Selective Hydrolysis of 4-Nitrophenyl Phosphate by a Dinuclear Cu(II) Complex

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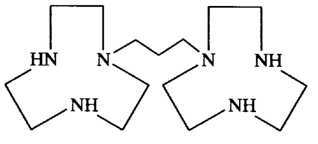
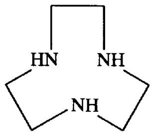
A flexible dinuclear Cu(II) complex with 1,3-bis(1,4,7-triaza-1-cyclononyl)propane, hydrolyzes 4-nitrophenyl phosphate under mild conditions, with 300 times rate enhancement over the corresponding mononuclear Cu(II) complex. In contrast, there is no difference in the reactivity in hydrolyzing bis(4-nitrophenyl)phosphate.

There have been considerable research efforts in developing efficient catalysts carrying two or three metal ions for phosphate ester hydrolysis.[1] In order to achieve a possible cooperativity among metal ions, it is crucial to design effective di- and trinucleating ligands. The common feature of the ligands in reported binuclear Zn(II), Cu(II), Co(III) complexes and trinuclear Zn(II) complexes is that the linkers connecting two or three monomeric units have functional groups such as phenyl, hydroxyphenyl and hydroxyalkyl groups to place metal ions in a close proximity.[2]

Here we report that a dinuclear Cu(II) complex with 1,3-bis(1,4,7-triaza-1-cyclononyl)propane(**L1**), hydrolyzes 4-nitrophenyl phosphate(NPP) efficiently under mild conditions. **L1** contains two triazacyclononane moieties that bind Cu(II) with a high stability and its equilibrium and structural data have been reported.[3] The Cu-L1 and the Cu-L2 complexes were prepared according to the known method.[4] Hydrolysis of NPP by the Cu-L1 and Cu-L2 complexes was performed at 30 ± 0.5 °C under 1:1 and 2:1 molar ratio of the metal complex and NPP.[5] The Cu complexes catalyzed hydrolysis of NPP to an inorganic phosphate, yielding 1 equivalent of 4-nitrophenol. Hydrolysis of NPP by the Cu-L1 complex was first-order in the complex concentration.[6] The second order rate constants for hydrolysis of NPP are listed in Table 1. The Cu-L1 complex is over two orders of magnitude more reactive than the Cu-L2 complex in hydrolyzing NPP.



L1



L2

Table 1. The second order rate constants($M^{-1}s^{-1}$) for the hydrolysis of NPP, BNPP and BDNPP by the Cu-L1 and Cu-L2 complexes at 30±0.5 °C[a]

	NPP	BNPP	BDNPP
Cu-L1[b]	2.2×10^{-2}	9.4×10^{-4}	9.3×10^{-2}
Cu-L2[c]	6.3×10^{-5}	4.2×10^{-4}	9.4×10^{-2}

[a] [Cu-L]=0. 2-0.7 mM, [NPP] and [BNPP]=0.25-0.5 mM, [BDNPP]=2.5-5.0 x 10^{-5}M. [b] At pH 6.5. [c] At pH 7.2(NPP, BNPP) and at pH 7.3(BDNPP).

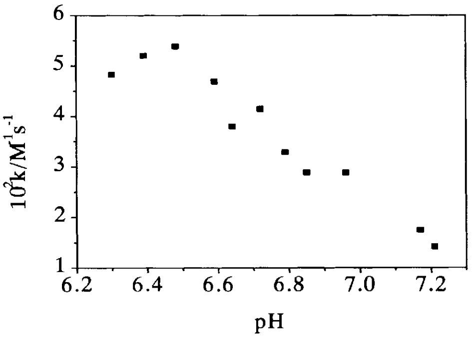


Figure 1. pH-rate profile for the Cu-L1 complex catalyzed hydrolysis of NPP at 30±0.5 °C. [Cu]=0.3-0.5 mM, MES, MOPS, and HEPES(20 mM), I=0.1 N $NaNO_3$.

