Rapid and highly base selective RNA cleavage by a dinuclear Cu(II) complex

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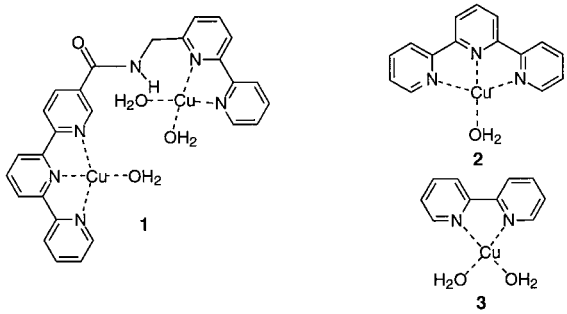
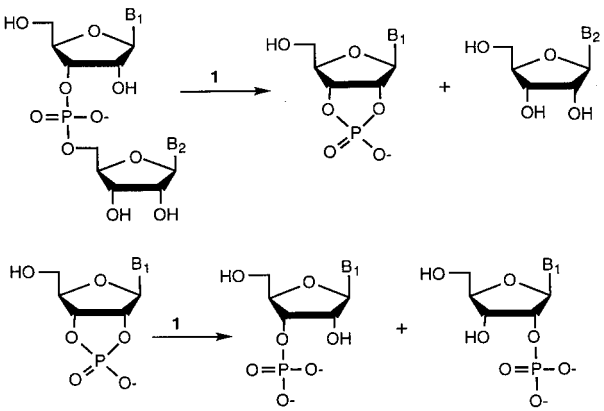
A bis-Cu(II) complex based on a covalently linked terpyridine and bipyridine ligand system is shown to rapidly cleave bis-ribonucleotides with remarkable selectivity for adenine bases.

Currently there is a great deal of interest in the design of catalysts for rapid and base selective RNA hydrolysis.[1,2] Such artificial ribonucleases may be useful as novel therapeutic agents for cancer and viral diseases[2f–i,3–5] or as chemical probes for RNA sequencing and structure mapping.[6] However, despite much effort none of the RNA cleaving agents developed so far has achieved the level of activity afforded by natural ribonucleases. Moreover, selective cleavage at specific base sites is a hallmark of the ribonucleases but is seen in few synthetic systems. We recently showed that dinuclear Cu(II) complex **1**



not only is more active than mononuclear complexes **2** and **3** but also exhibits remarkable base selectivity in promoting the hydrolytic cleavage of ribonucleoside 2′,3′-monophosphates, intermediates in RNA cleavage by natural ribonucleases.[7] We have now found that **1** is also highly active in promoting the hydrolysis of ribonucleotides and report here on this metal-based artificial ribonuclease that shows both potency and base selectivity.

Complex **1** was prepared as described previously.[8] The hydrolysis of RNA dimers in the presence of excess **1** was followed by HPLC.† The reaction proceeded by a transesterification–hydrolysis mechanism with ribonucleoside 2′,3′-monophosphates as intermediates (Scheme 1). All reactions followed first-order kinetics.The pH–rate profile in Fig. 1 shows



Scheme 1 Hydrolysis of RNA dimers in the presence of **1**–**3**. B_1 and B_2 are nucleobases.

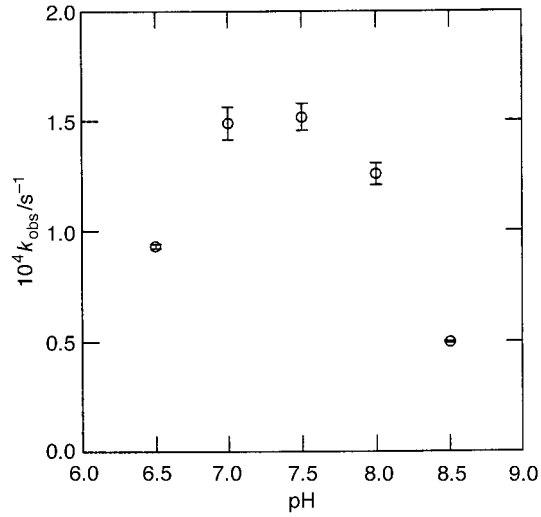


Fig. 1 pH–rate profile for the hydrolysis of ApA in the presence of **1** at 25 °C. [ApA] = 0.1 mM, [**1**] = 2.0 mM. The reaction media at pH 7.0, 7.5 and 8.0 are the HEPES buffers (0.05 M); that at pH 8.5 is the AMPSO buffer (0.05 M) and that at pH 6.5 is the MES buffer (0.05 M).

that **1** reaches its maximum activity at pH 7.5, consequently all other reactions were carried out at this pH. Table 1 summarizes the first-order rate constants and relative rates for the hydrolysis of six RNA dimers in the presence of dinuclear **1** and mononuclear **2**. All reactions with **2** as catalyst were carried out at pH 8.0 because previous work by Chin and coworkers found that related complexes reached their maximum activity near this pH in catalyzing the hydrolysis of ApA.[2c] The hydrolysis of RNA dimers in the presence of **3** was too slow to allow accurate determination of the first-order rate constants.

