

Short Communication

Hydrolysis of Uridine 2',3'-Cyclic Monophosphate and Uridyl-3',5'-uridine Promoted by Bimetallic Cu^{2+} and Zn^{2+} Complexes of BISDIEN

Satu Mikkola,* Qi Wang, Zoltan Jori, Mia Helkearo and Harri Lönnberg

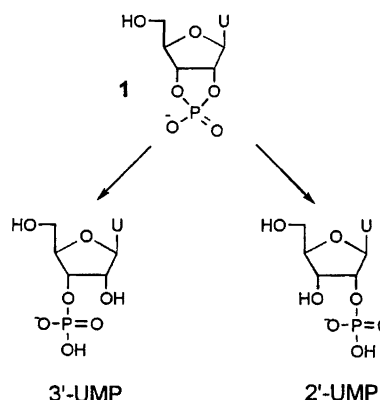
University of Turku, Department of Chemistry, FIN-20014 Turku, Finland

Mikkola, S., Wang, Q., Jori, Z., Helkearo, M. and Lönnberg, H., 1999. Hydrolysis of Uridine 2',3'-Cyclic Monophosphate and Uridyl-3',5'-uridine Promoted by Bimetallic Cu^{2+} and Zn^{2+} Complexes of BISDIEN. Acta Chem. Scand. 53: 453–456. © Acta Chemica Scandinavica 1999.

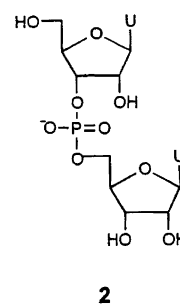
Metal-ion based catalysts for the hydrolytic cleavage of phosphodiester bonds is a subject that has attracted considerable interest over the last few decades.^{1–4} The motive behind such studies is to gain understanding of the action of ribozymes and other metalloenzymes, and more importantly to mimic the action of these by artificial cleaving agents. The catalytic activity of many single metal ion catalysts is relatively modest, and the search for active catalysts has recently become much focussed on bi- or tri-metallic systems.^{5–12}

A vast amount of work has been carried out on Co^{3+} complexes as metalloenzyme models, and the results have clearly shown that the bimetallic species are more efficient catalysts for phosphoester hydrolysis than catalysts containing only one Co^{3+} centre.^{13,14} The enhanced catalytic activity has been attributed to double Lewis acid activation of bimetallic systems;^{13,15} a marked rate acceleration takes place when both non-bridging oxygens bind an electrophile. Co^{3+} complexes differ from complexes of metal ions found in biological systems, such as Zn^{2+} or Cu^{2+} , in that they do not easily undergo ligand exchange. Enhanced catalytic activity has, however, also been observed with bimetallic systems containing substitutionally labile metal ions such as Zn^{2+} and Cu^{2+} compared with corresponding monometallic catalysts.^{5–12} The mechanism of catalysis may be somewhat different from that utilised by Co^{3+} catalysts: while Co^{3+} catalysts most likely provide a hydroxo ligand as the attacking nucleophile, in addition to Lewis acid activation,^{16–18} catalysis by the phosphate-bound Zn^{2+} and Cu^{2+} species is generally attributed to general acid/base catalysis by the hydrated metal ion.^{1–4}

In this paper, we report on the hydrolysis of 2',3'-cyclic monophosphate of uridine (**1**) and uridyl-3',5'-uridine (**2**) promoted by bimetallic Zn^{2+} and Cu^{2+} complexes of BISDIEN (**3**), referred to as $(\text{Zn}^{2+})_2$ -BISDIEN and $(\text{Cu}^{2+})_2$ -BISDIEN, respectively, in the following. Zn^{2+} and Cu^{2+} complexes of 1,4,7-triazacyclododecane ([9]aneN₃, **4**) were used for comparison as the corresponding mononuclear catalysts. The catalysts

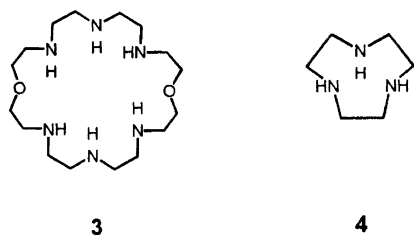


Scheme 1.



