Rapid Hydrolysis of 2′,3′-cAMP with a Cu(II) Complex: Effect of Intramolecular Hydrogen Bonding on the Basicity and Reactivity of a Metal-Bound Hydroxide

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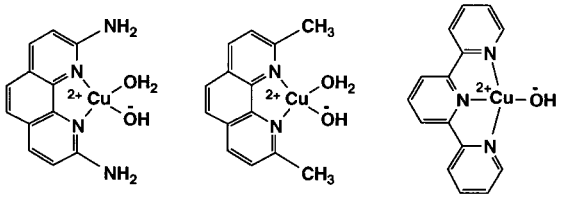
In general it is difficult to study the hydrolysis of biologically important phosphate diesters with poor leaving groups (e.g., DNA) because of their enormous stability. Consequently, activated phosphate diesters with good leaving groups (e.g., bis(2,4-dinitrophenyl)-phosphate (BDNPP), bis(*p*-nitrophenyl)phosphate (BNPP), 2-hydroxy-propyl *p*-nitrophenyl phosphate (HPNP)) are often used as substitutes for testing the reactivity of newly developed artificial phosphodiesterases. However, the mechanism for catalytic hydrolysis of phosphate diesters with good leaving is not always the same as that for those with poor leaving groups.[1] To this extent, 2′,3′-cyclic adenosine monophosphate (2′,3′-cAMP) is an ideal substrate for studying the effects of catalysts on hydrolyzing phosphate diesters with poor leaving groups since the cyclic phosphate hydrolyzes almost as rapidly as BDNPP under basic conditions due in large part to the five-membered ring strain (Table 1). Here we compare the reactivity of three Cu(II) complexes (**1**, **2**, and **3**) for hydrolyzing 2′,3′-cAMP.[2]



1 **2** **3**

2,9-Diamino-*o*-phenanthroline was synthesized according to a literature procedure.[3] [(2,9-Diamino-*o*-phenanthroline)$CuCl_2$] was prepared from a methanolic solution of $CuCl_2$ and 2,9-Diamino-*o*-phenanthroline. Copper complexes **1**, **2**, and **3** were freshly generated in water from the corresponding chlorides and 1 equiv NaOH. Hydrolysis of 2′,3′-cAMP was monitored by HPLC as described previously.[4] The reaction solutions were buffered by the metal complexes.[5] A typical HPLC plot for **1**-promoted cleavage of 2′,3′-cAMP (0.05 mM) at pH 5 and 25 °C is shown in Figure 1. It is evident from the HPLC plot that the cleavage reaction occurs hydrolytically producing 3′-AMP and 2′-AMP in a ratio of about 16 to 1. This represents the most regiospecific hydrolysis of the cyclic phosphate with a simple mononuclear metal complex. An interesting dinuclear Cu(II) complex that provides comparable regiospecificity for hydrolyzing 2′,3′-cAMP has been recently reported.[6] Surprisingly, **1** is over 100 times more reactive than even Ln(III) ions or complexes (Table 1).[7]

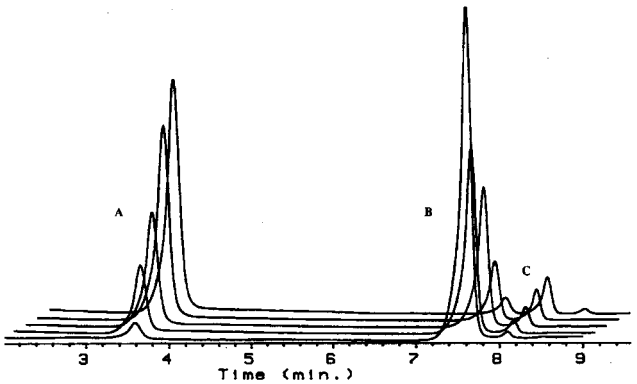
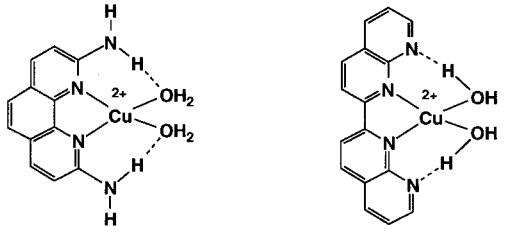


Figure 1. HPLC traces of **1** (1 mM) promoted cleavage of 2′,3′-cAMP (0.05 mM) at 25 °C, pH 5.0. Retention times are as follows: 3′-AMP = 3.5 min; 2′,3′-cAMP = 7.5 min; 2′-AMP = 8 min. Reaction times are from foreground to background, 6, 30, 120, 306, and 630 s.