The remarkable base selectivity of dinuclear complex **1** is evident from the relative rates for different RNA dimers in Table 1. As in the hydrolysis of nucleoside 2′,3′-cyclic monophosphate, **1** is highly selective for adenine, hydrolyzing ApA 12, 17 and 87 times faster than CpC, UpU and GpG, respectively. The intrinsic differences in hydrolysis rate for the different dinucleotides (caused presumably by intra- or intermolecular interactions) are much smaller, with purine–purine

Table 1 First-order rate constants (k_{obs}) and relative rates (k_{rel}) for the hydrolysis of RNA in the presence of dinuclear Cu(II) complex **1** and mononuclear Cu(II) complex **2**

	Cu(II) complex **1**[a]		Cu(II) complex **2**[b]	
Substrate	$10^6 k_{obs}/s^{-1}$	k_{rel}	$10^7 k_{obs}/s^{-1}$	k_{rel}
ApA	152 ± 5	87	45 ± 2	7.8
CpC	13.2 ± 0.2	7.5	18 ± 0.1	3.1
UpU	8.7 ± 0.2	5.0	5.8 ± 0.5	1.0
GpG	1.75 ± 0.19	1.0	11.8 ± 0.2	2.0
ApC	49 ± 0.3	28	—	—
CpA	53 ± 1.0	30	—	—

[a] In pH 7.5 HEPES buffer (0.05 M) at 25 °C; [RNA] = 0.1 mM, [**1**] = 2.0 mM. [b] In pH 8.0 HEPES buffer (0.05 M) at 25 °C; [RNA] = 0.1 mM, [**2**] = 2.0 mM.

Table 2 Relative rates (k_{rel}) for the hydrolysis of RNA and nucleoside 2′,3′-monophosphates in the presence of dinuclear Cu(II) complex **1**

Substrate	k_{rel}	Substrate	k_{rel}[a]
ApA	87	2′,3′-cAMP	31
CpC	7.5	2′,3′-cCMP	5.2
UpU	5.0	2′,3′-cUMP	3.0
GpG	1.0	2′,3′-cGMP	1.0

[a] Calculated from the first-order rate constants (k_{obs}) in ref. 7.

sequences (*e.g.* ApA) being among the slowest.[9] Replacement of either adenine group in ApA by a cytidine group diminished the rate of **1**-catalysed hydrolysis threefold. This level of base selectivity is unprecedented in a simple metal-based ribonuclease mimic that lacks any appended recognition elements (such as oligonucleotide strands). Dinuclear complex **1** is also highly active: at a concentration of 2.0 mM it provides over five orders of magnitude rate acceleration for the hydrolysis of its best substrate, ApA.[10] The data in Table 1 also show that mononuclear complex **2** has moderate selectivity for adenine. Apparently, attachment of a bipyridine–Cu(II) unit to **2** not only increases its activity but also amplifies its base selectivity.

We have suggested[7] that a strong π–π stacking interaction between adenine and the bipyridine–Cu(II) unit may be the principal reason for the high selectivity of **1** for adenine in **1**-catalyzed hydrolysis of nucleoside 2′,3′-cyclic monophosphates. This same interaction also appears to be responsible for the base selectivity shown by **1** in promoting the hydrolysis of RNA. First, the base selectivities for the dinucleotide and cyclic monophosphate substrates parallel each other, as can be seen in Table 2. Second, the high selectivity of **1** for adenine in the hydrolysis of RNA dimers is insensitive to the position of the adenine group relative to the phosphate bond to be cleaved since ApC and CpA have almost identical reactivity (Table 1). These observations are more consistent with association through a less specific π–π stacking rather than more directed interactions such as hydrogen bonding and metal coordination to the nucleobases as major sources of base selectivity. Face-to-face stacking provides an interaction that is adaptable to different nucleobase positions. If hydrogen bonding or metal coordination from **1** to the nucleobases were important in stabilizing the interaction, it is probable that different base selectivities between the RNA dimers and nucleoside 2′,3′-cyclic monophosphates would result due to the different orientations and flexibilities in the two sets of substrates. Likewise, ApC and CpA should also show quite different reactivities due to the different positions of adenine in the two substrates.

In conclusion, dinuclear Cu(II) complex **1** has been shown to function as a highly active artificial ribonuclease. In addition, a remarkable selectivity among the different nucleotide bases is seen with particularly effective cleavage of adenine-containing substrates. We are currently extending these dinuclear Cu(II) complex designs to enhance the level of activity and to alter the base selectivity.

