Nuclease Activity of a Hydroxamic Acid Derivative in the Presence of Various Metal Ions

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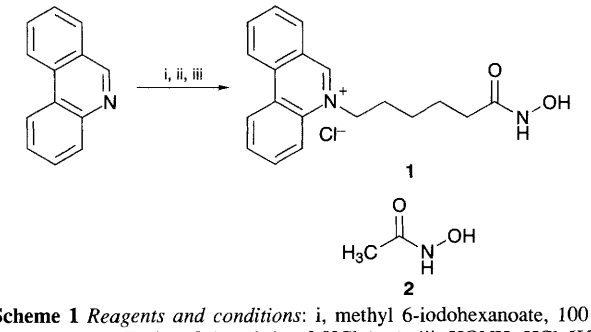
Phenanthridine-linked hydroxamic acid **1** effectively cleaves ColE1 DNA in the presence of transition (ferrous, ferric and vanadyl) or lanthanide(III) (lutetium, thulium and europium) metal ions; in the presence of the former, the cleavage is inhibited by free radical scavengers, but in the latter case it is not.

There is considerable interest in the development of artificial nucleases.[1–4] Artificial metallonucleases require ligands which effectively deliver the metal ion to the vicinity of the DNA strand, some of which mimic the metal chelation site of DNA-cleaving antibiotics or metalloproteins.[5,6] Hydroxamic acids constitute the ferric chelation site of the siderophores which exist in microorganisms.[7] Although many studies have been made of the chemical properties of hydroxamic acid–transition and –lanthanide metal complexes,[8] their interactions with DNA have not been reported. In order to investigate this point, we previously examined DNA cleavage by hydroxamic acid in the presence of copper(II) and found that the cleavage was promoted by the hydroxamic acids.[9] This result implied that this hydroxamic acid–metal complex would be a promising DNA cleaving agent. It has been reported that artificial metallonucleases whose metal complexes are linked to the intercalator *via* a linker effectively induce DNA cleavage.[10] We report here the molecular design of a metallonuclease which contains a phenanthridine ring as the intercalator, *N*-[5-(hydroxylaminocarbonyl)pentyl]phenanthridium chloride **1**, and the effective DNA cleavage by **1** in the presence of lanthanide and transition metal ions.

Compound **1** was synthesized according to Scheme 1. The structure of **1** was confirmed by a positive Fe^{III} test, which is characteristic of hydroxamic acid, and by spectroscopic examination.† The DNA cleavage by **1** and acetohydroxamic acid **2** were followed by monitoring the conversion of supercoiled ColE1 plasmid DNA (Form i) to the open circular form (Form ii).‡ Fig. 1(*a*) shows the agarose gel electrophoresis pattern of DNA cleavage by **1** in the presence of transition metal ions. Compound **1** (10 μmol dm^{-3}) effectively induced DNA cleavage in the presence of Fe^{II} or VO^{II} (10 μmol dm^{-3} each, lanes 4 and 8). Fe^{III}–**1** (50 μmol dm^{-3} each) was also effective, although the activity was lower than that of Fe^{II}–**1** (lanes 4 and 6). Cu^{II}–**1** was much less effective than Fe^{III}–**1** (data not shown). No significant DNA cleavage was observed in the presence of redox-inactive metal ions such as Mn^{II}, Co^{II}, Ni^{II} and Zn^{II}. Compound **1** alone showed no cleavage activity below 100 μmol dm^{-3} concentration (data not shown). DNA-cleaving metal complexes are usually activated in the presence of a reductant such as dithiothreitol or ascorbic acid to generate the reactive oxygen species responsible for DNA cleavage. We previously observed DNA cleavage by a hydroxamic acid–copper(II) complex without any reductant.[9] Hence, the fact that the cleavage by Fe^{III}–**1** proceeds without any reductant indicates that **1** can autoreduce Fe^{III} to Fe^{II} to generate the reactive oxygen species. As shown in Fig. 1(*b*), DNA cleavage by **1** was also examined in the presence of lanthanide metal ions. Compound **1** (30 μmol dm^{-3}) effectively induced DNA cleavage in the presence of Lu^{III}, Tm^{III} or Eu^{III} (30 μmol dm^{-3} each, lanes 4, 6 and 8). The cleavage activity increased in the order Eu^{III}–**1** > Tm^{III}–**1** > Lu^{III}–**1**. Ce^{III} was as effective as Lu^{III}, but La^{III} was less active in the DNA cleavage by **1** (data not shown). The results of DNA cleavage given in Fig. 1(*a*) and (*b*) clearly indicate that both the transition and the lanthanide metal ions are effective for DNA cleavage by **1**, and the hydroxamic acid moiety is thought to form a complex with the metal ions. Furthermore, the phenanthridine ring plays an important role in DNA binding of **1**§ since no cleavage activity was observed for Fe^{II} or Lu^{III}–**2** (lane 3).

