Metal–Ammonium Cooperativity in Phosphodiester Hydrolysis

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The copper(II) complex of the ammonium-functionalized ligand [6,6′-($Me_2HNCH_2C{\equiv}C)_2$bpy]$^{2+}$ **L**^{1} accelerates phosphodiester hydrolysis with greater efficiency compared with related complexes which do not contain such functional groups.

Understanding the functional principles of enzymatic phosphodiester hydrolysis should give fresh impetus to the rational design of efficient synthetic hydrolysis catalysts, which are of interest from various perspectives.[1] For some phosphodiesterases the catalytic cooperativity of a kinetically labile metal ion and a positively charged amino acid side chain in the active site was proposed, examples being nuclease S_1 (Zn^{2+} + Lys–ammonium)[2] and staphylococcal nuclease (Ca^{2+} + 2 Arg–guanidinium),[3] which hydrolyse the phosphodiester backbone of both RNA and DNA. The key mechanistic feature is a double electrophilic activation of the $P(OR)_2O_2^-$ moiety by the metal ion and the acidic group, so that a nucleophilic displacement reaction at phosphorus, possibly involving a coordinated hydroxide, is greatly facilitated.

To mimic a bifunctional, metal–ammonium promoted phosphodiester hydrolysis with a simple model system, we designed **L**^{1} which carries two NMe_2H^+ groups linked to a metal binding 2,2′-bipyridine (bpy) unit by alkyne spacers. Molecular modelling studies indicate that in the complexes the pendant ammonium groups are perfectly positioned to form hydrogen bonds with a phosphodiester coordinated to the metal. At the same time, the spacers prevent intramolecular metal chelation. We have reported that the zinc(II) complex of **L**^{1} hydrolyses the activated phosphodiester bis(*p*-nitrophenyl) phosphate (BNPP) *ca.* three times more efficiently than zinc nitrate.[4] In contrast, Zn^{2+} is deactivated when coordinated to **L**^{2}, in which the ammonium groups are replaced by alkyl substituents.

The observation that (bpy)Cu^{2+} catalyses the hydrolysis of BNPP[5] and promotes RNA hydrolysis[6] led us to investigate the reactivity of the copper(II) complex of **L**^{1}. Due to low stability of the complex in pure water, all investigations were performed in ethanol–water 19:1 (*v*/*v*). Mixing [**L**^{1}]$(NO_3)_2$ and $Cu(NO_3)_2{\cdot}3\ H_2O$ in this solvent yields green crystals of [(**L**^{1})Cu$(ONO_2)(OH_2)_2$]$(NO_3)_3$ in which the copper atom is square pyramidally coordinated by two bpy nitrogens, one nitrate oxygen and two water molecules.†

Hydrolysis of BNPP by the copper complex of **L**^{1} was studied at 20 °C and pH 6.1.‡ Initial rate constants of *p*-nitrophenol release using 2×10^{-4} mol dm^{-3} **L**^{1} at varying Cu^{2+} concentrations are shown in Fig. 1(*a*).§ Although the concentration of the (**L**^{1})Cu species in dilute solution is easily monitored at the 333 nm bpy π–π* band, this was not possible for the reaction solutions due to the strong UV absorbance of BNPP. However, when BNPP is replaced by diphenyl phosphate, which is less reactive and does not absorb above 300 nm, a UV titration of [**L**^{1}]$(NO_3)_2$ (10^{-4} mol dm^{-3}) with copper(II) nitrate can be performed in this closely related system. Titration data for Cu/**L**^{1} ratios > 0.5 are easily fitted to a simple 1:1 model with a formation constant $K = 1.56\ (\pm 0.07) \times 10^4$ dm^3 mol^{-1} for (**L**^{1})Cu. When a somewhat lower stability constant $K = 0.7 \times 10^4$ dm mol^{-1} is used to calculate [(**L**^{1})Cu] in the BNPP containing reaction solutions, the optimum linear fit of rate constant against complex concentration for Cu/**L**^{1} ratios between 0.5 and 3 is achieved [Fig. 1(*b*)]. This finding strongly suggests that (**L**^{1})Cu is the hydrolytically active species. For Cu/**L**^{1} ratios > 3 the rate decreases when the copper concentration is further increased.¶

The rate constants given in Fig. 1(*a*) are valid for reaction times < 10 min and correspond to a release of less than 0.5 equiv. *p*-nitrophenol per equiv. **L**^{1}. By ^{31}P NMR product analysis no free phosphate is detectable, only the monoester *p*-nitrophenyl phosphate (NPP). NPP is also slowly hydrolysed by (**L**^{1})Cu, but the rate is negligible when the monoester is present in concentrations < 10^{-4} mol dm^{-3}. With prolonged reaction times, k_{obs} for BNPP hydrolysis significantly decreases. This can be explained by product inhibition, since addition of 2×10^{-4} mol dm^{-3} NPP to the reaction solutions lowers the initial rate by at least 40%.

