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Letter

Lanthanide binuclear macrocyclic complexes as synthetic enzymes for the cleavage of DNA

Bing Zhu [a,*], Da-Qing Zhao [a], Jia-Zuan Ni [a], Dong Ying [b], Bai-Qu Huang [b], Zeng-Ling Wang [c]

[a] *Laboratory of Rare Earth Chemistry and Physics, Changchun Institute of Applied Chemistry Academy of Science, Changchun 130022, China*

[b] *Institute of Genetics and Cytology, Northeast Normal University, Changchun 130024, China*

[c] *Department of Chemistry, NanKai University, Tianjin, 300071, China*

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Abstract

Lanthanide binuclear complexes can accelerate the cleavage of pUC19 plasmid DNA, yielding predominantly linear form. The saturation kinetics of the cleavage of pUC19 was studied. The observed rates with lanthanide binuclear complexes showed the expected increase with the catalyst concentration. The rate of cleavage is greater than that of lanthanide ions alone.

Keywords: DNA cleavage; Supramolecular chemistry; Lanthanide complexes

1. Introduction

The hydrolysis of phosphate diesters of RNA and DNA has been attracting increasing interest mainly because it is essential for the preparation of artificial restriction enzymes [1–5]. A recent work by Chapman and Breslow [6] has shown that binuclear zinc complexes can accelerate the hydrolysis of phosphate esters but the rate of hydrolysis was low. In other recent studies with carefully designed binuclear complexes having peroxo ligands [7], zinc complexes were used for the hydrolysis of dinucleotides [8]. However, most of them take advantage of oxidative cleavage of deoxyribose at the target site, so hydrolytic cleavage systems showing high activities are needed. Trivalent lanthanide ions (Ln^{3+}) that efficiently promote RNA cleavage by phosphate ester transesterification [9–11] has been reported. Ragunatnan and Schneider [12] reported that lanthanide binuclear complexes can catalyze the hydrolysis of plasmid DNA, but the products of hydrolysis were only limited to nicked forms [12], and the formation of the linear form for hydrolyzing plasmid DNA by hydrolytic cleavage did not occur. Here, we report that lanthanide binuclear metal complexes hydrolytically cleave the plasmid DNA to the linear form at near neutral conditions and 37°C.

* Corresponding author: Department of Inorganic Chemistry, School of Pharmaceutical Science, Beijing Medical University, Beijing 100083, China. Fax: 010-62015584.

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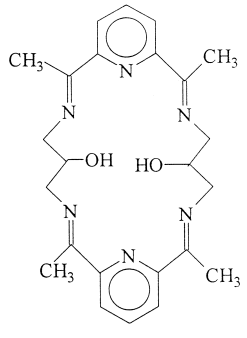
2. Discussion

Lanthanide complexes must be robust if they are to be useful as catalysts. Our efforts have focused on the synthesis of robust cationic Ln^{3+} complexes that may be effective catalysts in vivo. The kinetic inertness of the complexes to dissociation was studied by using excess Cu^{2+} as a trapping agent. The results showed the rate of dissociation is independent of Cu^{2+} concentration. Furthermore, the complexes give no precipitate on addition of KF or KOH. It indicates that the complexes remain undissociated in water and are kinetically stable to dissociation in vivo. In addition, it has been noted that the counter anions of lanthanide metal salts obviously affect the formation of the macrocyclic complexes. All of the lanthanide isothiocyanates (from La to Yb) can be used for the synthesis of binuclear macrocyclic complexes. However, only lanthanide binuclear macrocyclic complexes (from La to Eu) can be obtained with lanthanide nitrates as the template agent. This demonstrates that NCS^- anions have stronger coordination abilities than NO_3^- ions.

The synthesis and characterization of $Ho_2^{3+}L$ and $Er_2^{3+}L$ which possess NCS^- counter anions have been described by us [13]. The structure of ligand L is shown in Scheme 1. The addition of various complexes and lanthanides to supercoiled pUC19 resulted in an immediate reaction. Visual inspection of the gels shows that the linear form is the main products of cleavage. (Fig. 1). Electrophoresis and densitometry indicate that single cleavage of the supercoiled form yields 20.4% and 19.4% linear form by $Ho_2^{3+}L$ and $Er_2^{3+}L$ respectively (after correction for the linear form in the starting material). The amounts of supercoiled, linear and nicked pUC19 obtained in each experiment were quantitated by densitometry and listed in Table 1. This is to our knowledge the highest formation of linear form by hydrolytic cleavage yet reported for



Scheme 1. Structure of the ligand of L.

