Uracil-targeted Inhibition of Poly(A)–Poly(U) Hybridization by a Zinc(II)–cyclen Complex

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A zinc(II)–cyclen complex, which is a highly selective host for thymidine (dT) and uridine (U) in aqueous solution at physiological pH, inhibits both poly(A)–poly(U) hybridization and poly(U)–directed polyphenylalanine synthesis.

Metal complexes that can bind to specific nucleobases or sequences and compete with the control elements are currently attracting great interest.[1] Recently,[2,3] we discovered that Zn^{II}–cyclen complex 1 (cyclen = 1,4,7,10-tetraazacyclododecane)[4] is a highly selective host *in aqueous solution at physiological pH* for thymidine (dT) and uridine (U) among the DNA and RNA nucleosides {log K = 5.6 and 5.2, respectively, at 25 °C: K = [2]/([1]·[N(3)-deprotonated nucleoside]), dm^3 mol^{-1}}. In the resulting ternary complexes 2, the deprotonated imide nitrogen coordinates to Zn^{II} and both the adjacent carbonyl oxygens hydrogen bond with the cyclen NH groups positioned at complementary positions. This novel 'three-point' binding was established by the crystal structure of a ternary complex of 1 with AZT (3'-azido-3'-deoxythymidine).[2] Such a selective nucleobase receptor should inhibit or control some key processes of gene expression involving molecular recognition *via* base pairing. In accordance with this notion, we have examined the biochemical ability of 1 to regulate gene expression involving poly(U).

Synthetic homopolymers poly(A) and poly(U) associate together to form well-ordered double-stranded poly(A + U),[5–7] which is detectable by a decrease in the optical density at 260 nm (A_{260}).[5] The effect of Zn^{II}–cyclen 1 on this

hybridization was studied by the change in A_{260} in 10 mmol dm^{-3} NaCl and 5 mmol dm^{-3} tris-HCl (pH 7.6) (buffer I) at 25 °C. In a typical experiment, a mixture of poly(U) {[uracil]$_{poly(U)}$ = 50 μmol dm^{-3}} and 1 was equilibrated† before its addition to a solution of poly(A) {[adenine]$_{poly(A)}$ = 50 μmol dm^{-3}}. The A_{260} changed as a function of time as shown in Fig. 1(a). In the absence of 1 {r = [1]$_{added}$/[uracil]$_{poly(U)}$ = 0} a significant decrease in A_{260} was observed in accordance with poly(A + U) formation. In contrast, as the concentration of 1 was raised, the rate at which the absorbance lowered at 260 nm decreased. At $r > 1$, the poly(A)–poly(U) formation was almost completely inhibited.
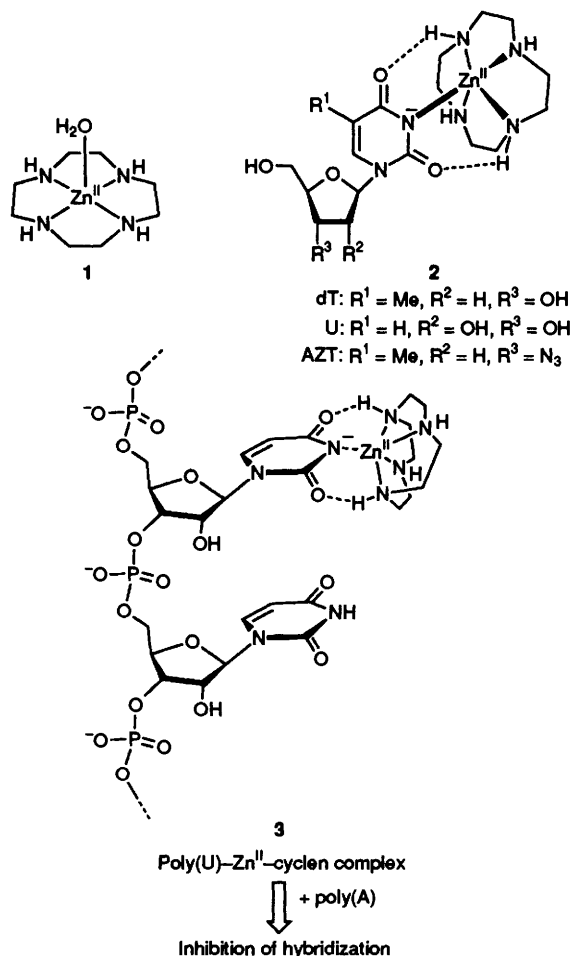
Melting point experiments [Fig. 1(b)] were continued by measuring the melting temperature on each sample of Fig. 1(a) to assess the effect of 1 on the degree of poly(A)–poly(U) hybridization. In the absence of 1 (r = 0) a single transition was observed in A_{260} at T_m 42 °C with hyperchromicity 31% [cf. 43 °C and 30% for the standard poly(A + U) sample (Pharmacia)]. Upon increasing 1, the break in the absorbance plot shifted to lower temperatures with smaller hyperchromicities; (T_m, hyperchromicity) = (40 °C, 30%), (36 °C, 7%) and (34 °C, ~3%) for r = 0.25, 0.5 and 1, respectively. This indicates that as more uracil bases are blocked by 1, there is less hybridization to form poly(A)–poly(U). At r = 2 there was no break at all in the absorbance plot which implies that no hybridization occurs at all at this ratio.‡

