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Cleavage of DNA by a New Mixed-ligand Cu(Ⅱ) Complex

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Abstract: Cleavage of DNA pUC19 by $[Cu(saldien)(NH_3)]Cl_2$ (here, saldien=bis(salicylicdene)-diethylenetriamine) was investigated at different pHs, times, concentrations, and 37℃. It was found that the copper(Ⅱ) complex could cleave DNA without added coreactants.

Keywords: Copper(Ⅱ) complex, cleavage of DNA, saldien.

Recently artificial metallonucleases are of particular interest[1-3]. Small metal complexes that are active enough to promote the hydrolysis of DNA under physiological conditions could be not only promising in biotechnology, but also elucidating the catalytic mechanism of natural enzyme. The preceding studies have shown that the water-soluble cations Schiff base complexes of Cu(Ⅱ) could interact strongly with DNA[4,5]. In this paper, we report our finding that a new mixed-ligand Cu(Ⅱ) complex, $[Cu(saldien)(NH_3)]Cl_2$, could effectively cleave DNA at pH 8 and 37℃.

Experimental

Elemental analysis was performed on a Perkin-Elmer 240c instrument, ^1H NMR spectra were obtained on a Bruker DRX-300 spectrometer, IR spectra were obtained on a Shimadzu FTIR-8300 spectrometer, Plasmid cleavage products were analyzed with a UVP GDS8000 complete gel documentation and analysis system. All other reagents were obtained commercially and used without further purification, Plasmid DNA (pUC19) was self-prepared, UV-Vis spectrometer was employed to check DNA purity(A_{260}:A_{280}>1.90) and concentration (ε =6600 L • $mol^{-1}cm^{-1}$at 260 nm).

A solution containing 1g(8.1 mmol) of salicylaldehyde in 10 mL ethanol was added to 0.4 g(4.0 mmol) of diethylenetriamine in 40 mL ethanol. The solution was stirred and heated to 80℃ for 30 min, a 6 mol/L HCl was added to the above reaction mixture. The yellowish precipitate was collected and dried in vacuum to afford saldien • HCl. ^1H NMR (D_2O, δ ppm): 2.75~3.25(m, 8H, CH_2-CH_2), 3.75(s, 2H, >CH=N-), 6.58∽7.75(m, 8H, Ar-H), Anal.calcd. for $C_{18}H_{21}N_3O_2$•HCl: C 62.14, H 6.33, N 12.09. Found: C61.64, H 6.30, N 12.05. IR (KBr, cm^{-1}) ν: 3446, 2694.4, 1635, 1575.7, 1394.1, 1278.7.

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Dissolution of $CuCl_2 \cdot 2H_2O$(0.34 g 2 mmol) in deionized water was accomplished with stirring and mild heating. To this solution was added concentrated NH_4OH. A deep blue $[Cu(NH_3)_4]^{2+}$ developed. A mixture containing saldien • HCl in 20 mL of ethanol was added to the above solution. The mixture was stirred for *ca*. 7 h. A green precipitate appeared, the solid was filtraled, washed with ethanol and dried in vacuum to afford $[Cu(saldien)(NH_3)]Cl_2$. Anal.calcd. for $C_{18}H_{24}N_4O_2Cl_2Cu \cdot 4H_2O$: C 40.41, H 5.99, N 10.48, C l13.28, Cu 11.88. Found: C 40.09, H 5.63, N 10.84, Cl 13.52, Cu 12.33. IR (KBr, cm^{-1}) ν: 3310.7, 3197.8, 3103.3, 1631.3, 1596.9, 1350.1, 1298.0, 831.3.

The molar conductivity of solution of 1 mmol/L Cu(Ⅱ) complex (210 $cm^2\ \Omega^{-1}\ mol^{-1}$) was measured with a DDS-11 conductivitymeter and was found in good agreement with the accepted value for 1:2 electrolytes in methanol[6].

The cleavage of DNA by Cu(Ⅱ) complex was accomplished by mixing 10 μL of 10 mmol/L Tris-HCl(containing 6.2 mmol/L NaCl) buffer, varying concentrations of Cu(Ⅱ) complex and 0.5 μL of DNA. After mixing, the DNA solutions were incubated at 37℃. At the appropriate times, the reactions were quenched by addition of EDTA and bromphenol blue and analyzed by gel electrophoresis. For examining if hydroxyl radicals were present, HO • scavengers (DMSO, glycerol, or methanol) were introduced to afford a final concentration of 0.4 or 2.5 mol/L before Cu(Ⅱ) complex addition.