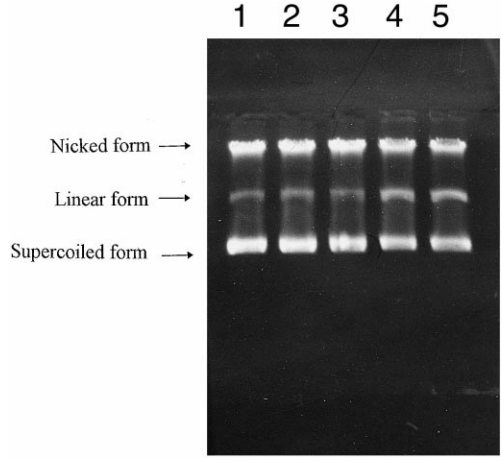


Fig. 1. Electrophoresis gel demonstrating cleavage of double stranded DNA after 3 h at 37°C and pH 7.0. For conditions see footnote a in Table 1. Lane 1: DNA alone; Lane 2: DNA and Ho^{3+}; Lane 3: DNA and Er^{3+}; Lane 4: DNA and Ho_2^{3+} L; Lane 5: DNA and Er_2^{3+} L.

Table 1
Cleavage of pUC19 with Ho_2^{3+} L, Er_2^{3+}, Ho^{3+} and Er^{3+}

Cleaving agents[a]	DNA form[b] (%)		
	Supercoiled	Nicked	Linear
	47	34.9	18.1
Ho_2^{3+} L	30.2 ± 0.6	31.3 ± 0.6	38.5 ± 0.6
Er_2^{3+} L	31.5 ± 0.6	31 ± 0.6	37.5 ± 0.6
Ho^{3+}	46.5 ± 0.4	34.1 ± 0.4	19.4 ± 0.4
Er^{3+}	46.4 ± 0.4	34.4 ± 0.4	19.2 ± 0.4

[a] Cleavage of double-stranded DNA is given as the percentage of the supercoiled, nicked and linear form. Incubation for 3 h at 37°C with plasmid DNA (pUC19; concentration 47 nM), $[M_2L] = 0.25$ mM and $[M^{3+}] = 0.25$ mM; HEPES buffer (10 mM), pH 7.0. The solutions of the metal complexes were prepared by dissolving the prepared complexes in deionized water. The concentration of the complexes were determined by inductively coupled plasma atomic emission spectroscopy (ICPAES); adding buffer, and adjusting the pH.

[b] The range of error given is three times the average deviation.

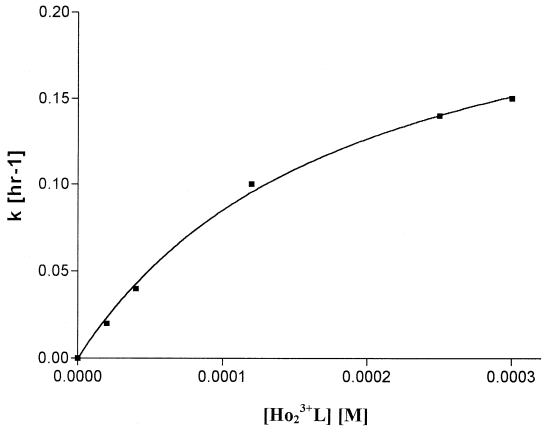


Fig. 2. Saturation kinetics of the cleavage of plasmid DNA by $Ho_2^{3+}L$. The theoretical curve corresponds to $K_M = 1.9 \times 10^{-4}$ M and $K_{cat} = 0.247\ h^{-1}$.

plasmid DNA. The kinetic analysis of plasmid DNA cleavage exhibited an excellent linear behaviour based on a pseudo first-order equation, the K_{obs} of cleaving pUC19 by $Ho_2^{3+}L$ (0.25 mM) and $Er_2^{3+}L$ (0.25 mM) is 0.156 h^{-1} and 0.150 h^{-1}. The results of Table 1 showed that the cleaving activity of binuclear lanthanide complexes is greater than that of metal ions alone.

In addition, the saturation kinetics of cleavage of pUC19 by $Ho_2^{3+}L$ was further studied. We applied pseudo first-order rate constants to derive Michaelis–Menten-type equilibrium and rate constants K_M and K_{cat}, respectively [14]. The corresponding saturation curve for $Ho_2^{3+}L$ (Fig. 2) with K_M around 1.9×10^{-4} M and K_{cat} around 0.247 h^{-1}, the observed rates with $Ho_2^{3+}L$ showed the expected increase with catalyst concentrations, but was limited by solubility problems at higher concentrations.

That 0.03 mM ethylenediaminetetraacetate (EDTA) has no effect on plasmid cleavage rates indicates the catalysis is not due to traces of free lanthanides. That the cleavage occurs by hydrolysis similar to that with enzymes is furthermore in line with the recent observation of Chin et al. [7] and Komiyama et al. [15]. Even redox-active Ce^{4+} ions act as hydrolytic catalysts. The present hydrolysis probably proceeds by coordination of the phosphate of plasmid DNA to the lanthanide ion in the complex followed by the metal ion toward the phosphorus atom. A similar mechanism has been proposed for the hydrolysis of activated aryl phosphates [16,17] and the P–O bond of nucleotides [18–20].

3. Conclusion

In conclusion, binuclear lanthanide complexes can accelerate the cleavage of pUC19 plasmid, yielding predominantly linear form. The rate of cleavage is greater than that of metal ions alone, the half-life of pUC19 is 4.4 h (with $Ho_2^{3+}L$ at 37°C and pH 7.0). Thus, binuclear lanthanide complexes may be useful as artificial restriction enzymes.

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