(**L**^{1})Cu in 10^{-4} mol dm^{-3} concentration (based on $K = 0.7 \times 10^4$ dm^3 mol^{-1}) accelerates the hydrolysis of BNPP 10^6-fold and is 1900 times more reactive than copper(II) nitrate under the same conditions (Table 1). For (**L**^{2})Cu the rate was not determined accurately, but any addition of **L**^{2} significantly reduces the activity of a copper nitrate solution. Coordination to 2,2′-bipyridine also deactivates Cu^{2+}, possibly due to the partial formation of the inactive hydroxy-bridge dimer [(bpy)Cu(OH)]$_2^{2+}$.[5]

Obviously, the extraordinary reactivity of (**L**^{1})Cu is related to the presence of the ammonium groups. The strong synergistic effect between a metal ion and an acidic group in a simple synthetic system is unique for phosphodiester hydrolysis. It has been reported that the functionalization of a macrocyclic zinc complex with an imidazole group results in a modest increase (20-fold) of phosphodiester transesterification rate by metal–base cooperativity.[9]

Indirect evidence for the presence of ternary (**L**^{1})Cu-(phosphodiester) species in the reaction solution is given by the

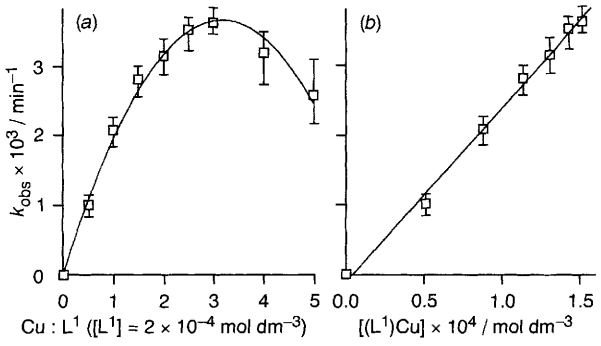
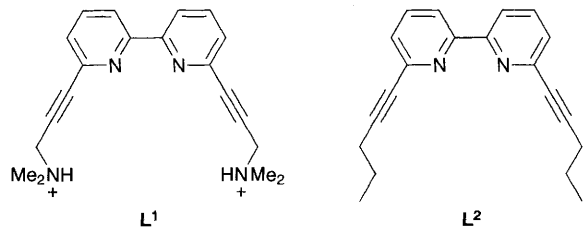


Fig. 1 (*a*) Initial pseudo-first-order rate constants for the hydrolysis of sodium BNPP (3×10^{-3} mol dm^{-3}) by 2×10^{-4} mol dm^{-3} [**L**^{1}]$(NO_3)_2$ at varying $Cu(NO_3)_2$ concentrations in ethanol–water 19:1 (*v*/*v*). T = 20(±0.5) °C, pH = 6.1(±0.1), buffer 2×10^{-3} mol dm^{-3} 2,4,6-trimethylpyridine, ionic strength 4.7(±0.7) $\times 10^{-3}$ mol dm^{-3}. Average values of three kinetic runs are given.

(*b*) Dependence of rate constant on (**L**^{1})Cu concentration for Cu/**L**^{1} ratios < 3 (correlation coefficient 0.997), derived from Fig. 1(*a*) using $K = 0.7 \times 10^{-4}$ dm^3 mol^{-1} to calculate [(**L**^{1})Cu] in the reaction solutions. BNPP hydrolysis by excess copper is negligible.



