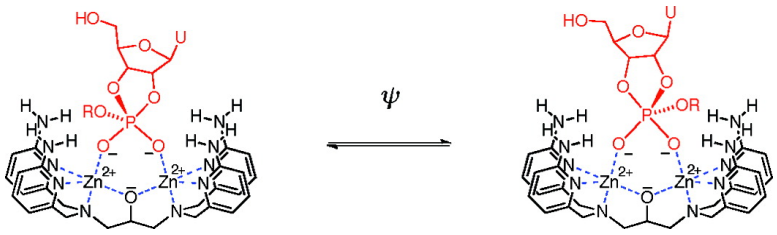
Communication

Cleavage and Isomerization of UpU Promoted by Dinuclear Metal Ion Complexes

Heidi Linjalahti, Guoqiang Feng, Juan C. Mareque-Rivas, Satu Mikkola, and Nicholas H. Williams

J. Am. Chem. Soc., **2008**, 130 (13), 4232-4233 • DOI: 10.1021/ja711347w



J|A|C|S
COMMUNICATIONS

Published on Web 03/07/2008

Cleavage and Isomerization of UpU Promoted by Dinuclear Metal Ion Complexes

Heidi Linjalahti,[†] Guoqiang Feng,[‡] Juan C. Mareque-Rivas,[§] Satu Mikkola,*[,†] and Nicholas H. Williams*[,‡]

Department of Chemistry, FI-20014 University of Turku, Turku, Finland, Centre for Chemical Biology, Department of Chemistry, University of Sheffield, Sheffield, U.K. S3 7HF, and School of Chemistry, University of Edinburgh, Edinburgh, U.K. EH9 3JJ

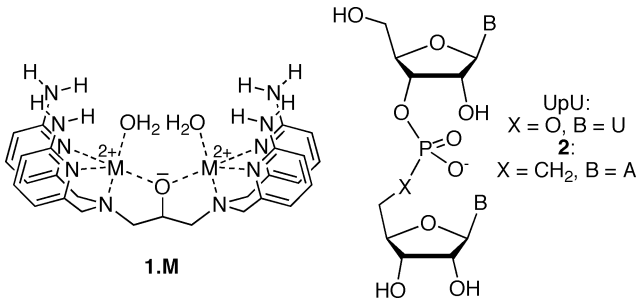
Received December 21, 2007; E-mail: satu.mikkola@utu.fi; N.H.Williams@Sheffield.ac.uk

The catalysis of phosphoryl transfer by metal ions has been intensively studied in biological, artificial, and theoretical systems, yet considerable debate still surrounds the status of the transient pentacoordinate phosphoryl species.[1] For potential di- or trianionic anionic intermediates, a central question is whether the reaction passes through a transition state or a formal intermediate and, perhaps more importantly, whether such intermediates are stable enough to allow the reaction pathways to follow an alternative course to direct in-line displacement at phosphorus. We report that dinuclear metal ion complexes **1.M** not only promote the cleavage of RNA fragments just as efficiently as the activated analogue 2-hydroxypropyl *p*-nitrophenyl phosphate (HPNPP) but also provide the first examples of metal ion catalyzed phosphate diester isomerization close to neutral pH (Chart 1). This observation implies that these complexes stabilize the formation of a phosphorane intermediate sufficiently to allow it to pseudorotate.

When UpU (0.05 mM) is incubated with 1 mM **1.Zn** (**1.M** with M = Zn; formed *in situ* from 1 mM ligand and 2 mM Zn(II) ions) at pH 6.5 at 25 °C, it is efficiently cleaved with a half-life of ~7 h. This corresponds to a rate acceleration of $\sim 10^6$-fold,[2] making this complex the most efficient Zn(II) based catalyst for promoting RNA cleavage under mild aqueous conditions reported to date.[3] The reaction proceeds *via* the usual intramolecular transesterification where the 2′ oxyanion on the ribose ring attacks the phosphate and expels the 5′OH of the nucleoside leaving group. The 2′,3′cUMP that initially forms is rapidly hydrolyzed to 2′UMP and 3′UMP monophosphates; we confirmed this by observing that, under the same conditions, an authentic sample of 2′3′cUMP was consumed within a few minutes. We ruled out alternative cleavage pathways by testing a 13-mer oligo-uridine substrate in which all but one site contained 2′OMe groups.[4] Two products were observed by capillary zone electrophoresis analysis, consistent with cleavage at the normal ribose linkage to give a leaving group 6-mer and a 7-mer terminated in a phosphorylated ribose. Furthermore, only the 7-mer product reacted when a sample was treated with alkaline phosphatase showing that it contained a terminal phosphomonoester group.