Table 1. Apparent Second-Order Rate Constants (M^{-1} s^{-1}) for Hydrolysis of Phosphate Diesters at 25 °C

	BDNPP (*k*)	BDNPP (rel)	2′,3′-cAMP (*k*)	2′,3′-cAMP (rel)
OH	3.2×10^{-3}	1	1.1×10^{-3}	1
3	$5.7(2) \times 10^{-4}$	1.8×10^{-1}	$5.1(2) \times 10^{-3}$	4.6
2	$8.0(2) \times 10^{-1}$	2.5×10^{2}	$6.2(2) \times 10^{-2}$	5.6×10
1	$2.0(1) \times 10$	6.3×10^{3}	$3.8(1) \times 10$	3.5×10^{4}

Potentiometric titration reveals that the pK_a values of the protonated metal−hydroxides in **1**, **2**, and **3** are 5.5, 7.0, and 8.2, respectively.[8] It is remarkable that the amino group in **1** lowers the pK_a of the metal-bound water molecule given that it is an electron-donating group. It appears that the amino group is acting as a hydrogen bond donor to the metal-bound water molecule, thereby lowering the pK_a (**1H**). If the amine nitrogen was acting as a hydrogen bond acceptor, the pK_a of the coordinated water molecule should have increased. To test this, we synthesized an analogue of **1H** with the two hydrogen bond donating amine groups replaced with two hydrogen bond accepting groups (**4H**). As anticipated, the pK_a of the metal-bound water in **4H** (7.7) is significantly higher than that of **1H** (or the conjugate acid of **2**).[9]



1H **4H**

To examine the possiblity of the intramolecular hydrogen bondings in **1H**, we determined the crystal structure of the

(1) (a) Jencks, W. P.; Kirsch, J. F. *J. Am. Chem. Soc.* **1964**, *86*, 837. (b) Menger, F. M.; Ladika, M. *J. Am. Chem. Soc.* **1987**, *109*, 3145. (c) Bashkin, J. K.; Jenkins, L. A. *J. Chem. Soc., Dalton Trans.* **1993**, 3631.

(2) For some other studies on hydrolysis of phosphates with Cu(II) complexes, see: (a) Kovari, E.; Kramer, R. *J. Am. Chem. Soc.* **1996**, *118*, 12704. (b) Morrow, J. R.; Trogler, W. C. *Inorg. Chem.* **1988**, *27*, 3387. (c) Stern, M. K.; Bashkin, J. K. *Inorg. Chim. Acta*, **1997**, *263*, 49. (d) Liu, S.; Hamilton, A. D. *Tetrahedron Lett.* **1997**, *38*, 1107. (e) Burstyn, J. N.; Deal, K. A. *Inorg. Chem.* **1993**, *32*, 3585.

(3) Ogawa, S.; Yamaguchi, T.; Gotoh, N. *J. Chem. Soc., Perkin Trans. 1.* **1974**, 976.

(4) Linkletter, B.; Chin, J. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 472.

(5) We were not able to find a buffer system that does not decrease the activity of **1** for cleaving 2′,3′-cAMP. The pH of the reaction solution did not change by more than 0.1 unit during the cleavage reaction.

(6) Liu, S.; Luo, Z.; Hamilton, A. D. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 2678.

(7) Breslow, R.; Huang, D.-L. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 4080.

(8) The pK_a values of the two amino groups in **1** (or **1H**) are expected to be much higher than 5.5. For example, the pK_a of the amino group in the Co(III) complex of dipyridylamine has been shown to be about 5. Wong, Y. J.; Petersen, J. D.; Geldard, J. F. *Inorg. Chem.* **1985**, *24*, 3352.