fact that L^1 and diphenyl phosphate bind to Cu^{2+} with positive cooperativity. By addition of 30 equiv. sodium diphenyl phosphate to a 1 : 2 L^1–Cu mixture (5×10^{-5} mol dm^{-3}, L^1) at pH 6.1 the complex concentration (initially 2.3×10^{-5} mol dm^{-3}) is increased 1.6-fold. Approximately the same effect is achieved by only 1 equiv. of the monoester phenyl phosphate, which as a dianion may bind strongly to the $(L^1)Cu^{4+}$ fragment. In contrast, addition of both diphenyl phosphate and phenyl phosphate to a L^2–Cu mixture decreases the concentration of the (L^2)Cu complex. These observations indicate that one or both ammonium group(s) of (L^1)Cu are involved in phosphate ester binding. In part the very different reactivities of (L^1)Cu and (L^2)Cu might be attributed to different equilibrium constants for BNPP binding.‖

The hydrolytic reactivity of the L^1–Cu^{2+} system is limited to pH values greater than 4.5 and reaches a maximum at pH 6.1. Above pH 6.6 copper(II) salts precipitate. The observed pH dependence might be related to the generation of a nucleophilic Cu–OH species. The first pK_a value of the H_2O molecules in $(bpy)Cu(OH_2)_2^{2+}$ is 7.1 in aqueous solution,[10] and the proximity of the positively charged ammonium groups should make a water molecule coordinated to (L^1)Cu even more acidic. Since the ammonium groups of (L^1)Cu might be partially deprotonated under the reaction conditions (pK_a $[L^1(NO_3)_2]$ = 7.2 in water), NMe_2 should also be considered as a potential nucleophile.[11] However, an intramolecular nucleophilic attack of NMe_2 at the phosphorus atom of a coordinated phosphodiester appears to be sterically highly disfavoured according to molecular modelling investigations.

The mechanism proposed in Scheme 1 includes a double electrophilic activation of the phosphodiester by coordination to the metal and hydrogen bonding to one of the ammonium groups. The substrate is fixed in a suitable position for the nucleophilic attack at phosphorus by a copper-coordinated OH^-. Intramolecular attack of M–OH to a coordinated phosphodiester has been postulated for (bpy)Cu[5] and other metal complexes[12] with two available *cis*-orientated coordination sites. Also, general acid catalysis by the NR_2H^+ group plays a crucial role in phosphodiester hydrolysis promoted by di- and poly-amines.[11,13,14] Scheme 1 shows how these two features are combined in the same molecule.

In conclusion, ammonium-functionalization is a strategy to considerably increase the hydrolytic reactivity of a metal complex with phosphodiesters. We have successfully modelled the efficient catalytic cooperativity of a metal ion and a positive acidic group, a functional motif of nuclease enzymes.

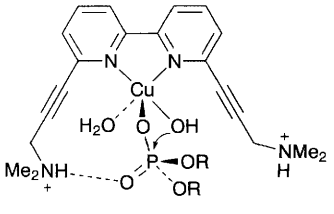
This work was supported by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie.

Received, 17th February 1995; Com. 5/00970G

Table 1 Observed and relative initial pseudo-first-order rate constants for BNPP hydrolysis by various copper(II) compounds (average values for three kinetic runs, reproducibility given in parentheses)

Compound (10^{-4} mol dm^{-3})	k_{obs}/min^{-1}	k_{rel}
$[(L^1)Cu]$	2.4×10^{-3}[b]	10^6
$[(L^2)Cu]$	$< 1.3 \times 10^{-6}$[c]	< 540
(bpy)Cu[d]	$4.3\ (\pm 0.7) \times 10^{-7}$	180
$Cu(NO_3)_2$	$1.3\ (\pm 0.2) \times 10^{-6}$	540
$[L^1](NO_3)_2$	$2.6\ (\pm 0.9) \times 10^{-9}$	1.1
none	$2.4\ (\pm 0.7) \times 10^{-9}$	1

[a] For reaction conditions see Fig. 1(*a*). [b] Based on $K = 0.7 \times 10^4$ dm^3 mol^{-1} for (L^1)Cu in the reaction solutions. [c] For various L^2/Cu ratios < 2 at $Cu(NO_3)_2 = 10^{-4}$ mol dm^{-3}. [d] bpy : $Cu(NO_3)_2$ 1 : 1.



Scheme 1 Possible mechanism of BNPP hydrolysis by (L^1)Cu (R = *p*-nitrophenyl). A square pyramidal copper coordination is assumed.

Footnotes

† X-ray crystallographic details for $[(L^1)Cu(ONO_2)(OH_2)_2](NO_3)_3$ will be published elsewhere. We have already described the crystal structure of the chloro-bridged complex $[(L^2)Cu(Cl)(\mu\text{-}Cl)]_2$.[7] Both complexes display a square pyramidal Cu coordination with an in-plane NO_3 (NCl_3, respectively) donor set, with the second bpy nitrogen in the apical position.