Varying the concentration of **1.Zn** (1−5 mM) has no effect on the rate of the cleavage of 3′,5′UpU at this pH. This suggests that the complex binds the monoanionic phosphate very strongly, with a binding constant > 10^4 M^{-1} so that saturation is achieved at the millimolar concentrations used in our experiments. The final monophosphate products (2′UMP and 3′UMP), which under the experimental conditions are dianionic, should bind even more strongly. This was confirmed by adding 3′UMP to the reaction mixture and observing that it efficiently inhibits catalysis by **1.Zn**. This much tighter binding compared to similar mononuclear complexes suggests that the phosphates bridge the two metal ion centers, as we have previously reported.[3b]

Chart 1. Metal Ion Complex and Substrates



Previous studies have shown that the catalytic activity of transition metal ion complexes generally depends on the identity of the metal ion, with complexes of Cu(II) usually being the most active catalysts,[5,6] so we screened several late transition metal ions for activity. However, the rate constants collected in Table 1 show that, of the complexes studied in this work, **1.Co** and **1.Zn** are the most efficient catalysts. Zn(II) complexes have frequently been studied as potential catalysts for the cleavage of RNA, but few reports on the catalysis by Co(II) exist despite the fact that Co(II) can substitute for Zn(II) in many metalloenzyme catalyzed reactions *in vitro*.[7] As simple aquo ions, Zn(II) and Co(II) only modestly promote the cleavage of UpU, with the effect of Co(II) being only one-tenth of that of Zn(II).[8] Bound in complex **1.M**, this reactivity is greatly enhanced and the difference is reversed to give a 4-fold advantage in favor of Co(II).

Remarkably, in addition to efficiently promoting the cleavage of the 5′ phosphodiester bond in 3′5′UpU, we observed that 2′,5′UpU appeared in the chromatograms, showing that **1.M** also catalyzes isomerization of the diester. This reaction is slower than cleavage, and the signal area of the 2′,5′ isomer remained low throughout the whole reaction (2′5′UpU cleavage is also promoted by the complex), so reliable rate constants for isomerization of UpU could not be calculated directly from these data. The observation was therefore verified using a nucleoside phosphonate (**2**) as a substrate. This substrate cannot be cleaved by intramolecular transesterification, but previous studies have shown that it isomerizes in the same way as dinucleoside monophosphates, that is, with pH-independent and acid-catalyzed components but without base catalysis.[9,10] The rate constants for isomerization of **2** are also shown in Table 1 and are consistent with the extent of 2′5′UpU that appears in the original experiments. The relative rate enhancement is modest in comparison to that observed for the cleavage reaction but

[†] FI-20014 University of Turku.
[‡] University of Sheffield.
[§] University of Edinburgh.

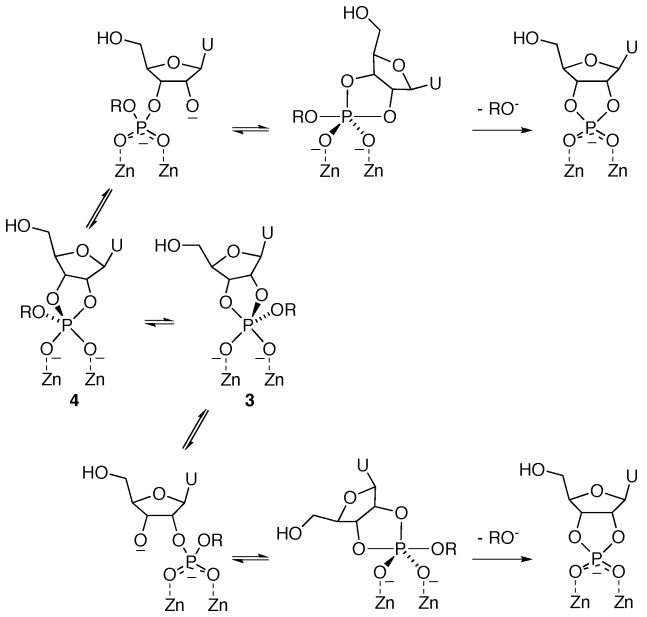
10.1021/ja711347w CCC: $40.75

Table 1. Rate Constants for Reactions Promoted by **1.M**[a]