(9) The pK_a of the metal bound water in the Cu(II) complex of 5,5′-diaminobipyridyl (5.65) is comparable to that of **1H**.

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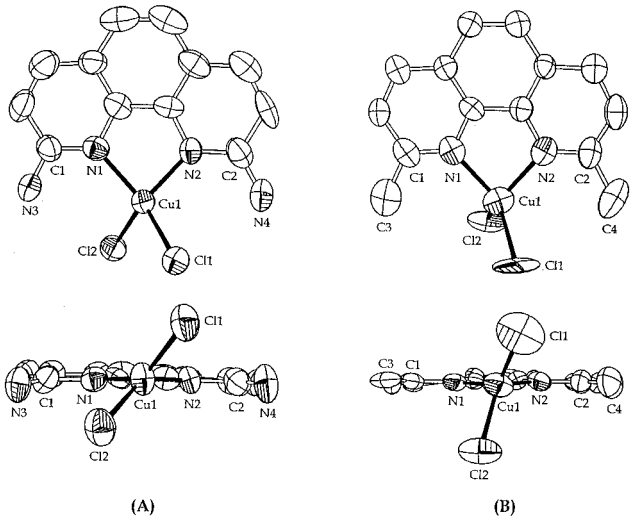


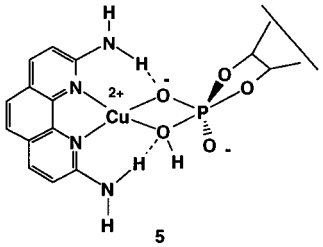
Figure 2. Crystal structures (ORTEP representation; ellipsoids at 50% probability level) of the dichloro complex of **1** (A) and **2a** (B). The two phenyl groups of bathocuproine in **2a** have been omitted for comparison.

dichloro complex of **1** (Figure 2A).[10] The range of distances required between a nitrogen donor and a chlorine acceptor for a hydrogen bond in the solid state is 3.10−3.26 Å[11] and both N−Cl distances in Figure 2 are well within this range (3.13 and 3.17 Å). For comparison, a crystal structure of a Cu(II) complex where the amino groups have been replaced by methyl groups has been obtained. In the copper chloride complex of bathocuproine (**2a**, Figure 2B)[12] there is no possibility of hydrogen bonding interactions and the distances between the chlorine atoms and the methyl carbon atoms are considerably longer (3.62 and 3.53 Å, respectively).

The reactivities of the free hyroxide and the three metal−hydroxide complexes for hydrolyzing BDNPP and 2′,3′-cAMP are listed in Table 1. At solution pH below the pK_a of the coordinated water molecules, complex **1** is about 2×10^4 times more reactive than **2** and about 4×10^6 times more reactive than **3** for hydrolyzing 2′,3′-cAMP. Above the pK_a of the coordinated water molecules, complex **1** is over 6×10^2 times more reactive than **2** and over 7×10^3 times more reactive than **3** (Table 1). The greater reactivity of **1** cannot be due to an increase in the equilibrium constant for complexation of the phosphate diester since dimethyl phosphate does not significantly inhibit the reaction up to 0.1 M. The simple mononuclear Cu(II) complex (**1**) is about 100-fold more reactive than the recently reported dinuclear Cu(II) complex for hydrolyzing the cyclic phosphate.[6]

It is well-known that *cis*-aquahydroxy metal complexes dimerize and that the dimers are not reactive for hydrolyzing phosphates.[2e,4] The rate of hydrolysis of 2′,3′-cAMP increases linearly with increase in concentration of **1** or **2**, indicating that the metal complexes do not dimerize significantly under our experimental conditions.[4] Potentiometric titrations of **1**, **2**, and **4** at various concentrations also reveal that the metal complexes do not dimerize.