‡ The pH was measured with a glass electrode and corresponds to pH meter reading +0.8 pH units. This correction value for ethanol–water 19 : 1 (*v*/*v*) solutions was derived by extrapolation of literature data.[8] pH 6.1 corresponds to a 1.1×10^{-3} mol dm^{-3} 2,4,6-trimethylpyridine–0.9×10^{-3} mol dm^{-3} 2,4,6-trimethylpyridine·HNO_3 buffer composition in ethanol–water 19 : 1 (*v*/*v*) at 4×10^{-3} mol dm^{-3} $NaNO_3$.

§ At pH 6.1 in ethanol–water 19 : 1 (*v*/*v*) *p*-nitrophenol in effect does not dissociate to *p*-nitrophenolate and protons. To detect the increase in *p*-nitrophenol concentration in the reaction solutions (10 ml), at least five 0.5 ml samples were taken at appropriate time intervals and mixed with 2.5 ml 40 mmol dm^{-3} NaOH in ethanol–water 2 : 3 containing 0.2 mmol dm^{-3} EDTA. The produced *p*-nitrophenolate (ε_{400} = 19100 dm^3 mol^{-1} cm^{-1}) was detected photometrically. An approximately linear increase of *p*-nitrophenolate concentration with time was observed in all cases (correlation coefficients > 0.99).

¶ A possible explanation for the deactivation of (L^1)Cu by Cu^{2+} in large excess is the formation of inactive, hydroxy bridged $(L^1)Cu(\mu\text{-}OH)_2Cu$ species.

‖ The determination of rate constants (k_{cat}) for BNPP hydrolysis under substrate saturation conditions is complicated by the sensitivity of (L^n)Cu (n = 1, 2) concentration to changes in phosphodiester concentration.

References

1 D. S. Sigman, A. Mazumder and D. M. Perrin, *Chem. Rev.*, 1993, **93**, 2310; J. K. Bashkin, in *Bioinorganic Chemistry of Copper*, ed. K. D. Karlin and Z. Tyeklár, Chapman & Hall, New York, 1993, pp. 132–139; F. M. Menger, *Angew. Chem.*, 1991, **103**, 1109; *Angew. Chem., Int. Ed. Engl.*, 1991, **30**, 1091.
2 H. Witzel, W. Berg, O. Creutzenberg and A. Karreh, in *Zinc Enzymes*, ed. I. Bertini, C. Luchinat, W. Maret and M. Zeppezauer, Birkhäuser, Boston, 1986, pp. 295–306.
3 C. B. Anfinsen, *Science*, 1973, **181**, 223; F. A. Cotton, E. E. Hazen Jr. and M. J. Legg, *Proc. Nat. Acad. Sci. USA*, 1979, **76**, 2551.
4 E. Kövári and R. Krämer, *Chem. Ber.*, 1994, **127**, 2151.
5 J. R. Morrow and W. C. Trogler, *Inorg. Chem.*, 1988, **27**, 3387.
6 M. K. Stern, J. K. Bashkin and E. D. Sall, *J. Am. Chem. Soc.*, 1990, **112**, 5357.
7 E. Kövári and R. Krämer, *Z. Naturforsch., B. Chem. Sci.*, 1994, **49**, 1324.
8 H. Galster, *pH Messung*, VCH, Weinheim, 1990, pp. 56–61; 231.
9 R. Breslow, D. Berger and D.-L. Huang, *J. Am. Chem. Soc.*, 1990, **112**, 3686.
10 R. L. Gustafson and A. E. Martell, *J. Am. Chem. Soc.*, 1959, **81**, 525.
11 M. Komiyama and K. Yoshinari, *J. Chem. Soc., Chem. Commun.*, 1989, 1880.
12 P. Hendry and A. M. Sargeson, *Progr. Inorg. Chem.*, 1990, **38**, 201; J. Chin, *Acc. Chem. Res.*, 1991, **24**, 145.
13 K. N. Dalby, A. J. Kirby and F. Hollfelder, *Pure and Appl. Chem.*, 1994, **66**, 687.
14 K. Yoshinari, K. Yamazaki and M. Komiyama, *J. Am. Chem. Soc.*, 1991, **113**, 5899.