Ribonuclease Mimic: Zn^{2+} Promoted Cleavage of C8-Histamino-r(UpA) proceeds through 2',3'-cUMP as Intermediate†

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The designed ribodinucleotide **1** containing a proximal imidazole, in the presence of $ZnCl_2$, undergoes self hydrolysis faster than unmodified r(UpA); like its enzymatic counterpart, this reaction goes through the same steps of initial transesterification to 2',3'-cUMP followed by hydrolysis to 2'- or 3'-UMP.

Hydrolytic fission of phosphodiester linkages in nucleic acids is chemically challenging[1] and has recently attracted much attention.[2] Extensive studies on the mechanism of ribonuclease A catalysis[3] have led to the development of nuclease mimics.[4] The active site of ribonuclease is composed of two histidines (His-12 and 119) and a lysine (Lys-41) as chemical operators involved in catalysis. Model studies have shown that RNA can be cleaved by acid–base catalysis in an imidazole buffer *via* a sequential bifunctional catalysis.[5] In an attempt to mimic the phosphate cleavage by taking advantage of rate acceleration in the intramolecular reaction,[6] we synthesised the ribodinucleotide **1** which incorporates the catalytic imidazole spatially close to the phosphate and 2'-hydroxy groups.[7] In this communication, we report successful self-cleavage of the internucleotide phosphodiester bond in **1** promoted by Zn^{2+} with a significant rate enhancement compared to the unmodified r(UpA) **2**. Importantly, the reaction is a true mechanistic mimic of ribonuclease action; it goes through the same steps of initial transesterification followed by hydrolysis of the intermediate 2',3'-cyclic phosphodiester.

Upon incubation of **1** with $ZnCl_2$ (1 mmol dm^{-3}) in Hepes buffer (pH 7.0) at 40 °C, two fission products were seen by HPLC analysis [Fig. 1(*a*,*b*)]. Of the two products, the one eluting at 10.35 min was identified as **3** by comparison with a standard, and the formation of this product indicated that cleavage was taking place at the P–O5' bond rather than the O3'–P bond. At 70 °C, the reaction was complete within 10–12 h and gave **3** as one of the products [Fig. 1(*d*,*e*)]. However, the other product was different from the corresponding product of the reaction at 40 °C. A time course of the reaction at 70 °C, followed by HPLC, indicated that the initially formed product at 1.99 min [Peak I, Fig. 1(*c*)] was slowly and completely transformed to a second product eluting at 2.64 min [Peak II, Fig. 1(*d*,*e*)]. By comparison with an authentic sample, coinjection (HPLC) and ^{31}P NMR, (δ 20.27), peak I was identified as 2',3'-cyclic UMP **4** which underwent further hydrolysis to a mixture of 2'- and 3'-monophosphates **5** (Peak II). Peak I of the 70 °C reaction was identical to the early eluting peak at 40 °C. Thus reaction of **1** with $ZnCl_2$ at 40 °C does not go beyond 2',3'-cUMP formation while at 70 °C the

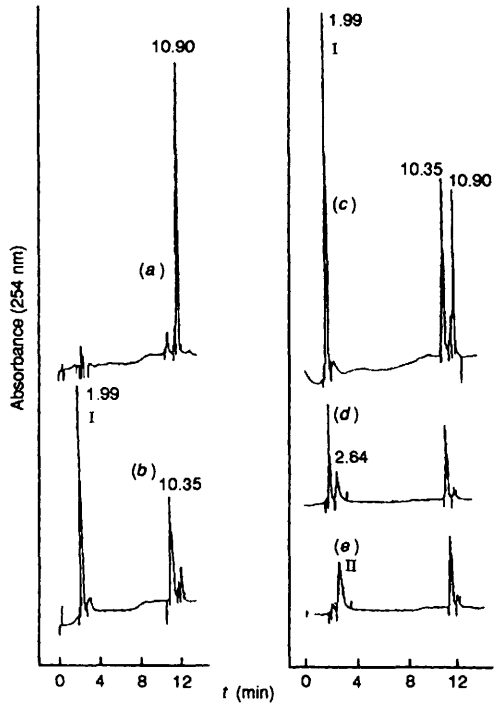

Fig. 1 HPLC analysis of cleavage of **1**: (*a*) 40 °C, 0 h; (*b*) 40 °C, 207.5 h; (*c*) 70 °C, 4 h; (*d*) 70 °C, 17 h; (*e*) 70 °C, 49 h

