group. One oxygen atom from the latter forms a single-atom bridge between two copper ions, a geometry that to our knowledge has not been previously encountered for any phosphodiester-bridged dimetallic center. Dinuclear $[Ni_2(\mu\text{-OAc})_2(BPAN)(H_2O)](ClO_4)_2$ (**3**) and tetranuclear $[Co_4(\mu\text{-OH})_4(BPAN)_2](ClO_4)_4$ (**4**) also readily assemble with BPAN (Scheme 1).[10]

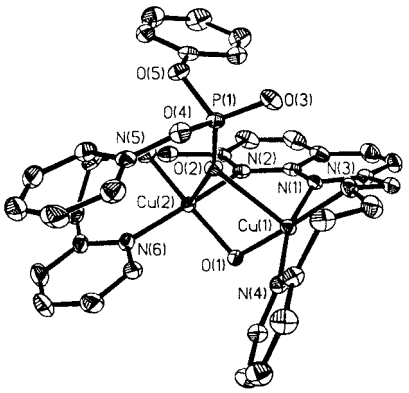
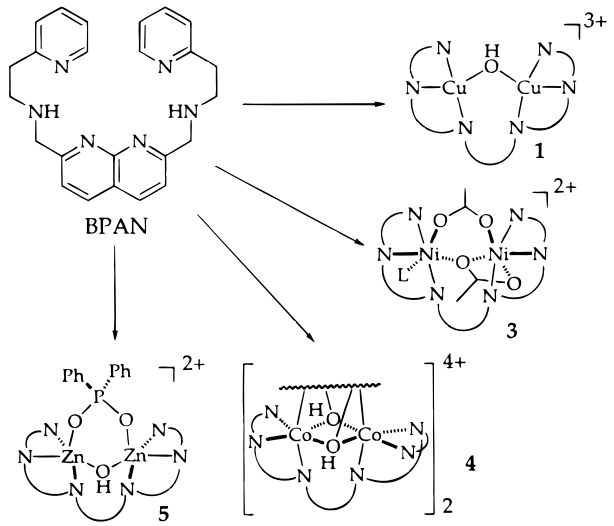


Figure 1. ORTEP diagram of $[Cu_2(\mu\text{-OH})\{\mu\text{-}(PhO)_2PO_2\}(BPAN)](ClO_4)_2$ (**2**) showing the 40% probability thermal ellipsoids for all non-hydrogen atoms.

Scheme 1



Several laboratories[11−19] have attempted to mimic the action of the carboxylate-bridged dizinc(II) unit, which is employed in many metallohydrolases to hydrolyze phosphate esters and other biological substrates.[1,2] We were therefore interested in preparing a dizinc(II) derivative of BPAN to evaluate its potential for promoting this chemistry. The compound $[Zn_2(\mu\text{-OH})(\mu\text{-}Ph_2PO_2)$-

(1) Sträter, N.; Lipscomb, W. N.; Klabunde, T.; Krebs, B. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2024−2055.
(2) Lipscomb, W. N.; Sträter, N. *Chem. Rev.* **1996**, *96*, 2375−2433.
(3) Wilcox, D. E. *Chem. Rev.* **1996**, *96*, 2435−2458.
(4) Feig, A. L.; Lippard, S. J. *Chem. Rev.* **1994**, *94*, 759−805.
(5) Tikkanen, W. R.; Krüger, C.; Bomben, K. D.; Jolly, W. L.; Kaska, W. C.; Ford, P. C. *Inorg. Chem.* **1984**, *23*, 3633−3638.
(6) Boelrijk, A. E. M.; Neenan, T. X.; Reedijk, J. *J. Chem. Soc., Dalton Trans.* **1997**, 4561−4570.
(7) Fahrni, C. J.; Pfaltz, A. *Helv. Chim. Acta* **1998**, *81*, 491−506.
(8) Fahrni, C. J.; Pfaltz, A.; Neuburger, M.; Zehnder, M. *Helv. Chim. Acta* **1998**, *81*, 507−524.
(9) Chandler, C. J.; Deady, L. W.; Reiss, J. A.; Tzimos, V. *J. Heterocycl. Chem.* **1982**, *19*, 1017−1019.
(10) See Supporting Information for structural and synthetic details.
(11) Chapman, W. H.; Breslow, R. *J. Am. Chem. Soc.* **1995**, *117*, 5462−5469.
(12) Koike, T.; Inoue, M.; Kimura, E.; Shiro, M. *J. Am. Chem. Soc.* **1996**, *118*, 3091−3099.
(13) Molenveld, P.; Kapsabelis, S.; Engbersen, J. F. J.; Reinhoudt, D. N. *J. Am. Chem. Soc.* **1997**, *119*, 2948−2949.
(14) Molenveld, P.; Stikvoort, W. M. G.; Kooijman, H.; Spek, A. L.; Engbersen, J. F. J.; Reinhoudt, D. N. *J. Org. Chem.* **1999**, *64*, 3896−3906.
(15) Rossi, P.; Felluga, F.; Tecilla, P.; Formaggio, F.; Crisma, M.; Toniolo, C.; Scrimin, P. *J. Am. Chem. Soc.* **1999**, *121*, 6948−6949.
(16) Bazzicalupi, C.; Bencini, A.; Bianchi, A.; Fusi, V.; Giorgi, C.; Paoletti, P.; Valtancoli, B.; Zanchi, D. *Inorg. Chem.* **1997**, *36*, 2784−2790.
(17) Jurek, P.; Martell, A. E. *Inorg. Chim. Acta* **1999**, *287*, 47−51.
(18) Vance, D. H.; Czarnik, A. W. *J. Am. Chem. Soc.* **1993**, *115*, 12165−12166.
(19) Young, M. J.; Chin, J. *J. Am. Chem. Soc.* **1995**, *117*, 10577−10578.