2

* To whom correspondence should be addressed.



used in this study differ markedly from the bimetallic species reported previously in the sense that within the BISDIEN ligand the metal ions are bridged through aquo ligands, whereas the bimetallic systems used in previous studies consist of two separate metal ion chelates linked to each other. The speciation of the BISDIEN complexes of Zn^{2+} and Cu^{2+} is quite well known; their composition as a function of pH has been established by potentiometric titrations,¹⁹ and they are known to bind tightly to phosphate.²⁰ As mentioned above, the metal ions complexed with a single BISDIEN molecule are forced rather close to each other, and they have been suggested to form bridged hydroxo complexes.¹⁹ A similar hydroxo bridge has been shown to exist between Zn^{2+} and Fe^{3+} or two Fe^{3+} ions in purple acid phosphatase enzyme.¹² A catalytically active monohydroxy bridged dimer has also been suggested to be formed in solution, where the structure of the monomeric Cu^{2+} units prevents the formation of an inactive dihydroxy dimer.²¹

The hydrolysis of 2',3'-cyclic monophosphate of uridine (2',3'-cUMP, **1**) to a mixture of 3'- and 2'-monophosphates (3'- and 2'-UMP, respectively) (Scheme 1) was followed at 30 °C by reversed-phase HPLC. The catalysts employed were the 2:1 (M^{2+} :L) Zn^{2+} and Cu^{2+} complexes of BISDIEN (**3**), which have been shown to be the predominant species under the experimental conditions.¹⁹ The cleavage of only 10–15% of the substrate was followed, since the reactions catalysed by bimetallic catalysts, $(Cu^{2+})_2$ -BISDIEN particularly, showed a clear tendency to slow down on prolonged treatment, probably due to the aggregation of the catalysts.

The cleavage of 2',3'-cUMP promoted by both $(Cu^{2+})_2$ -BISDIEN and $(Zn^{2+})_2$ -BISDIEN is hydrolytic: the only products observed were 2'- and 3'-UMP; no products that could have resulted from oxidative cleavage, such as uracil base, could be detected. As has been shown to be the case with other bimetallic catalysts,⁹ $(Cu^{2+})_2$ -BISDIEN also produced the 2'- and 3'-UMP products in a ratio different from that of the solvent-induced reaction.^{22,23} While a solvent-induced reaction gives a 2:1 mixture of 3'- and 2'-monophosphates, a 1:2 ratio was obtained in the presence of $(Cu^{2+})_2$ -BISDIEN. This result was verified by spiking the reactions with samples of 2'- and 3'-UMP obtained from Sigma. The altered product distribution caused by $(Cu^{2+})_2$ -BISDIEN seems to result from the preferred cleavage of the 3'-O–P

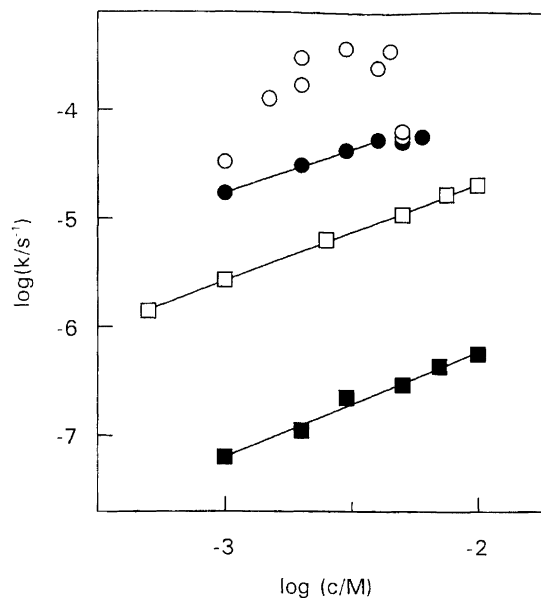


Fig. 1. Logarithmic rate constants of hydrolysis of 2',3'-cUMP as a function of the catalyst concentration at 30 °C. Notation: ○, $(Cu^{2+})_2$ -BISDIEN at pH 8.5 and $I=0.15$ M; ●, $(Zn^{2+})_2$ -BISDIEN at pH 7.0 and $I=0.1$ M; □, Cu^{2+} -[9]aneN₃ at pH 8.5 and $I=0.15$ M; ■, Zn^{2+} -[9]aneN₃ at pH 7.0 and $I=0.1$ M. The pH was adjusted with HEPES buffers and the ionic strength with $NaNO_3$.

bond; the catalyst does not enhance the interconversion of 2'- and 3'-UMP. The $(Zn^{2+})_2$ -BISDIEN complex and the monomeric catalysts, in contrast, produced the normal 2:1 mixture of 3'- and 2'-UMPs.

The rate constants of the hydrolysis of 2',3'-cUMP as a function of the catalyst concentration are shown in Fig. 1. The measurements were made at an optimal pH for the reaction. The limiting factor in the case of the Zn^{2+} complex was the poor solubility/stability of the catalyst under alkaline conditions. At pH 7.0, however, no precipitation was observed, and the reaction seemed to follow first-order kinetics. With $(Cu^{2+})_2$ -BISDIEN, in contrast, the cleavage seemed to slow down, although no precipitate was observed, and as can be seen, the results obtained are rather scattered. The measurement in 5 mM $(Cu^{2+})_2$ -BISDIEN solution was repeated several times, and the result seems to be reproducible.

