Hydrolytic cleavage of DNA by a novel copper(ii) complex with *cis,cis*-1,3,5-triaminocyclohexane

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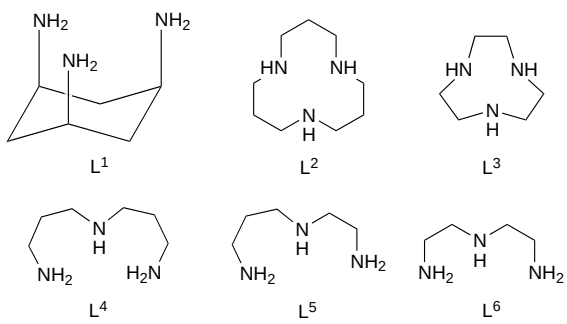
Copper(II) complex with *cis,cis*-1,3,5-triaminocyclohexane effectively promotes the hydrolytic cleavage of phage DNA with a rate constant of 4.34 ± 0.77 h^{-1} at pH 8.1 and 35 °C.

The development of reagents which hydrolytically cleave nucleic acids under mild conditions is currently attracting great interest in the field of artificial nucleases,[1–3] as such a process has the advantage that it produces fragments which may be religated enzymatically. Recently, it has been demonstrated that many chelate compounds of transition metals (Fe^{2+}, Co^{3+}, Cu^{2+}, Zn^{2+}) and lanthanides (La^{3+}, Ce^{4+}, Eu^{3+}, Tm^{3+}, Yb^{3+}, Lu^{3+}) are capable of promoting the hydrolytic cleavage of RNA[2,4] and DNA;[2,5–7] however, DNA hydrolysis is much slower compared with RNA hydrolysis. This is mainly because the phosphate diester linkage of DNA is kinetically more stable than that of RNA toward hydrolysis.[8] The best enhanced rate constant for DNA hydrolysis by a metal chelate is in the range 0.18–0.25 h^{-1},[7] and a more active metal chelate is therefore required.

Recently, Burstyn and coworkers reported that copper(ii)–macrocyclic triamine (L^3) complexes promote the hydrolytic cleavage of plasmid DNA,[6] however, the rate constant is estimated to be 0.04–0.09 h^{-1} at 50 °C from the half life of the hydrolysis.[6a] In order to improve the DNA hydrolysis capacity of copper(ii) complexes, we have studied the hydrolysis of lithium 2,4-dinitrophenyl ethyl phosphate (DEP) and phage φx174 DNA† by copper(ii) complexes of triamine ligands (L^1–L^6), and found that a novel copper(ii) complex of *cis,cis*-1,3,5-triaminocyclohexane[9,10] (CuL^1) has a high potential for DNA hydrolysis: the hydrolysis rate constant reached 4.3 h^{-1} at 35 °C.

Fig. 1 shows agarose gel electrophoresis patterns for the cleavage of φx174 DNA after treated with CuL^1 at pH 8.1 and 35 °C for 5 h. The initial concentration of DNA was set at 7 μmol dm^{-3} base pairs, and the concentration of CuL^1 was varied from 0 to 70 μmol dm^{-3}. The conversion of form I (supercoiled) to form II (nicked) was observed with the increase in concentration of CuL^1, and the formation of form III appeared in the presence of 35 and 70 μmol dm^{-3} CuL^1 (lanes 6 and 7). The complete disappearance of form I was observed in lanes 5–7 (14–70 μmol dm^{-3} CuL^1). On the other hand, CuL^2–CuL^6 were mostly ineffective under the conditions employed, indicating that only CuL^1 effectively enhances the cleavage of φx174 DNA.



