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DNA Cleavage of the Copper(II) Complexes with a Polyamine Ditopic Ligand

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Abstract: A new binuclear complex $[Cu_2L(OH)](ClO_4)_3 \cdot 2H_2O$ has been synthesized and characterized, where L=2,6-bis{[bis-(2-aminoethyl)amino]methyl}-benzene. In the presence of 0.5 mmol/L complex at pH 8.10 and 37°C, the complex can efficiently cleavage pBR322 DNA with a rate constant kobs of 1.35×10^{-4} s^{-1}. The cleavage occurred by a non-oxidative mechanism showing activity to be dependent on pH.

Keywords: Hydrolytic cleavage, copper complex, binuclear.

The investigations on simple and efficient reagents, which can cleave nucleic acids under mild conditions have been attracted considerable attentions[1,2]. Generally, in order to eliminate the possibility of significant cytotoxic side effect of reactive oxygen species, pathways that result in DNA cleavage by hydrolysis mechanisms are preferable[3,4]. We have reported two novel metal complexes magnesium (II)-diethylenetriamine (dien) and copper (II)-L-histidine systems that both can effectively promote the double-stranded cleavage of plasmid DNA under physiological pH and temperature[5,6]. One of the characteristics of the two systems is that the metal centers both have flexible coordination atmosphere, which is different from the copper (II)-1,10-phenanthroline system. In nature, many phosphoesterases are activated by two or more metal ions, so the binuclear metal complexes are of current interest[7,8]. Numerous model studies have been devoted to understanding how metalloenzymes hydrolyze phosphate diester. Among them bimetal central structures of dinuclear Cu (II) complexes have been reported to be the active center[9]. In this paper, we reported a binuclear copper metal complex with 2,6-bis{[bis-(2-aminoethyl)amino]methyl}- benzene (L, **Scheme 1**) which includes two dien moieties. Experimental results show that copper (II)-L complexes could effectively promote the cleavage of pBR322 DNA at mild conditions.

Synthesis

Complex $[Cu_2L(OH)](ClO_4)_3 \cdot 2H_2O$ (Cu_2(II)-L): L·6HCl[10] (5.95 g 0.01 mol) and $Cu(ClO_4)_2$ (8.15 g, 0.02 mol) were dissolved in water (30 mL). The pH of the solution

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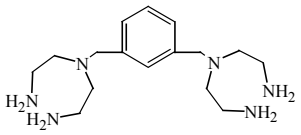
Scheme 1 The structure of L, 2, 6-bis{[bis-(2-aminoethyl)amino]methyl}benzene



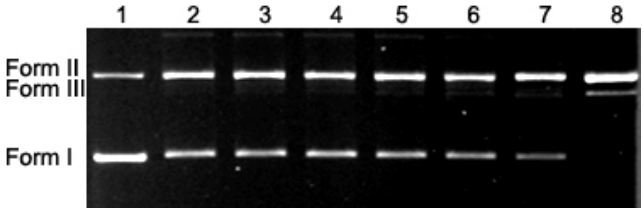
was adjusted to 8.0 with 1 mol/L NaOH aqueous solution. The resulting solution was refluxed for 15 min. The blue precipitate was collected and washed with MeOH and diethyl ether, and vacuum dried. Yield: 5.34 g (73.4%). The content of copper for complex was determined by EDTA titration. Anal. Calcd. for $C_{16}H_{37}N_6O_{15}Cl_3Cu_2$: Cu, 17.57; C, 26.91; H, 5.02; N, 11.21. Found: Cu, 17.66; C: 26.76; H: 5.16; N: 11.46. IR (KBr disk):3232br, 1120vs, 1087s, 626w, 690w, 734w. Visible spectra [λ_{max}, nm (ε, M^{-1} cm^{-1})]: 632 (209) in H_2O.

Results and Discussions

Figure 1 shows the Agarose gel electrophoresis patterns for the cleavage of pBR 322 DNA by the complex Cu_2(II)-L at pH 7.0 and 37°C for 3 h in Tris-HCl buffer (10 mmol/L Tris, 6.2 mmol/L NaCl). Compared with the control lane, the conversion from Form I (supercoiled) to Form II (nicked) was observed at lower concentration of 25 μmol/L. Form III (linear) began to appear in the presence of 100 μmol/L. At the concentration of 0.5 mmol/L, Form I was completely inverted to form II and Form III.