The $(Zn^{2+})_2$ -BISDIEN promoted reaction shows a clear first-order dependence on the catalyst concentration at low catalyst concentration. At higher concentration the dependence begins to level off. With $(Cu^{2+})_2$ -BISDIEN as a catalyst, the tendency is clearer, and the reaction actually becomes slower as the concentration of the catalyst is increased to 5 mM. The abrupt rate decrease at higher concentrations suggests that the catalyst undergoes aggregation at high concentrations, which would also explain the observed rate decrease with longer reaction times.

Figure 1 also contains the data obtained with the monometallic species. The rate of hydrolysis promoted by both Zn^{2+} and Cu^{2+} chelates of [9]aneN₃ (**4**) shows

a first-order dependence on catalyst concentration without any indication of saturation. In both cases the bimetallic complex is a more efficient catalyst than its monometallic counterpart. The rate constants obtained with 1 mM $(\text{Zn}^{2+})_2\text{-BISDIEN}$ and $(\text{Cu}^{2+})_2\text{-BISDIEN}$ are 30 and 150 times those obtained with 2 mM $\text{Zn}^{2+}[9]\text{aneN}_3$ and $\text{Cu}^{2+}[9]\text{aneN}_3$ complexes. While the difference observed between $(\text{Cu}^{2+})_2\text{-BISDIEN}$ and $\text{Cu}^{2+}[9]\text{aneN}_3$ can most probably be attributed to the higher catalytic ability of the bimetallic system, this may not be entirely the case with the Zn^{2+} complexes. The rate constants for reactions with Cu^{2+} based catalysts were obtained at a pH optimal for cleavage, and hence, to the maximal catalytic activity. Similar comparisons cannot be made with Zn^{2+} complexes, because of the poor solubility of $(\text{Zn}^{2+})_2\text{-BISDIEN}$. Comparison of the catalytic activity of the free metal ions and the BISDIEN complexes, in contrast, cannot be made, since under the experimental conditions employed in this study, the metal aquo ions precipitate as hydroxides.

The catalytic activity of $(\text{Zn}^{2+})_2\text{-BISDIEN}$ and $(\text{Cu}^{2+})_2\text{-BISDIEN}$ is quite high. At pH 8.5, the rate constant of the OH^- -catalysed cleavage of 2',3'-cUMP is $1.0 \times 10^{-7} \text{ s}^{-1}$ at 30 °C, which means that the catalysis by 1 mM $(\text{Cu}^{2+})_2\text{-BISDIEN}$ is about greater by a factor of 1000 under these conditions. Assume a linear dependence of the solvent-induced cleavage rate on the hydroxide ion concentration, and the catalytic activity of $(\text{Zn}^{2+})_2\text{-BISDIEN}$ at pH 7.0 appears to be even larger, about 5000-fold. A comparison of the rate constants obtained in this study with those reported by Liu *et al.* for the bimetallic system consisting of two Cu^{2+} terpyridine complexes,⁹ shows that the catalytic activities are rather comparable, the BISDIEN complexes being maybe about one order of magnitude less active.

$(\text{Zn}^{2+})_2\text{-BISDIEN}$ also seems to be a good catalyst for the hydrolysis of internucleosidic phosphodiester bonds. At pH 7, the rate constant of the cleavage of 3',5'-UpU (**2**) promoted by 2 mM $(\text{Zn}^{2+})_2\text{-BISDIEN}$ was $2 \times 10^{-7} \text{ s}^{-1}$, which suggests that an approximately 1500-fold rate enhancement was observed. This result is quite promising, as the catalytic activity seems to be approximately ten times as high as that of a Zn^{2+} based mononuclear catalyst.²⁴ The $(\text{Cu}^{2+})_2\text{-BISDIEN}$ complex, in contrast, seems to be less able to catalyse the hydrolysis of an acyclic phosphodiester. The hydrolysis of 3',5'-UpU (**2**) appears to be promoted only by a factor of 20 in 2 mM $(\text{Cu}^{2+})_2\text{-BISDIEN}$ at pH 8.5 and 30 °C. The clear preference for the cyclic monophosphate could possibly be attributed to the size of the cavity of the macrocycle, and the tight fit of the phosphate ion inside it. While the cyclic phosphate function fits snugly inside, the cavity is less able to accommodate an acyclic phosphodiester bearing two relatively bulky nucleoside moieties, as in 3',5'-UpU.

In conclusion, the BISDIEN complexes of Zn^{2+} and Cu^{2+} are efficient catalysts for the hydrolysis of cyclic phosphodiester. $(\text{Zn}^{2+})_2\text{-BISDIEN}$ also shows a high

catalytic activity towards the hydrolysis of internucleosidic phosphodiester bonds.