In order to examine whether the DNA cleavage is induced by hydrolysis of the phosphodiesters of DNA or a radical reaction to decompose the sugar moiety, reactions with inhibitors were carried out and the results are shown in Table 1. Free radical scavengers such as peroxidase (H_2O_2 scavenger), thiourea and potassium iodide (·OH scavenger) inhibited both the cleavage by Fe^{II}–**1** and VO^{II}–**1**, although the inhibitory effect against the cleavage by VO^{II}–**1** was higher than against that of Fe^{II}–**1**. Reactive oxygen species are suggested to be involved in these reactions. On the other hand, any inhibitors except EDTA had little inhibitory effect on the reaction by Lu^{III}–**1**. This result



Scheme 1 *Reagents and conditions*: i, methyl 6-iodohexanoate, 100 °C; ii, $AgNO_3$–DMF, then 0.4 mol dm^{-3} HCl (aq.); iii, $HONH_2$·HCl–KOH–MeOH, room temp.

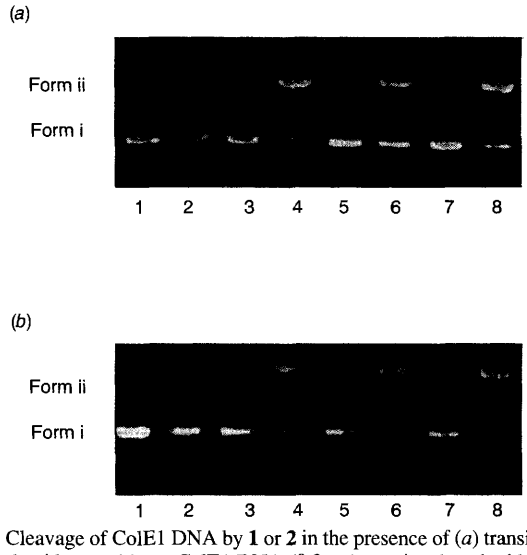


Fig. 1 Cleavage of ColE1 DNA by **1** or **2** in the presence of (*a*) transition or (*b*) lanthanide metal ions. ColE1 DNA (0.3 μg) was incubated with **1** or **2** in the presence of metal ions in 40 mmol dm^{-3} TRIS-HCl buffer (pH 8.0, total volume 15 μl) at 37 °C for (*a*) 1.0 h or (*b*) 2.0 h. Lane assignment: (*a*) lane 1, DNA control; lane 2, 10 μmol dm^{-3} $FeSO_4$: lane 3, 10 μmol dm^{-3} $FeSO_4$ + 100 μmol dm^{-3} **2**; lane 4, 10 μmol dm^{-3} $FeSO_4$ + 10 μmol dm^{-3} **1**; lane 5, 50 μmol dm^{-3} $FeCl_3$; lane 6, 50 μmol dm^{-3} $FeCl_3$ + 50 μmol dm^{-3} **1**; lane 7, 10 μmol dm^{-3} $VOSO_4$; lane 8, 10 μmol dm^{-3} $VOSO_4$ + 10 μmol dm^{-3} **1**. (*b*) lane 1, DNA control; lane 2, 30 μmol dm^{-3} $LuCl_3$; lane 3, 30 μmol dm^{-3} $LuCl_3$ + 100 μmol dm^{-3} **2**; lane 4, 30 μmol dm^{-3} $LuCl_3$ + 30 μmol dm^{-3} **1**; lane 5, 30 μmol dm^{-3} $TmCl_3$; lane 6, 30 μmol dm^{-3} $TmCl_3$ + 30 μmol dm^{-3} **1**; lane 7, 30 μmol dm^{-3} $EuCl_3$; lane 8, 30 μmol dm^{-3} $EuCl_3$ + 30 μmol dm^{-3} **1**.