10.1021/ja993125g CCC: $19.00 © 2000 American Chemical Society
Published on Web 12/18/1999

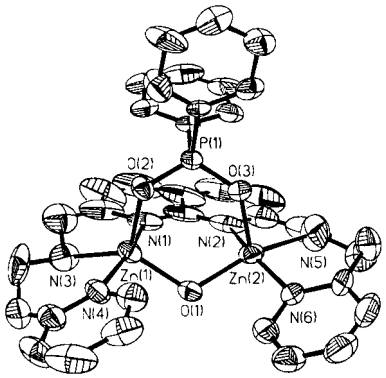


Figure 2. ORTEP diagram of $[Zn_2(\mu\text{-}OH)(\mu\text{-}Ph_2PO_2)(BPAN)](ClO_4)_2$ (**5**) showing the 40% probability thermal ellipsoids for all non-hydrogen atoms.

$(BPAN)](ClO_4)_2$ (**5**) was synthesized by allowing 2 equiv of $Zn(OTf)_2$ to react with BPAN, Ph_2PO_2H, and $LiOH{\cdot}H_2O$ in a 2:1 $EtOH/H_2O$ solution of $NaClO_4$ under neutral conditions. The structure of **5,** depicted in Figure 2, comprises two zinc ions, 3.287(5) Å apart, bridged by the naphthyridine fragment of BPAN as the carboxylate analogue, a hydroxide ion, and a diphenyl phosphonate group as a substrate analogue. The Zn(1) atom has distorted trigonal bipyramidal coordination geometry and Zn(2) is square pyramidal. The structure of **5** closely resembles those of the dizinc cores in many metallohydrolases. The formation of a hydroxide bridge under neutral conditions in aqueous solution suggests that the pK_a value of zinc(II)-bound water is significantly lower owing to the use of the dinucleating ligand BPAN.

A potentiometric titration of **5** displayed two end points corresponding to pK_a values of 6.85 ± 0.05 and 8.70 ± 0.05. The first deprotonation constant is attributed to the bridging water molecule, a situation encountered in many biological systems where a hydroxide group is generated from water by bridging two zinc(II) ions under neutral conditions. The second pK_a value of **5** may correspond to formation of a terminally bound hydroxide ion or to a second bridging hydroxide that replaces the diphenyl phosphonate ligand.

The ability of **5** to catalyze the transesterification of the RNA model substrate 2-hydroxypropyl-4-nitrophenyl phosphate (HPNP) was evaluated by adding a 10-fold excess of substrate to the dizinc(II) complex in buffered aqueous solutions.[20] A mononuclear reference compound, prepared by stoichiometric addition of $Zn(ClO_4)_2{\cdot}6H_2O$ to the ligand *N*,*N*-bis(2-pyridylmethyl)-*tert*-butylamine (bpta), was similarly investigated. The rate of transesterification cleavage of HPNP catalyzed by **5** was 6.4-fold faster than that of the mononuclear reference compound under neutral aqueous conditions.[21] The reaction with **5** was followed at 10 different pH values to generate a pH−rate profile that revealed an inflection point at 6.97 (Figure 3). Since this value corresponds to the first pK_a obtained in the potentiometric titration experiment, we conclude that the bridging hydroxide is most likely the base that catalyzes the transesterification cleavage of the substrate HPNP.