Experimental

Synthesis of 3. The synthesis of **3** was carried out as described before.²⁵ The NMR data for these compounds and the final products were consistent with the values reported previously.²⁵ The authenticity of the final product was further verified by EI MS ($M+H^+$ 347).

Kinetic measurements. The complexes of **3** were prepared by mixing 2 equiv. of Zn^{2+} or Cu^{2+} (as nitrate) and 1.1 equiv. of the ligand. The solution was allowed to stand at least overnight prior to use. The reaction solutions were prepared by mixing the chelate and the buffer and salt stock solutions. The pH was checked and adjusted if necessary. The details of the kinetic analysis have been described before.²² The reaction was quenched by addition of an excess of EDTA to the aliquots. The HPLC analysis was carried out on Hypersil ODS (250 × 4 mm, 4 μm particle size) column with 0.1 M formic acid buffer as the eluent (pH 3.3, containing 0.25 M Me_4NCl). The first-order rate constants were calculated for the decrease of the mole fraction of 2',3'-cUMP or 3',5'-UpU in the reaction mixture by using an integrated form of the first-order rate law.

Acknowledgements. Funding from the Academy of Finland is gratefully acknowledged.

References

- Morrow, J. R. *Metal Ions Biol. Syst.* 33 (1996) 561.
- Kuusela, S and Lönnberg, H. *Metal Ions Biol. Syst.* 32 (1996) 271.
- Kuusela, S and Lönnberg, H. *Curr. Top. Solution Chem.* 2 (1997) 29.
- Trawick, B. N., Daniher, A. T. and Bashkin, J. K. *Chem. Rev.* 98 (1998) 939.
- Wall, M., Hynes, R. C. and Chin, J. *Angew. Chem., Int. Ed. Engl.* 32 (1993) 1633.
- Yashiro, M., Ishikubo, A. and Komiyama, M. *J. Chem. Soc., Chem. Commun.* (1995) 1793.
- Chapman, J. W. Jr. and Breslow, R. *J. Am. Chem. Soc.* 117 (1995) 5462.
- Young, M. A. and Chin, J. *J. Am. Chem. Soc.* 117 (1995) 10577.
- Liu, S., Luo, Z. and Hamilton, A. D. *Angew. Chem., Int. Ed. Engl.* 36 (1997) 2678.
- Molenveld, P., Kapsabelis, S., Engebets, J. F. J. and Reinhoudt, D. N. *J. Am. Chem. Soc.* 119 (1997) 2948.
- Yashiro, M., Ishikubo, A. and Komiyama, M. *J. Chem. Soc., Chem. Commun.* (1997) 83.
- Sträter, N., Libscomb, W. N., Klabunde, T. and Krebs, B. *Angew. Chem., Int. Ed. Engl.* 35 (1995) 2024.
- Jones, D. R., Lindoy, L. F. and Sargeson, A. M. *J. Am. Chem. Soc.* 106 (1984) 7807.
- Vance, D. A. and Czarnik, A. W. *J. Am. Chem. Soc.* 105 (1993) 12165.
- Williams, N. H. and Chin, J. *J. Chem. Soc., Chem. Commun.* (1996) 131.

SHORT COMMUNICATION

16. Anderson, B., Milburn, R. M., Harrowfield, J. MacB., Roberts, G. B. and Sargeson, A. M. *J. Am. Chem. Soc.* 99 (1977) 2652.
17. Harrowfield, J. MacB., Jones, D. R., Lindoy, L. F. and Sargeson, A. M. *J. Am. Chem. Soc.* 102 (1983) 7733.
18. Jones, D. R., Lindoy, L. F. and Sargeson, A. M. *J. Am. Chem. Soc.* 105 (1983) 7327.
19. Motekaitis, R. J., Martell, A. E., Lecomte, J.-P. and Lehn, J.-M. *Inorg. Chem.* 22 (1983) 609.
20. Motekaitis, R. J. and Martell, A. E. *Inorg. Chem.* 31 (1992) 5534.
21. Wahnou, D., Hynes, R. C. and Chin, J. *J. Chem. Soc., Chem. Commun.* (1996) 1441.
22. Oivanen, M. and Lönnberg, H. *J. Org. Chem.* 54 (1989) 2556.
23. Kuusela, S. and Lönnberg, H. *J. Phys. Org. Chem.* 5 (1992) 803.
24. Kuusela, S. and Lönnberg, H. *J. Chem. Soc., Perkin Trans 2* (1994) 2301.
25. Comarmond, J., Plumerc, P., Lehn, J.-M., Agnus, Y., Louis, R., Weiss, R., Kahn, O. and Morgenstern-Badarau, I. *J. Am. Chem. Soc.* 104 (1982) 6330.

Received November 9, 1998.