Bis-imidazolyl cleft-shaped mimic of the active site of ribonuclease A

Teru Kato,[a] Toshifumi Takeuchi[b] and Isao Karube*[a]†

[a] *Research Center for Advanced Science and Technology, The University of Tokyo, 4-6-1 Komaba, Meguro-ku, Tokyo 153, Japan*
[b] *Laboratory of Synthetic Biochemistry, Faculty of Information Sciences, Hiroshima City University, 151-5 Ozuka, Numata-cho, Asaminami-ku, Hiroshima 731-31, Japan*

A newly synthesized cleft shaped mimic of the active site of ribonuclease A accelerates the cleavage of (3′–5′) adenylyl adenosine at pH 7.0–8.0.

Ribonucleases and their molecular mimics are of considerable interest to chemists.[1–3] They can be used as probes for the investigation of RNA structure[4] and for the specific inactivation of target genes[5] by the cleavage of the respective transcribed mRNA. In this paper, compounds mimicing the active site of bovine pancreatic ribonuclease A (RNase A) are described and their RNA cleavage activities demonstrated.

The structure and catalytic mechanism of RNase A have been well characterized.[6] The catalytic site contains two key histidine residues (His119 and His12), which act as a proton donor (imidazolium) and a proton acceptor (imidazole) respectively.[7] Compound **1**‡ was designed to provide a cleft shaped[8] topographical and functional mimic of the two essential imidazole groups of the histidines of bovine RNase A. The strategy behind the design of compound **1** was to space the imidazole groups at a suitable distance and orientation, such that they would act as general bifunctional acid and base catalysis elements similar to the histidines in the natural RNase A (Fig. 1). Based upon CPK model examinations, we selected a dipicolinoyl group as a rigid spacer between the two imidazole moieties.

The cleavage activity of compound **1** was compared to that of imidazole and compound **2** containing only one imidazole moiety. Adenylyl (3′–5′) adenosine (ApA)§ and related derivatives were used as substrates for the evaluation of cleavage activities.¶ The typical products of the reactions with compounds **1** and compound **2** under the conditions employed (50 °C, pH 7.5 and 4 d) are shown in Fig. 2. Compound **1** degraded 30% of the initial ApA to yield adenosine 3′-phosphate (A3′p), adenosine 2′-phosphate (A2′p), adenosine 2′,3′-cyclic phosphate (A > p) and adenine (A). Compound **2** and imidazole (data not shown) barely degraded ApA, even at two fold higher concentration. Moreover, no substrate cleavage was observed in the absence of compounds **1**, **2** and imidazole. The formation of A2′p and A > p indicates that the degradation of ApA proceeds *via* nucleophilic attack of the 2′-hydroxy group of ribose to phosphodiester, and not by simple basic hydrolysis of the phosphodiester followed by the formation of A3′p and A5′p. As the reported reaction mechanism of RNase A[6] involves the formation of 2′,3′-cyclic phosphate, followed by hydrolysis to yield 3′-phosphate, the A > p formation would therefore arise from the first step in the RNase A-induced reaction.

The cleavage activity of a DNA dimer, deoxyadenylyl adenosine [d(ApA)] was similarly examined. Experiments showed, however, that compounds **1**, **2** and imidazole could not degrade d(ApA) using the same reaction conditions as above. This observation provides additional support for the cleavage reaction proceeding *via* a transesterification process. The formation of comparable amounts of A2′p and A3′p also rules out the possibility of contamination with natural RNase enzymes classified into two types: one yields only ribonucleoside 3′-phosphate and the other yields only ribonucleoside 5′-phosphate.