A typical time course for DNA cleavage by CuL^1 is shown in Fig. 2. The cleavage products were separated by agarose gel electrophoresis and quantified by densitometry after staining with ethidium bromide. The decrease of form I fitted well to a single exponential decay curve. The increase of form II also fitted to a single exponential curve, although the deviation of form II was somewhat larger than that of form I. From these curve fits, the hydrolysis rate constants at a complex concentration of 70 μmol dm^{-3} were estimated to be 1.46 ± 0.22 h^{-1} (R^2 = 0.954) for the decrease of form I and 1.82 ± 0.33 h^{-1} (R^2 = 0.939) for the increase of form II. Similarly, the hydrolysis rate constants for φx174 DNA in the presence of 7, 14, 140, 210 and 280 mmol dm^{-3} CuL^1 were obtained, and are plotted in Fig. 3.

The plots of the rate constants against the concentrations of CuL^1 allowed a Michaelis–Menten analysis for φx174 DNA, and gave k = 4.34 ± 0.77 h^{-1}, K_m = 9.55 × 10^{-5} mol dm^{-3} for the decrease of form I, and k = 4.16 ± 0.44 h^{-1}, K_m = 5.75 × 10^{-5} mol dm^{-3} for the increase of form II. The obtained rate constants were similar to each other, and are about twenty times higher than the highest values reported previously for the rate

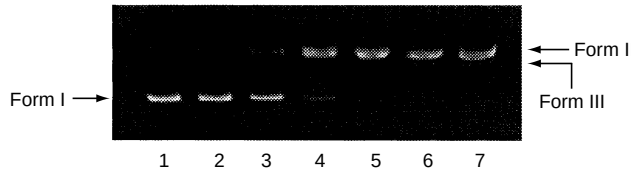


Fig. 1 Agarose gel electrophoresis patterns for the cleavage of φx174 DNA by CuL^1. Conditions: 7 μmol dm^{-3} DNA base pairs; 0–70 μmol dm^{-3} CuL^1; 10 mmol dm^{-3} HEPES buffer; pH 8.1; 35 °C; reaction time, 5 h. Lane 1: DNA control. Lane 2: DNA + 0.7 μmol dm^{-3} CuL^1. Lane 3: DNA + 3.5 μmol dm^{-3} CuL^1. Lane 4: DNA + 7 μmol dm^{-3} CuL^1. Lane 5: DNA + 14 μmol dm^{-3} CuL^1. Lane 6: DNA + 35 μmol dm^{-3} CuL^1. Lane 7: DNA + 70 μmol dm^{-3} CuL^1.

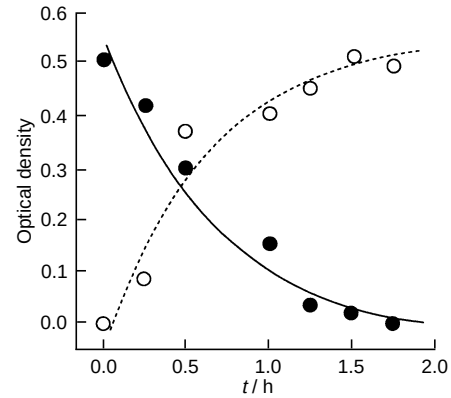


Fig. 2 Time course of DNA cleavage by CuL^1 at pH 8.1 (10 mmol dm^{-3} HEPES buffer) and 35 °C in the presence of 7 μmol dm^{-3} φx174 DNA and 70 μmol dm^{-3} CuL^1: (●) form I; (○) form II; solid line, single exponential curvefit for the decrease of form I; dashed line, single exponential curvefit for the increase of form II

constant for DNA hydrolysis by a metal chelate,[7] although the metal and experimental conditions, including employed DNA, were not necessarily the same as those of our experiment.

In Table 1 are summarized the results of the hydrolysis of the phosphate diester DEP by CuL^1–CuL^6. The hydrolysis of DEP was followed by monitoring the increase in the visible absorbance at 400 nm caused by the release of 2,4-dinitrophenolate ion at pH 8.1 (50 mmol dm^{-3} HEPES buffer) and 25 °C, and analysed by using a reference method.[10] The hydrolysis rate was first order with respect to the concentration of both DEP and CuL. The apparent second order rate constants thus obtained are listed in Table 1.