Results and Disscusion

The cleavage of DNA by Cu(Ⅱ) complex were investigated in varied concentrations. **Figure 1** showed the cleavage of plasmid DNA after being treated with Cu(Ⅱ) complex at pH 8 (5 mmol/L Tris-HCl, 3.1 mmol/LNaCl) and 37℃ for 5h. The initial concentration of DNA was set at 0.325 mmol/L, and the concentration of Cu(Ⅱ) complex was varied from 0 to 9 mmol/L. The conversion of form Ⅰ to form Ⅱ was observed with the increase in concentration of Cu(Ⅱ) complex. Form Ⅰ was barely observable in lane 7(1 mmol/L). At higher concentrations of the Cu(Ⅱ) complex, band weakening and slow migration of the plasmid DNA was observed, indicating the binding of the complexes to the DNA, higher concentrations led to precipitation of the plasmid DNA as a white solid, due to charge neutralization caused by extensive binding of the Cu(Ⅱ) complex.

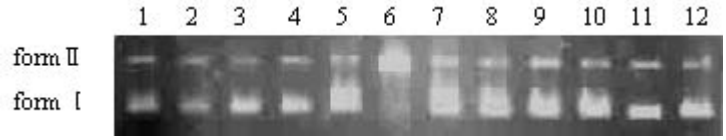
The pH-dependence of the catalytic activity of Cu(Ⅱ) complex on the cleavage of DNA was tested by using agarose gel electrophoresis. The cleavage of the DNA (form Ⅰ) was followed by its conversion to the open circular form(form Ⅱ). Typical gels were shown in **Figure 2**. The higher activity was observed at pH 8,where almost all supercoiled(form Ⅰ) DNA was cleaved to form Ⅱ. At other pHs, the activity was observed by forming form Ⅱ at around pH 8, showing gradual decrease in higher or lower pHs.

Cleavage reactions of plasmid DNA(pUC19) by Cu(Ⅱ) complex were also examined in different time. A time course of a gel pattern of DNA cleavage during the reaction in the presence of 5 mmol/L Cu(Ⅱ) complex and at pH 7.22 and 37℃ were shown in **Figure 3**. It has been concluded that the cleavage efficiency of DNA by that complex also corresponds relationship with reaction time.

Figure 1 The cleavage of DNA(0.325 mmol/L) by various concentrations of Cu(Ⅱ) complex at pH 8 (5 mmol/L Tris-HCl, 3.1 mmol/L NaCl) and 37℃ for 5h.

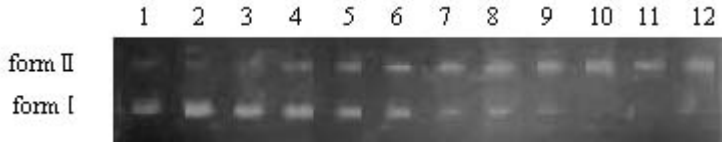
Lane 1:DNA control; Lanes 2~11: 0.1, 0.3, 0.5, 0.7, 0.9, 1, 3, 5, 7, 9 mmol/L, respectively.

Figure 2 Cleavage of DNA(0.325 mmol/L) by Cu(Ⅱ) complex (1 mmol/L) as a function of pH.



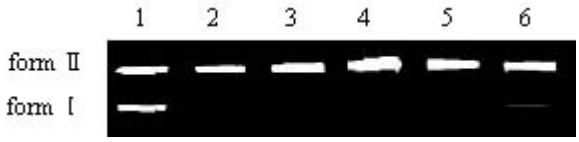
Lane 1, 8 control DNA at pH 6 and 9. Lanes 2~7, 9~12, pH 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, respectively. Lanes 1~3, 5 mmol/L MES-NaOH buffer containing 3.1 mmol/L NaCl; Lanes 4~7, 5 mmol/L Tris-HCl buffer containing 3.1 mmol/L NaCl; Lanes 8~12, 5 mmol/L HEPES-NaOH buffer containing 3.1 mmol/LNaCl; 37℃ for 5 h.

Figure 3 The time course of DNA (0.325 mmol/L) cleavage by Cu(Ⅱ) complex(5mmol/L).



Lane 1: DNA control(6 h); Lanes 2~12: 5, 15, 30, 60, 90, 120, 180, 150, 240, 300, 360 min.

Figure 4 Cleavage of DNA(0.325 mmol/L) by Cu(Ⅱ) complex(2.5 mmol /L) in the absence or presence of HO • scavengers and the presence 1 mmol/L H_2O_2. Cleavage conditions: 5 mmol/L Tris-HCl buffer; 3.1 mmol/L NaCl; pH 8; 37℃ for 5 h.



Lane 1: DNA control, Lane 2: Cu(Ⅱ) complex, Lane 3: + 1 mmol/L H_2O_2, Lane 4: + 2.5 mmol/L MeOH, Lane 5: + 0.4 mol/L DMSO, Lane 6: + 0.4 mol/L glycerol.

Cu(Ⅱ) complex-mediated DNA cleavage mechanism were considered. Addition of hydrogen peroxide does not change the cleavage rates within the error, nor acts as HO •scavengers like DMSO, glycerol or MeOH(**Figure 4**). The observations suggested that Cu(Ⅱ) complex-mediated cleavage reaction did not proceed *via* radical cleavage.

Acknowledgments

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