No change in either optical density or melting point were observed upon addition of 1 (up to r = 10) to the standard poly(A + U) sample, suggesting that once completely hybridized, the poly(A + U) can not be readily dissociated by 1. Moreover, this implies that disproportionation did not occur from double-helical poly(A + U) to triple-helical poly(U)–poly(A)–poly(U) [+ poly(U)] as reported in the presence of huge excess Mg^{2+}.[8]

To determine the ratio [1]$_{complexed}$/[uracil]$_{poly(U)}$ required for complete inhibition of hybridization, the apparent affinity constant of 1 for the uracil base in poly(U) {K_{app} = [1]$_{complexed}$/([uracil]$_{poly(U), uncomplexed}$·[1]$_{uncomplexed}$)} at pH 7.6 was determined to be log K_{app} = 4.1 ± 0.1§ by a UV titration method in buffer I at 25 °C.[2] From this value, it is estimated that at r = 2 nearly half of the total uracil bases in poly(U) are in the form of a ternary complex 3 to inhibit the hybridization. Moreover, as expected from the little affinity of 1 for dA, dC and dG,[2] no significant interactions were observed between 1 and poly(A), poly(G) and poly(C) in the same UV titration studies.

Additional evidence for the inhibition of poly(A)–poly(U) hybridization by 1 was obtained by a ^{31}P NMR study. When a solution containing 1 (2 mmol dm^{-3}) and poly(U) {[uracil]$_{poly(U)}$ = 1 mmol dm^{-3})} was equilibrated in buffer I at 25 °C, the spectrum displayed only a broad singlet at −0.11 ppm (85% H$_3$PO$_4$ as the external reference) which is assigned to the ternary complex of 1 with poly(U). Upon addition of poly(A) {[adenine]$_{poly(A)}$ = 1 mmol dm^{-3}} an additional broad singlet at −0.39 ppm, an identical signal for the free poly(A), was observed and there was no further change over a 15 h period. The standard poly(A + U) showed only one broad singlet at −0.19 ppm.

The biochemical inhibitory effect of 1 on *in vitro* polyphenylalanine [poly(Phe)] synthesis in cell-free extracts was preliminarily studied. In this experiment, poly(U) was used as
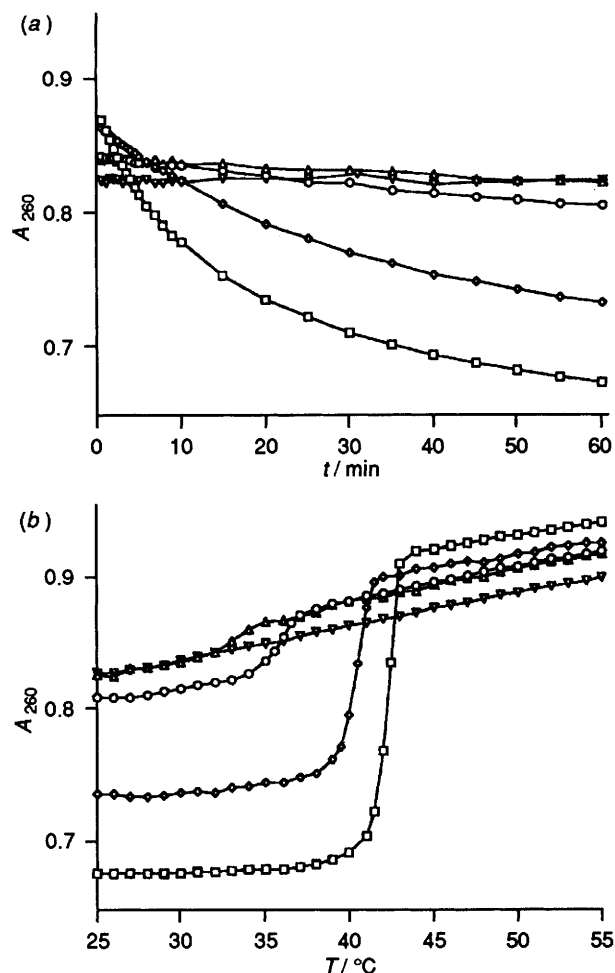