metal ion **M**	Zn(II)	Co(II)	Cu(II)	Ni(II)
$k_{obs}/10^{-6}$ s^{-1} (3′,5′ UpU cleavage)	26 ± 3	95 ± 2	3.9 ± 0.1	0.70 ± 0.05
$k_i/10^{-6}$ s^{-1} (isomerization of **2**)	1.25 ± 0.02[b] 0.87 ± 0.03[c]	0.92 ± 0.02[b] 1.05 ± 0.03[c]	0.35 ± 0.01[b] 0.31 ± 0.04[c]	ND

[a] 1 mM ligand, 2 mM metal ion, 50 mM MOBS buffer, pH 6.5, 25 °C. [b] Interconversion of 3′ isomer to 2′ isomer. [c] Interconversion of 2′ isomer to 3′ isomer.

Scheme 1. Possible Mechanism for Cleavage and Isomerization of UpU Promoted by **1.Zn**[a]



[a] **1.Zn** is only represented by the Zn ions for clarity.

represents (at least) a 150-fold rate enhancement over the uncatalyzed reaction and represents the first substantial catalytic acceleration of this process.

Although both metal and hydroxide ions accelerate the cleavage of nucleotides, neither catalyzes isomerization.[10] For metal ions, this can be rationalized by suggesting that the ion stabilizes the formation of a dianionic phosphorane intermediate or closely related transition state. Isomerization would require both the formation and pseudorotation of the phosphorane intermediate. Even if the dianionic phosphorane was sufficiently stable to be a true intermediate, pseudoration is expected to be slow, since it would require that a negatively charged oxygen ligand adopts an apical position, which is energetically unfavorable.[11]

We show a possible mechanism for the reactions involved in Scheme 1. After initial attack at phosphorus by the 2′O anion,[11] cleavage can be achieved by direct breakdown of the initial phosphorane if the leaving group occupies an axial position. Alternatively, if phosphorane **4** forms (i.e., with an axial oxyanion, breaking one of Westheimer's rules[13]), pseudorotation to **3** and expulsion of the 3′O leads to 2′5′UpU. For isomerization to proceed by interconverting the phosphoranes involved in the cleavage pathway, then three pseudorotation steps would be required, still passing through both **3** and **4**.

The rate enhancement for isomerization and cleavage observed in the present work presumably results from the combination of the Lewis acid effect of the Zn ions and hydrogen bond donors of the ligand stabilizing the anionic oxygens of the phosphorane. The approximate upper limit of a rate enhancement that can be achieved by neutralization of the charge on the phosphate, without additional catalysis of the nucleophilic attack by the alkoxy anion or of the departure of the leaving group, can be estimated from the reactions of neutral phosphotriesters. Previous results obtained with nucleoside phosphotriesters have shown that the spontaneous cleavage of nucleoside phosphotriesters under neutral conditions is about 10^5 fold faster than that of corresponding phosphodiesters.[14] Considering that **1.M** binds the phosphate through two coordinate bonds and four hydrogen bonds, the rate enhancement observed could well be attributed to strong interactions between the catalyst and the phosphate group. However, data obtained with nucleoside phosphotriesters also show that under neutral conditions nucleophilic attack on a neutral phosphate results in extremely fast phosphate migration relative to cleavage, via a monoanionic phosphorane. In the present case the catalysis of the cleavage is efficient, whereas the isomerization is only modestly enhanced. This shows that, in addition to electrophilic catalysis, the complexes of ligand **1a** direct the reaction of the phosphorane toward the cleavage products, but it is not possible to say whether this results from catalysis of the cleavage reaction or from the inhibition of pseudorotation. Enzymes or ribozymes that catalyze RNA cleavage do not appear to catalyze isomerization as well, but presumably in these cases, the confines of the active site even more firmly direct the reaction toward cleavage if similar phosphoranes are involved. These data do clearly demonstrate that dinuclear metal ion complexes with second sphere hydrogen bond donors stabilize a phosphorane intermediate enough to allow pseudorotation.