Table 1 shows that all of the investigated CuL complexes are capable of hydrolysing the phosphate diester linkage, and that CuL^1 has a high hydrolysing capacity.‡ In addition, we observed that DNA cleavage by CuL^1 is not affected by the presence or absence of O_2. This fact indicates that no oxidative process is involved.[6] Hence, these data strongly support the suggestion that DNA cleavage by CuL^1 proceeds *via* a hydrolysis mechanism. On the other hand, we found the following discrepancies between DEP and DNA hydrolysis: (i) the former is first order-dependent on complex concentration, but the latter is saturation-dependent on complex concentration; (ii) the former is greatly enhanced by not only CuL^1 but also CuL^2 and CuL^3, but the latter is specific toward CuL^1.

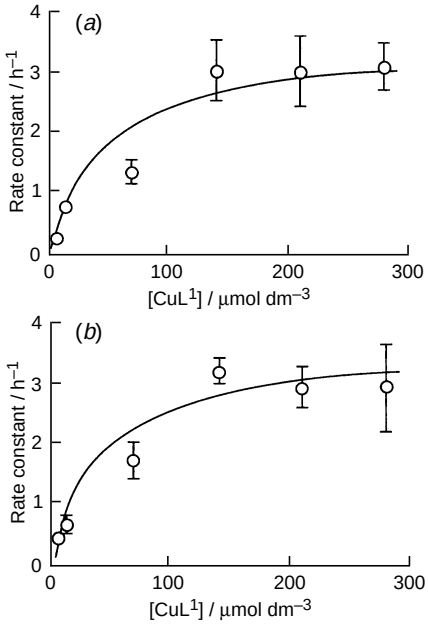


Fig. 3 Saturation kinetics for cleavage of φx174 DNA by 7–280 μmol dm^{-3} CuL^1 at pH 8.1 and 35 °C: (*a*) decrease of form I; (*b*) increase of form II

Table 1 Rate constants (k_{app}) of hydrolysis of DEP by CuL^1–CuL^6 at pH 8.1 (50 mmol dm^{-3} HEPES), ionic strength = 0.1 m ($NaClO_4$) and 25 °C

Complex	k_{app}/dm^3 mol^{-1} s^{-1}
CuL^1	$(1.2 \pm 0.1) \times 10^{-2}$
CuL^2	$(1.4 \pm 0.1) \times 10^{-2}$
CuL^3	$(7.1 \pm 0.2) \times 10^{-3}$
CuL^4	$(0.4 \pm 0.1) \times 10^{-4}$
CuL^5	$< 10^{-4}$
CuL^6	$< 10^{-4}$

Although we have no clear explanation for these data, one explanation is the possibility of a polymer effect for DNA, with specific reaction sites for CuL^1.

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Footnotes

† Phage φx174 RFI DNA was purchased from Sigma Co. Ltd.

‡ We also confirmed the following facts: (i) free copper(ii) ions produce almost no cleavage of φx174 DNA at concentrations below 2 μmol dm^{-3} of copper(ii) ion under the employed conditions, and (ii) the concentration of free copper(ii) ion is less than 1.1 μmol dm^{-3} under our experimental conditions [copper(ii) ion exists as over 97.5% CuL^1 and CuL^1 (OH) species] on the basis of the calculation using the following deprotination and stability constants determined by potentiometric titration at 35 °C; $K_1 = [H^+][H_2L^{2+}]/[H_3L^{3+}] = 10^{-6.94}$, $K_2 = [H^+][HL^+]/[H_2L^{2+}] = 10^{-8.69}$, $K_3 = [H^+][L]/[HL^+] = 10^{-9.75}$, $K_{ML} = [CuL^{2+}]/[Cu^{2+}][L] = 10^{10.55}$, $K_{MHL} = [CuHL^{3+}]/[Cu^{2+}][HL^+] = 10^{6.47}$, $K_a = [CuL(OH)^+][H^+]/[CuL^{2+}] = 10^{-8.40}$.

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