It should be pointed out that Cu_2(II)-L complex cleavages pBR322 DNA without any co-reactant such as H_2O_2 and ascorbic acid, which is different from some artificial nucleases by an oxidative pathway. In addition, control experiments were carried out in order to investigate the effect of oxygen in aerobic condition and the possibility of that DNA cleavage occurred *via* a hydroxyl radical-based mechanism. Experimental results showed that the cleavage was not influenced by addition of radical scavenger such as 4 mol/L DMSO, glycerol or 25 mol/L MeOH, whereas, the oxidative degradation of DNA by $Fe(EDTA)^{2-}/H_2O_2$ was almost totally inhibited by those radical scavenges at ten times lower concentration[11]. The cleavage ability of Cu_2(II)-L complexes was hardly prohibited under anaerobic condition. In the case of hydrolysis reaction, the activity

Figure 1 Agarose gel electrophoresis patterns for the cleavage of pBR322 DNA by the Cu_2(II)-L complex with different concentration (μmol/L), lane 1: DNA control, lanes 2-8: 10, 25, 50, 100, 150, 250, 500



increases linearly with pH showing some activity-pH dependence relation[12]. In **Figure 2**, it was illustrated that the cleavage activity shows a slight pH dependence relation. With pH value increasing, the complex cleavages more Form II to Form III DNA. All those results suggest that the DNA double-strand cleavage induced by Cu_2(II)-L complex was not occur through a oxidative mechanism.

Furthermore we investigated the kinetics of DNA double-strand scission by quantitation of form I, form II and form III DNA. In **Figure 3** was plotted a time course of form I DNA during a reaction in the presence of 0.5 mmol/L Cu_2(II)-L complex at pH 8.10 and 37°C, and a inset of electrophoresis profile. The data for the mass fraction of form I DNA were fit to a single-exponential decay curve (R=0.983). A straight line was obtained in log plot of form I mass fraction *vs* time, indicating that the cleavage follows pseudo first-order kinetics. The rate constant K_{obs} of 1.35×10^{-4} s^{-1} was determined.

Figure 2 Agarose gel electrophoresis patterns for the cleavage of pBR322 DNA by the Cu2(II)-L complex (0.5 mmol/L) with varying pH value, lane 1: DNA control, lanes 2-8: 5.00, 6.01, 7.00, 7.62, 8.20, 9.14, 9.70

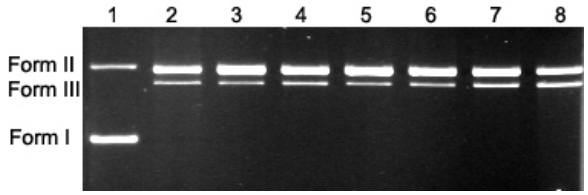
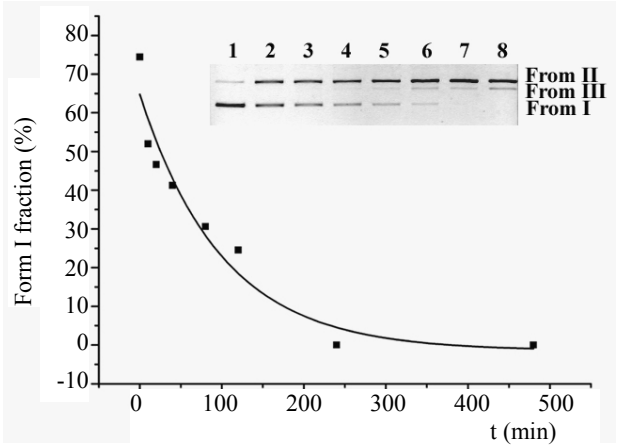


Figure 3 A plot of the time course of form I DNA mass fraction during cleavage reaction of pBR322 DNA by Cu_2(II)-L complex (0.5 mmol/L, 37°C, pH 8.10), inset: cleavage profile for increasing time 0-480 min.



In summary, this paper reports that the Cu_2(II)-L complex can effectively cleavage DNA in mild condition *via* a non-oxidative mechanism. Based on our experiment results, we may predict that the Cu_2(II)-L complex would be another potential DNA hydrolytic cleavage agent, even though that need be validated by T4 DNA ligase experiment. The further study such as determination of active species, detailed mechanism and the binding mode of complex to DNA are in progress.

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