We propose that the mechanism for **1**- and **2**-promoted hydrolysis of 2′,3′-cAMP involves intramolecular nucleophilic attack by the metal−hydroxide on the coordinated phosphate diester.[2] The reason **1** is more reactive than **2** for hydrolyzing 2′,3′c-AMP may be because the amino group hydrogen bonds to the OH group in **5** (as in **1H**).[13] This hydrogen bonding should acidify the OH group, making it easier for this group to deprotonate and thus facilitate the expulsion of the leaving group. This deprotonation may well be coupled to solvent-mediated protonation of the leaving group oxygen. The extent of this proton switch at the transition state is expected to be greater for phosphate diesters with poorer leaving groups.[14] Hence the difference in the reactivity of **1** and **2** for hydrolyzing BDNPP is only about 20-fold while that for 2′,3′-cAMP is about 600-fold (Table 1). The lowering of the pK_a in **1H** is expected to lower the nucleophilicity of the metal−hydroxide. However, this appears to be more than compensated for by the subsequent ease in the deprotonation of the OH group in **5**.



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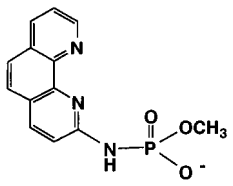
It is unlikely that the amino group is acting as a general base catalyst to directly deprotonate the OH group in **5** since its basicity is expected to be too weak (For example, the pK_a value of the conjugate acid of 2-aminopyridinium is only −7.6.[15]). It is also unlikely that the amino group is acting as a nucleophilic catalyst for hydrolyzing 2′,3′-cAMP since **6** does not hydrolyze to any appreciable extent under the same experimental conditions used to hydrolyze 2′,3′-cAMP to completion.



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In conclusion, **1** provides over (1×10^9)-fold rate acceleration over the background hydroxide rate (1 mM metal complex at pH 6, 25 °C) for hydrolyzing 2′,3′-cAMP by a novel mechanism. Complex **1** is by far the most reactive transition-metal complex reported to date for hydrolyzing 2′,3′-cAMP.[16]

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(10) Crystal structural data for brown thin plates of **1**: monoclinic, space group *P*-1, $a = 6.9839(13)$ Å, $b = 10.4179(14)$ Å, $c = 18.806(3)$ Å, $\alpha = 84.683(11)°$, $\beta = 80.406(13)°$, $\gamma = 77.085(12)°$, $Z = 4$; $R = 0.1000$, GOF = 1.095. Crystal structural data for red crystals of **2b**: monoclinic, space group *P*-1, $a = 14.300(3)$ Å, $b = 15.759(3)$ Å, $c = 11.8256(15)$ Å, $\alpha = 100.423(13)°$, $\beta = 102.141(12)°$, $\gamma = 107.165(13)°$, Z = 4; $R = 0.069$, GOF = 3.30.

(11) Macgillavry, C. H., Rieck, G. D., Eds. *International Tables for X-Ray Crystallography*; The Kynoch Press: Birmingham, 1962; vol 3.

(12) Linkletter, B. PhD dissertation, *Hydrolytic Cleavage of RNA by Metal Complexes*; Department of Chemistry, McGill University, Canada, 1995.

(13) Consistent with this interpretation, compound **4H** is not active for hydrolyzing 2′,3′-cAMP under our experiemental conditions up to pH 8.

(14) Extent of bond making and breaking in transition state **5** is not indicated. This reaction may well be concerted (Hengge, A. C.; Cleland, W. W. *J. Am. Chem. Soc.* **1990**, *112*, 7421) since the metal−hydroxide is expected to be as good a leaving group as *p*-nitrophenol. Unfortunately, kinetic solvent isotope effect could not be measured because **1** is not soluble in D_2O down to 0.1 mM, whereas it is soluble in H_2O up to 2 mM. It may be that the intramolecular hydrogen bond in **1H** is weakened in D_2O.

(15) Jencks, W. P.; Regenstein, J. *Handbook of Biochemistry*; Chemical Rubber Company: Cleveland, OH, 1970; pp J-150.

(16) Ligand free Ce(IV) is more reactive than **1**. See, for example: (a) Takasaki, B. K.; Chin, J. *J. Am. Chem. Soc.* **1994**, *116*, 1121. (b) Sumaoka, J.; Miyama, S.; Komiyama, M. *J. Chem. Soc., Chem. Commun.* **1994**, 1755.