Fig. 3 shows the pH dependence of the RNA cleavage activity of compounds **1**, **2** and imidazole. Compound **1** exhibited high cleavage activity in a pH range of approximately 7–8, whilst compound **2** and imidazole showed almost no activity in a pH range of 6–9, even at two fold higher concentration. The optimum pH for the catalytic reaction with compound **1** was 7.6 with about 35% cleavage of ApA under the given conditions. The rate acceleration by compound **1** (1 equiv. compared to ApA) was estimated to be approximately 50 fold over compound **2** (2 equiv. compared to ApA) at pH 7.6. The pH dependence of the cleavage reaction shows a bell shaped profile similar to that of RNase A enzyme catalysed reactions. These results suggest that the two imidazole groups of **1** are crucial for its cleavage activity. From a preliminary titration study, pK_as of 6.1 and 8.5 were estimated for the two imidazole groups. It is most likely that a mono-cationic species, *i.e.* the protonation of only one imidazole group of compound **1**, is present in the active pH range (pH 7.0–8.0). Accordingly, the bell shaped

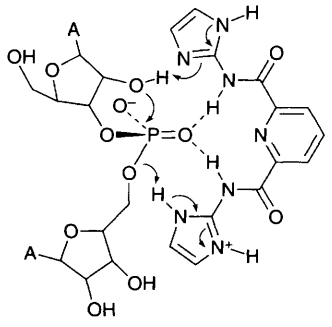

Fig. 1 Possible RNA cleavage mechanism by the mimic **1**

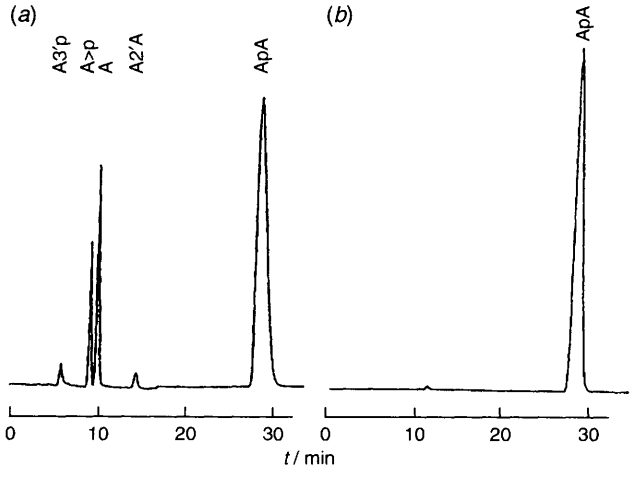

Fig. 2 A typical chromatogram of the reaction products of the cleavage of ApA: (*a*) with 10 mmol dm^{-3} of **1**; (*b*) with 20 mmol dm^{-3} of **2**. Reaction conditions: 50 °C, 4 d, 10 mmol dm^{-3} of ApA.

pH-cleavage activity profile would be dependent upon the concentration of the mono protonated species.

The two imidazole groups in the active site of RNase A[6] are suitably positioned and oriented for hydrolytic catalysis. Owing to the possibility of the formation of intramolecular hydrogen bonds between proton donating and accepting groups, previously reported RNase mimics[3] having these two groups showed no catalytic activity without the help of affinity groups for the RNA substrate. Thus those mimics often required an elevated concentration in order to achieve efficient cleavage activity.[2] By using a rigid dipicolinic spacer, as in compound **1**, the formation of an intramolecular hydrogen bond between the imidazole and the imidazolium group is prevented.