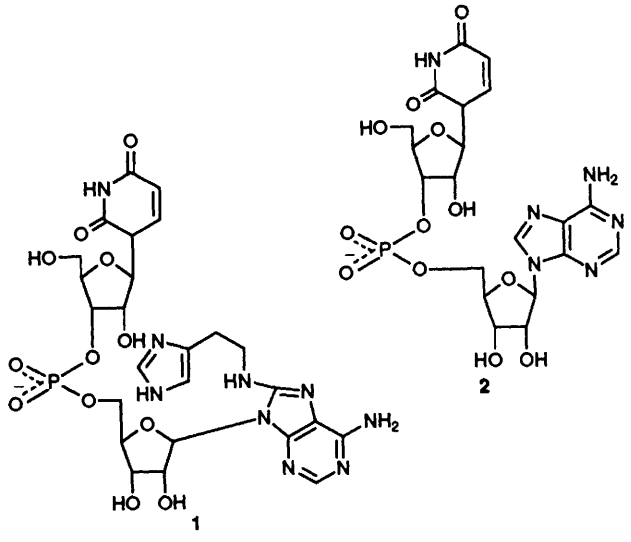

Table 1 Pseudo-first-order rate constants for $ZnCl_2$ promoted hydrolysis[a] of **1** and **2**

T/°C	$10^3 k$ (**1**)/h^{-1}	$10^3 k$ (**2**)/h^{-1}	k(**1**)/k(**2**)
40	12.90 ± 0.65	0.89 ± 0.01	14.50
55	70.60 ± 1.20	7.24 ± 0.43	9.60
70	316.60 ± 2.50	30.37 ± 1.13	10.10
55[b]	74.00 ± 1.45	1.20 ± 0.06	61.70

[a] Typical analysis: the substrate **1** (1.7×10^{-5} mol dm^{-3}) in Hepes buffer (10 mmol dm^{-3}, 440 μl, pH 7.0) containing $ZnCl_2$ (1 mmol dm^{-3}) was heated at 55 °C and aliquots (40 μl) were withdrawn and quenched by addition of EDTA (1 mmol dm^{-3}) before injecting into HPLC for analysis on C18 column. Solvent: Buffer A = 0.1 mol dm^{-3} NEt_4O_2CMe, Buffer B = 0.1 mol dm^{-3} NEt_4O_2CMe, in 30% acetonitrile, A to B in 20 min. In case of **2** similar analysis was done but in presence of imidazole (0.5 mmol dm^{-3}). [b] Hydrolysis reaction in presence of 10 mmol dm^{-3} $ZnCl_2$.

Table 2 Thermodynamic parameters[a] for hydrolysis of **1** and **2**

Dinucleotide	E_a/ kcal mol^{-1}	$\Delta H°$/ kcal mol^{-1}	$\Delta G°$/ kcal mol^{-1}	$\Delta S°$/cal mol^{-1} K^{-1}
1	23.6	22.9	26.5	−10.6
2	27.1	26.4	28.1	−5.1

[a] E_a was calculated from a plot of log K *vs.* T^{-1}. Standard error: ± 0.025 ± 0.4 kcal mol^{-1}. 1 cal = 4.184 J.

reaction leads to the formation of 2′- or 3′-monophosphate through the detectable intermediate cyclic phosphate species. In both cases no 2′,5′-isomerised product was formed. The unmodified ribodinucleotide r(UpA) **2** behaved similarly in the presence of $ZnCl_2$ (1 mmol dm^{-3}) and imidazole (0.5 mmol dm^{-3}), but at much slower rates. Other metal salts such as $MgCl_2$, $CoCl_2$, $NiCl_2$ and $CuCl_2$ were ineffective in inducing cleavage even at higher temperature (70 °C).

The pH–rate profile of the fission reaction of **1** showed a bell-shape with a maximum at pH 7.0. This is characteristic of acid–base catalysis by imidazole.[8] It also rules out the alternative possibility of catalysis by a direct nucleophilic attack of Zn-bound hydroxide as this would lead to enhanced rates with increasing pH.