1

2

dT: R^1 = Me, R^2 = H, R^3 = OH
U: R^1 = H, R^2 = OH, R^3 = OH
AZT: R^1 = Me, R^2 = H, R^3 = N$_3$

3

Poly(U)–Zn^{II}–cyclen complex

⇩ + poly(A)

Inhibition of hybridization

Fig. 1 (a) The time dependence of A_{260} for a 1 : 1 mixture of poly(A) and poly(U) at various concentrations of Zn^{II}–cyclen 1. $r =$ $[1]_{added}/[uracil]_{poly(U)}$. (b) Melting-temperature profiles of each sample succeeding the experiments of Fig. 1(a). For (a) and (b): $\square$, $r = 0$; $\Diamond$, $r = 0.25$; $\bigcirc$, $r = 0.50$; $\triangle$, $r = 1.0$; ∇, $r = 2.0$.

a messenger RNA (where a UUU sequence is a codon for Phe). Experimental conditions¶ have been described previously.[9] When 0.56 mmol dm⁻³ ($r = 1$) and 5.6 mmol dm⁻³ of 1 ($r = 10$) were added to the reaction mixture, the poly(Phe) synthesis underwent 15% and 26% inhibition, respectively. This inhibition process may possibly involve the formation of the ternary complex of 1 with uracil base in poly(U) (see 3). In addition, it is most likely that 1 was blocked significantly by other ligands such as 2-mercaptoethanol and Cl⁻ etc. and that therefore the observed inhibitions of the poly(Phe) synthesis may be lower limits for the effect of 1. In other words, 1 is more effective than the experimental results indicate.

These results strongly demonstrate that 1 is potentially a new type of chemical agent that may control some gene expression processes involving molecular recognition via base pairing.

Footnotes

† The formation of the ternary complex of 1 with the uracil base in poly(U) is accompanied by a decrease in A_{260}. This equilibrium was reached within a few minutes. It is to be noted that once the hybridization of poly(A)–poly(U) starts, 1 ($r < 2$) can not hinder its process, as demonstrated by the observation: when poly(U) was added to a mixture of 1 and poly(A), poly(A)–poly(U) hybridization occurred without hindrance. Hence, prior binding of 1 to poly(U) is necessary for the inhibition of hybridization.

‡ Under these conditions, hydrolysis of poly(U) by 1 was too slow to detect even at 55 °C.

§ In this calculation, each uracil base in poly(U) was taken as an independent base. The interaction between 1 and monoanionic phosphate groups was neglected, as reported earlier (T. Koike and E. Kimura, J. Am. Chem. Soc., 1991, 113, 8935). For reference, the affinity constant of 1 for each N(3)-deprotonated uracil base in poly(U) was determined to be log $K = 5.1 \pm 0.1$ {$K = [1]_{complexed}/$ ([N(3)-deprotonated uracil]$_{poly(U)}$, uncomplexed·[1]$_{uncomplexed}$) dm³ mol⁻¹} by a pH titration method at $I = 0.10$ (NaClO₄) and 25 °C (cf. log $K = 5.2 \pm 0.1$ for monomeric U, see ref. 2).

¶ The reaction mixture (125 μl) containing 60 mmol dm⁻³ tris-HCl (pH 7.8), 8 mmol dm⁻³ Mg(AcO)₂, 80 mmol dm⁻³ NH₄Cl, 6 mmol dm⁻³ 2-mercaptoethanol, 0.4 mmol dm⁻³ spermidine, 0.9 mmol dm⁻³ ATP, 0.2 mmol dm⁻³ GTP, 4 mmol dm⁻³ phosphoenolpyruvate, 2 μg pyruvate kinase, 0.05 μCi L-[U-¹⁴C]Phe (100 μCi/μmol; 3.7 MBq/μmol), 4 μmol dm⁻³ each of 19 amino acids except Phe, 25 μg E. coli MRE 600, poly(U) {[uracil]$_{poly(U)}$ = 0.56 mmol dm⁻³}, 60 μg 70S ribosomes and 240 μg S-150 fraction was incubated at 37 °C for 30 min with or without 1.

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