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Notes and references

† Hydrolysis of RNA dimers by **1** was followed by HPLC (Ranin). The following procedure is typical: 2.85 mL of **1** (2.1 mM) in a buffer solution was mixed with 0.15 mL of an RNA dimer (2.0 mM) in deionized H_2O. Aliquots (300 μL) of the reaction mixture were quenched with 50 mM EDTA (300 μL). After filtration, the quenched solution (15 μL) was injected onto a C-18 reversed-phase column and eluted for 10–15 min with 0–15% MeCN in H_2O containing 0.1% CF_3CO_2H (flow rate = 1.0 mL min^{-1}). The eluent was monitored at λ_{max} of the nucleobase (260 nm for adenine and uracil, 252 nm for guanine and 268 nm for cytidine) by a Ranin UV detector. The first-order rate constants (k_{obs}) were obtained as slopes of plots of $\ln(A_0/A_t)$ *vs.* *t*, where A_0 and A_t are the integrations of the areas of the HPLC peaks for the RNA dimer at $t = 0$ and *t*, respectively.

1 Reviews: B. N. Trawick, A. T. Daniher and J. K. Bashkin, *Chem. Rev.*, 1998, **98**, 939; M. Komiyama, *J. Biochem.*, 1995, **118**, 665; J. R. Morrow, *Adv. Inorg. Biochem.*, 1994, **9**, 41; M. W. Gobel, *Angew. Chem.*, 1994, **106**, 1201; *Angew. Chem., Int. Ed. Engl.*,1994, **33**, 1141; D. S. Sigman, A. Mazumder and D. M. Perrin, *Chem. Rev.*, 1993, **93**, 2295; J. Chin, *Acc. Chem. Res.*, 1991, **24**, 145.

2 Recent examples: (*a*) P. Hurst, B. K. Takasaki and J. Chin, *J. Am. Chem. Soc.*, 1996, **118**, 9982; (*b*) M. J. Young and J. Chin, *ibid.*, 1995, **117**, 10 577; (*c*) B. Linkletter and J. Chin, *Angew. Chem.*, 1995, **107**, 529; *Angew. Chem., Int. Ed. Engl.*, 1995, **34**, 472; (*d*) W. H. Chapman, Jr. and R. Breslow, *J. Am. Chem. Soc.*, 1995, **117**, 5462; (*e*) M. Yashiro, A. Ishikubu and M. Komoyama, *Chem. Commun.*, 1997, 83; (*f*) M. Yashiro, A. Ishikubu and M. Komiyama, *J. Chem. Soc., Chem. Commun.*, 1995, 1793; (*g*) M. Komiyama, N. Takeda, M. Irisawa and M. Yashiro, in *DNA and RNA Cleavers and Chemotherapy of Cancer and Viral Diseases*, ed. B. Meunier, Kluwer Academic Publishers, Boston, 1996, p. 321; (*h*) J. K. Bashkin, J. Xie, A. T. Daniher, L. A. Jenkins and G. C. Yeh, *ibid.*, p. 355; (*i*) R. Haner, J. Hall, D. Husken and H. E. Moser, *ibid.*, p. 307; (*j*) D. Magda, R. A. Miller, M. Wright and J. Rao, *ibid.*, p. 337; (*k*) J. R. Morrow and V. M. Shelton, *New J. Chem.*, 1994, **18**, 371; (*l*) R. Ott and R. Krämer, *Angew. Chem., Int. Ed.*, 1998, **37**, 1957.

3 C. A. Stein and J. S. Cohen, *Cancer Res.*,1988, **48**, 2659.

4 E. Uhlmann and A. Peyman, *Chem. Rev.*, 1990, **90**, 543.

5 J. F. Milligan, M. D. Matteucci and J. C. Martin, *J. Med. Chem.*, 1993, **36**, 1923.

6 For the techniques currently available for RNA sequencing and structure mapping see: T. D. Tullus, in *Bioorganic Chemistry: Nucleic Acids*, ed. S. M. Hecht, Oxford University Press, New York, 1996, p. 1244.

7 S. Liu, Z. Luo and A. D. Hamilton, *Angew. Chem., Int. Ed. Engl.*, 1997, **36**, 2678.

8 S. Liu and A. D. Hamilton, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 1779.

9 T.Koike and Y. Inoue, *Chem. Lett.*, 1972, 569.

10 The background rate of hydrolysis of ApA at pH 7.5 is *ca.* 1.6×10^{-10} s^{-1}, see Y. Matsumoto and M. Komiyama, *J. Chem. Soc., Chem. Commun.*, 1990, 1050; K. Yoshinari and M. Komiyama, *Chem. Lett.*, 1990, 519.

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