The time-progression of the reactions of **1** and **2** at different temperatures (40, 55 and 70 °C) was monitored by HPLC, and Table 1 shows the rate constants for the first transesterification step, calculated by fitting the data into pseudo-first order kinetics. It is seen that the presence of the internal imidazole in **1** causes 10–15 fold rate accelerations compared with unmodified **2**. The enhancement factor was even larger (62) at higher $ZnCl_2$ concentration (10 mmol dm^{-3}).‡ The kinetic data at 70 °C also indicated that 2′,3′-cUMP **4** hydrolysed 12 times slower than the initial transesterification. Table 2 shows the thermodynamic parameters computed for the reactions of **1** and **2** from the temperature dependence of the reaction rates. The energy of activation E_a for **1** is lower by 4 kcal mol^{-1} (1 cal = 4.184 J) than that of **2**, implying catalysis by the linked imidazole in **1**. A catalytically favourable lower enthalpy of activation ($\delta\Delta H$ 3.5 kcal mol^{-1}) was accompanied by a large negative entropy contribution ($\delta\Delta S$ −5 cal mol^{-1} K^{-1}).

The spatial proximity of anchored imidazole thus causes a significant acceleration of the first step (transesterification) leading to formation of intermediate 2′,3′-cyclic phosphate **4**. The magnitude of the observed rate enhancement corresponds to an effective molar concentration of 10–15 mol dm^{-3} which is reasonable for local concentration effects in intramolecular reactions.[6,8] The imidazole attached to C8 of dA, though it increases the local effective concentration, may not have a suitable orientation to enhance the catalysis dramatically. Its spatial predisposition is influenced by the preferred conformation of the rigid dinucleotide[7] and such orientational effects may contribute to unfavourable entropic factors.

Based on the above analysis, the sequence of events taking place in the transesterification–hydrolysis of **1** may be shown as in Scheme 1. This is similar to that occurring in the ribonuclease A[3,9] reaction with the difference that Zn^{2+} performs the role of the imidazolium (His-119) ion. It is possible that the spatial predisposition of imidazole may also assist cleavage by protonation of the departing 5′-alkoxide group, similar to Lys-41 in ribonuclease A. Thus self cleavage–hydrolysis of **1** in the presence of $ZnCl_2$ is conceptually a mechanistic mimic of the ribonuclease function, and future efforts are focussed on further tuning of the catalysis by variation in spacer chain and introduction of electrophilic cations such as guanidinium, which is a good phosphate receptor,[10] into the model.

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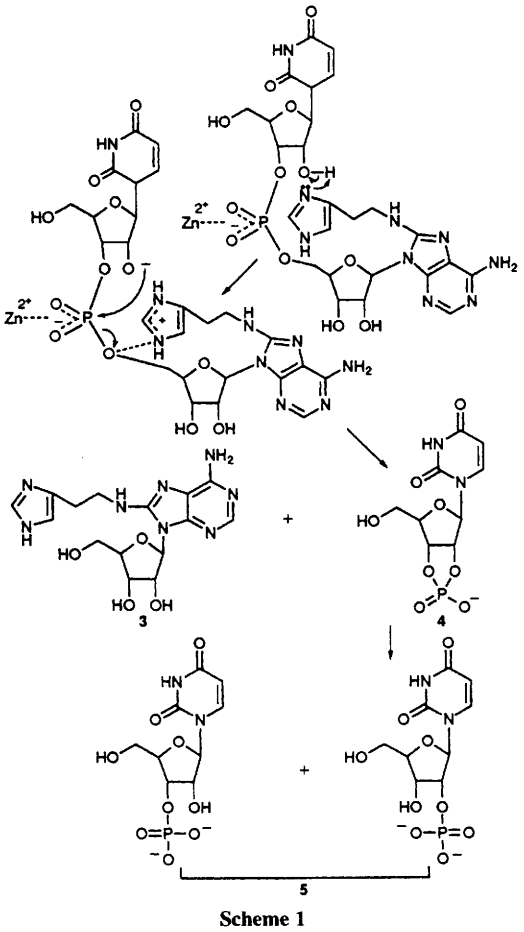

Scheme 1

Footnotes

† N. C. L. Communication number: 5937.

‡ An increase in the $ZnCl_2$ concentration inhibits hydrolysis of r(UpA) since it complexes away the catalytic imidazole. See Breslow *et al.*[3b] Such was not the case with **1**.

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