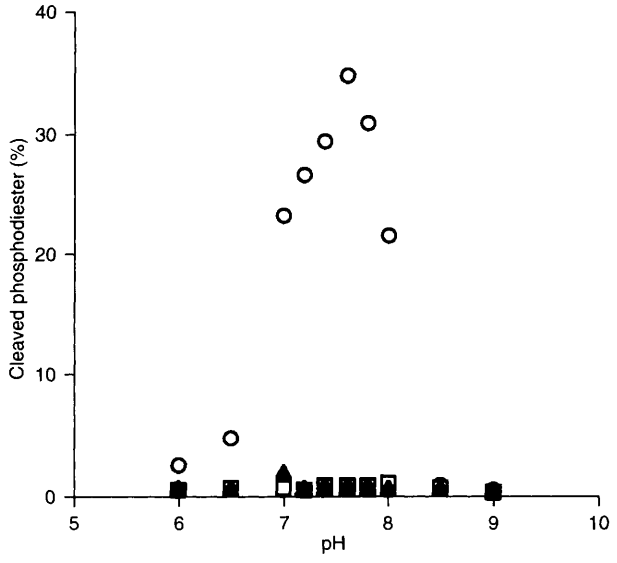

Fig. 3 Effects of pH on the cleavage of ApA by 10 mmol dm^{-3} of **1** (○), 20 mmol dm^{-3} of **2** (□) and 20 mmol dm^{-3} of imidazole (▲). ApA: 10 mmol dm^{-3}.

This work was supported by The Ministry of Education, Science, Sports and Culture: Large-scale Research Projects under the New Program in Grants-in-Aid for Scientific Research.

Footnotes

† E-mail: karube@bio.rcast.u-tokyo.ac.jp

‡ Compound **1** was synthesized from pyridine-2,6-dicarboxylic acid dichloride and 2-aminoimidazole toluene-*p*-sulfonate in a single step. δ_H ([$^2H_6$]Me_2SO) 6.07 (d, *J* 25 Hz, 4-H, H-4, 5 Im), 7.52 (m, 3 H, H-3, 4, 5 Py), 11.14 (br, s, 2 H, CONH), 11.52 (br s, 2 H, H-1 Im).

§ Though ApA is not a substrate of natural RNase A, we used it as a substrate of cleavage reactions because we focused on the catalytic mechanism of the active site of RNase A.

¶ All reactions were analysed on a YMC R-ODS-5 column eluted with 20 mmol dm^{-3} triethylammonium acetate buffer pH 6.0–acetonitrile (93 : 7). The extent of RNA hydrolysis was determined from the ratio of substate and product peaks integrals at 260 nm.

References

1 B. Barbier and A. Brack, *J. Am. Chem. Soc.*, 1992, **114**, 3511; R. Breslow, *Acc. Chem. Res.*, 1991, **24**, 317; M. P. Mertes and K. B. Mertes, *Acc. Chem. Res.*, 1990, **23**, 413.

2 M. Komiyama and K. Yoshinari, *J. Chem. Soc., Chem. Commun.*, 1989, 1880; K. Yoshinari, K. Yamazaki and M. Komiyana, *J. Am. Chem. Soc.*, 1991, **113**, 5899.

3 M. A. Podyminogin, V. V. Vlassov and R. Giegé, *Nucleic Acids Res.*, 1993, **21**, 5950.

4 A. Garcia, R. Giegé and J. P. Behr, *Nucleic Acids Res.*, 1990, **18**, 89.

5 C. Giovannangeli, N. T. Thuong and C. Hélène, *Proc. Natl. Acad. Sci. USA.*, 1993, **90**, 10013.

6 H. W. Wyckoff, D. Tsernoglou, A. W. Hanson, J. R. Knox, B. Lee and F. M. Richards, *J. Biol. Chem.*, 1970, **245**, 305; R. Blackburn and S. Moore, *The Enzymes*, Academic Press, New York, 1982, vol. 15, ch. 12, pp. 317–433.

7 E. Anslyn and R. Breslow, *J. Am. Chem. Soc.*, 1989, **111**, 4473.

8 F. Chu, L. S. Flatt and E. V. Anslyn, *J. Am. Chem. Soc.*, 1994, **116**, 4194; P. Tecilla, S. K. Chang and A. D. Hamilton, *J. Am. Chem. Soc.*, 1990, **112**, 9586; V. Julian, R. P. Dixon and A. D. Hamilton, *J. Am. Chem. Soc.*, 1992, **114**, 1120; J. Smith, K. Ariga and E. V. Anslyn, *J. Am. Chem. Soc.*, 1993, **115**, 362.

Received, 20th December 1995; Com. 5/08260I