The rate constants were measured at the pH near the maximum rate obtained from pH-rate profile shown in Figure 1.[7] In contrast to the NPP hydrolysis, the Cu-L1 complex hydrolyzed bis(4-nitrophenyl)phosphate(BNPP) and bis(2,4-nitrophenyl) phosphate(BDNPP) in almost the same rates as the Cu-L2 complex did. Hydrolysis of BNPP by the two complexes was carried out under the same conditions used above. Hydrolysis of BDNPP was performed under the pseudo-first order conditions with the metal complexes in excess. As a result of the hydrolysis of BDNP, two equivalents of 2,4-dinitrophenol were released. The second order rate constants for BNPP and BDNPP hydrolysis by the copper complexes are also listed in Table 1. The Cu-L2 complex is slightly more efficient in hydrolyzing BNPP and BDNPP than the Cu-L1 complex. The results indicate that the two copper ions in the Cu-L1 complex act independently as like two equivalents of the Cu-L2 complex in hydrolyzing phosphate diesters, such as BNPP and BDNPP.

Participation of two metal ions in hydrolyzing phosphate monoesters has been reported by several research groups. Czarnik and Vance reported that a rigid Co(III) dimer showed about 10 fold rate enhancement in hydrolyzing NPP, but not in hydrolyzing BNPP.[8] Recently, Kimura et al. showed that a Zn(II)-cryptate complex was able to selectively recognize and hydrolyze NPP over other phosphate di- and triesters.[9] In the above cases, the suggested mechanisms involved a bridging of phosphate monoesters to the two metal centers. The efficiency of this double Lewis acid activation has been estimated in hydrolyzing phosphate diesters.[1a] In the present study, it is quite surprising that a selective recognition of phosphate monoesters by the Cu-L1 complex is observed.

The remarkable selectivity of NPP over BNPP by Cu-L1 complex could be due to the higher basicity of NPP than BNPP as Breslow pointed out in his dinuclear Zn complex system.[10] However the differences in the basicity can not explain the observed 300 fold rate enhancement in this study.[11] We recently have shown that a dinuclear Zn(II)L complex(L=1,3-bis(1,5,9-triaza-1-cyclododecyl)propane) hydrolyzed NPP only 4 times faster than the corresponding mononuclear complex did.[12] The hydrolysis of NPP by the Cu-L1 complex most likely proceeds via bridging NPP to the two Cu ions followed by the attack of available nucleophiles, yielding an inorganic phosphate. The similar mechanism has been suggested in the case of ApA and 2,3-cyclic AMP hydrolysis by a rigid Cu(II) dimer.[11] The bell-shaped pH-rate profile indicates that the monohydroxy form of the dinuclear complex or its kinetic equivalent is the active species in hydrolyzing NPP.[13]

In conclusion, the Cu-L1 complex efficiently and selectively hydrolyzes phosphate monoester NPP. Two metal ions in the Cu-L1 complex interact cooperatively, where about 300 fold rate enhancement is observed over its corresponding mononuclear Cu-L2 complex. The present results may well be relevant to dinuclear metal centers in phosphomonoesterases such as an alkaline phosphatase.

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References and Notes

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5 In a typical kinetic run, 2 mL of the Cu complex (0.2-1 mM) in a buffer solution [MES (pH 6-6.6), MOPS (pH 6.7-7.4)] of an appropriate pH (20 mM, I=0.1 M KNO_3) was incubated at 30 ± 0.5 °C for 5 min and to which was added 5-10 μL of NPP or BNPP (0.1 M stock solution). The reaction was followed by monitoring the increase in the absorbance at 400 nm. The concentration of 4-nitrophenol released was calculated from the known coefficient (ε = 18,800 $M^{-1}cm^{-1}$), with the absorbance corrected for pH. The rates were obtained by the initial slopes method (0.5-3% completion, correlation coefficient R>0.99), and each kinetic run was reproducible within 5% error. The second rate constants were obtained by dividing the rate with substrate and catalyst concentrations.

6 A plot of log k (s^{-1}) versus log of the catalyst concentration gives a slope of 0.91.

7 According to the pH-rate profile for the Cu-L2 complex catalyzed hydrolysis of NPP, the rate reaches its maximum near pH 7.3.

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13 In titration experiments, the apparent pK_a values of the Cu-L1 and Cu-L2 complexes were 6.4 and 7.3, respectively. These are not the pK_a of the coordinated water, but rather a composite of multiple pH-dependent equilibria (ref. 3a and 3f). The kinetic pK_as of the Cu-L1 and Cu-L2 complexes are consistent with those from the titration experiment. Titration was performed with 0.5 mM solution of the Cu complex and 0.5 M KOH in water. The ionic strength was maintained at 0.15 M KCl.