Acknowledgment. We thank the BBSRC for financial support and Dr I. Rosenberg for generously providing compound **2**.

Supporting Information Available: Experimental methods for kinetic analysis and representative chromatograms showing reaction progress for isomerization of **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Perreault, D. M.; Anslyn, E. V. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 432. (b) Oivanen, M.; Kuusela, S.; Lönnberg, H. *Chem. Rev.* **1998**, *98*, 961. (c) Range, K.; McGrath, M. J.; Lopez, X.; York, D. M. *J. Am. Chem. Soc.* **2004**, *126*, 1654. (d) Kluger, R.; Taylor, S. *J. Am. Chem. Soc.* **1991**, *113*, 5714.
(2) Li, Y.; Breaker, R. R. *J. Am. Chem. Soc.* **1999**, *121*, 5364.
(3) (a) Morrow, J. R.; Iranzo, O. *Curr. Opin. Chem. Biol.* **2004**, *8*, 192. (b) Feng, G.; Natale, D.; Prabaharan, R.; Mareque-Rivas, J. C.; Williams, N. H. *Angew. Chem., Int. Ed.* **2005**, *45*, 7056.
(4) Kaukinen, U.; Lyytikäinen, S.; Mikkola, S.; Lönnberg, H. *Nucleic Acids Res.* **2002**, *30*, 468.
(5) (a) Molenveld, P.; Engbersen, J. F. J.; Reinhoudt, D. N. *Chem. Soc. Rev.* **2000**, *29*, 75. (b) Chin, J. *Curr. Opin. Chem. Biol.* **1997**, *1*, 514.
(6) Although Brown's recent work in methanol shows much greater activity for Zn(II) complexes than Cu(II) (Lu, Z. L.; Liu, C. T.; Neverov, A. A.; Brown, R. S. *J. Am. Chem. Soc.* **2007**, *129*, 11642; Neverov, A. A.; Lu, Z. L.; Maxwell, C. I.; Mohamed, M. F.; White, C. J.; Tsang, J. S.; Brown, R. S. *J. Am. Chem. Soc.* **2006**, *128*, 16398).
(7) Maret, W.; Vallee, B. L. *Methods Enzymol.* **1993**, *226*, 52.
(8) Kuusela, S.; Lönnberg, H. *J. Phys. Org. Chem.* **1993**, *6*, 347.
(9) Lönnberg, T.; Kralikova, S.; Rosenberg, I.; Lönnberg, H. *Collect. Czech. Chem. Soc.* **2006**, *71*, 859.
(10) (a) Beckmann, C.; Kirby, A. J.; Kuusela, S.; Tickle, D. C. *J. Chem. Soc., Perkin Trans. 2* **1997**, 573. (b) Virtanen, N.; Nevalainen, V.; Lehtinen, T.; Mikkola, S. *J. Phys. Org. Chem.* **2007**, 72.
(11) (a) Lopez, X.; Dejaegere, A.; Leclerc, F.; York, D. M.; Karplus, M. *J. Phys. Chem. B* **2006**, *110*, 11525. (b) López, C. S.; Faza, O. N.; de Lera, A. R.; York, D. M. *Chem.—Eur. J.* **2005**, *11*, 2081.
(12) The reactive tautomer of the substrate bound to the complex, as proposed by ourselves (Feng, G.; Mareque-Rivas, J. C.; Torres Martín de Rosales, Williams, N. H. *J. Am. Chem. Soc.* **2005**, *127*, 13470) and Richard and Morrow (Yang, M.-Y.; Iranzo, O.; Richard, J. P.; Morrow, J. R. *J. Am. Chem. Soc.* **2005**, *127*, 1064; O'Donoghue, A.; Pyun, S. Y.; Yang, M.-Y.; Morrow, J. R.; Richard, J. P. *J. Am. Chem. Soc.* **2006**, *128*, 1615).
(13) Westheimer, F. H. *Acc. Chem. Res.* **1968**, *1*, 70.
(14) Kosonen, M.; Lönnberg, H. *J. Chem. Soc., Perkin Trans. 2* **1995**, 1203.

JA711347W