(20) Containing 1% acetonitrile which was used to make a concentrated stock solution of **5** at 25 ± 0.2 °C.

(21) The experiments were performed by allowing substrate HPNP (2.0 mM) to react with either **5** (0.2 mM) or bpta-Zn(II) (0.4 mM) in 19 mM aqueous buffered solutions (HEPES) containing 1% CH_3CN at pH 7 at 25 ± 0.2 °C. The observed pseudo-first-order rate constants k_{obs} (s^{-1}) are 1.6×10^{-5} for **2** and 0.25×10^{-5} for bpta-Zn(II). Under these experimental conditions, a control experiment revealed that the presence of the $Ph_2PO_2^-$ ion does not interfere with catalysis to within experimental error.

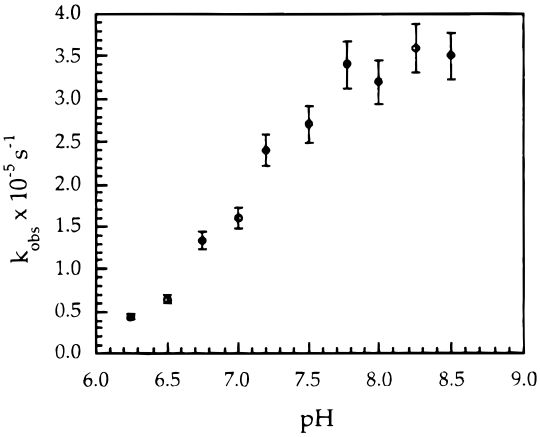


Figure 3. pH−rate profile for the transesterification cleavage of 2.0 mM HPNP by 0.2 mM $[Zn_2(\mu\text{-}OH)(\mu\text{-}Ph_2PO_2)(BPAN)](ClO_4)_2$ (**5**) in buffered aqueous solutions containing 1% of CH_3CN, 19 mM HEPES for pH 7.00−8.50 and 19 mM MES for pH 6.25−6.75, at 25 ± 0.2 °C.

The greater activity of **5** compared to the mononuclear reference compound could result from the double Lewis acid activation of the substrate by coordination to two zinc(II) ions in proximity to the bridging hydroxide group. pK_a values >8.0 are typically observed for mononuclear zinc(II)-bound water.[22,23] By positioning two zinc ions close to each other with the BPAN ligand, the pK_a of the zinc(II)-bound water is lowered significantly, allowing formation of a hydroxide group under neutral conditions. This bridging hydroxide appears to be the reactive base for the transesterification cleavage of HPNP, based on the pH−rate profile. A less likely, alternative mechanism cannot be excluded, however, in which the hydroxyl group of HPNP is deprotonated upon binding to zinc, and then attacks the phosphate group, also activated through coordination to zinc. Turnover was observed without significant loss of reactivity. A substrate concentration dependence study indicated weak binding of HPNP to catalyst **5** in aqueous media, with a dissociation constant of $K_d = 10.0 \pm 1.9$ mM.

In conclusion, 1,8-naphthyridine appears to function well as a masked version of the bridging carboxylate ligand widely distributed in nature. The BPAN ligand effectively promotes the formation of dinuclear metal complexes. Novel structures can be accessed with this ligand as illustrated. In addition, the catalytic transesterification cleavage of HPNP under neutral aqueous conditions by **5**, which proceeds at a rate severalfold higher than that of the corresponding mononuclear compound, indicates that interesting reactivity can also be obtained with complexes of this ligand system. This result further demonstrates that BPAN is a very effective ligand in aqueous media for synergistic action of two metal ions.

Acknowledgment. This work was supported by a grant from the National Science Foundation. C.H. was the recipient of a Merck/MIT graduate fellowship. We thank A. M. Barrios for assistance with the potentiometric titration studies and J. Du Bois for helpful discussions.

Supporting Information Available: Details of the synthetic procedures, X-ray crystallographic tables and physical characterization of **1**−**5**, and potentiometric titration and kinetic data for **5** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA993125G

(22) Koike, T.; Kimura, E. *J. Am. Chem. Soc.* **1991**, *113*, 8935−8941.

(23) Kimura, E.; Shiota, T.; Koike, T.; Shiro, M.; Kodama, M. *J. Am. Chem. Soc.* **1990**, *112*, 5805−5811.