Table 1 Effects of added inhibitors on ColE1 DNA cleavage induced by **1** in the presence of transition or lanthanide metal ions[a]

Inhibitor	Concentration	Inhibition(%) of Form ii DNA production[b]		
		1 + Fe^{II}	**1** + VO^{II}	**1** + Lu^{III}
No inhibitor	—	0.0	0.0	0.0
SOD[c]	50 μg ml^{-1}	1.2	−38.3[d]	1.6
Peroxidase	50 μg ml^{-1}	60.3	67.9	0.8
Peroxidase	10 μg ml^{-1}	22.9	73.3	1.1
Thiourea	50 mmol dm^{-3}	25.8	77.7	4.8
KI	50 mmol dm^{-3}	40.8	68.3	6.1
NaN_3	50 mmol dm^{-3}	53.2	46.1	5.0
EDTA	0.1 mmol dm^{-3}	94.3	98.7	91.6

[a] Reactions performed under the same conditions as shown in Fig. 1 except for the addition of inhibitors. Concentrations of **1** and metal ions were as follows: 5 μmol dm^{-3} $FeSO_4$ + 5 μmol dm^{-3} **1**; 10 μmol dm^{-3} $VOSO_4$ + 10 μmol dm^{-3} **1**; and 25 μmol dm^{-3} $LuCl_3$ + 25 μmol dm^{-3} **1**. [b] Percentage of Form ii DNA was measured by fluorometric densitometry after ethidium bromide staining, and inhibition (%) of Form ii DNA production was calculated as {1 − Form ii (%, with inhibitor)/Form ii (%, no inhibitor)} × 100. [c] SOD = superoxide dismutase. [d] See footnote ¶.

indicates that the reactive oxygen species should not be involved in this reaction. Recently, the groups of Komiyama and Chin reported that lanthanide(III) ions induce DNA cleavage by hydrolysis of the phosphodiester of DNA.[11,12] These reports strongly support that the DNA cleavage by lanthanide(III)–**1** may proceed through the hydrolytic mechanism. Detailed examinations are now in progress in our laboratory to elucidate the mechanism.

In conclusion, both hydrolytic and oxidative cleavage of DNA have been accomplished by use of the hydroxamic acid–metal ion systems. This simple cleavage system may be useful for the development of artificial metallonucleases, especially artificial hydrolytic nucleases.

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Footnotes

† Compound **1**: ^1H NMR (400 MHz, CD_3OD) δ 1.57 (2H, m), 1.75 (2H, m), 2.12–2.21 (4H, m), 5.14 (2H, t), 8.08–8.17 (3H, m), 8.39 (1H, dt), 8.58 (2H, d), 9.08 (1H, d), 9.14 (1H, dd), 10.15 (1H, s); IR (KBr), ν/cm^{-1} 3180br, 2930s, 1670s, 1620s, 1525m; FAB-MS: $[M - Cl]^+$ *m/z* 309.

‡ The relative amounts of supercoiled (Form i) and open circular (Form ii) DNA were analysed on 0.9% horizontal agarose gel in 40 mmol dm^{-3} TRIS-HCl–5 mmol dm^{-3} sodium acetate–1 mmol dm^{-3} EDTA (pH 8.0). The electrophoresis was carried out at 100 V for 1.0 h. The gels were photographed on a UV transilluminator after ethidium bromide staining at 0.5 mg ml^{-1}.

§ Viscometric titration of sonicated calf thymus DNA with **1** showed the viscosity increase typical of the intercalator.

¶ Similar promotion by SOD of DNA cleavage has been observed with thiolate–haemin complexes (ref. 13).

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