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Interaction between Bifunctional Dioxo Macrocyclic Polyamine Co(II) Complex and DNA in Metallomicellar System

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Abstract: The cleavage of DNA (pUC_{18}) by lipophilic macrocyclic polyamine Co(II) complex was investigated in metallomicellar system. The results showed that the conversion of plasmid DNA (pUC_{18}) increases with the increase of the concentration of complex under physiological conditions.

Keywords: Bifunctional dioxo macrocyclic polyamine, complex, micellar system, cleavage of DNA.

Artificial enzymes are non-protein molecules that are more simple than natural enzymes, but they also possess high efficiency and specificity. In recent years, the study of enzyme models is one of the most active fields[1-2], especially chemical nuclease. Micelles, dynamic colloidal aggregates formed by amphipathic surfactant molecules, can mimic the hydrophobic structure of active site of enzymes because they offer a hydrophobic microenviornment which is similar to the important part located at the active site of enzymes. After simple chemical nuclease was led into micellar systems, catalytic hydrolysis reactions can be accelerated by the comicellar system because of the formation of supramolecular assembly between the lipophilic complex and general micelle.

Nucleic acid is the material foundation of genetic variation in organisms. DNA is the carrier of genetic information in most life-organisms. Non-enzymatic hydrolysis of DNA and RNA has attracted much interest, mainly because it is essential for further developments in biotechnology, molecular biology, therapy and related fields. In current biotechnology, the DNA of bacteria and viruses is manipulated using naturally-occurring enzymes[3]. The catalytic hydrolysis research of DNA is not only of momentous current significance in clarifying the relationship between structure of biomacromolecules and their functions, but also a spading work at designing of novel pharmaceuticals possessing anticancer activity. However, the phosphodiester linkages in DNA are enormously stable (the half-life of the phosphodiester linkage in DNA at pH 7 and 30°C is estimated to be 200 million years), and (until recently) can not be easily hydrolyzed without using natural enzymes[4]. We have recently reported the catalytic

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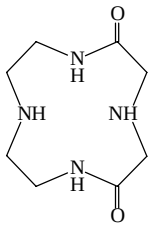
hydrolysis of phosphate diester and lipophilic amino-acid esters with metal complexes of macrocyclic tetraamine in comicellar solution[5,6]. In this paper, we report our work on the interaction between bifunctional dioxo macrocyclic polyamine Co(II) complex and DNA (pUC_{18}) in metallomicellar system. To our knowledge, this is the first paper to study DNA cleavage in micellar system.

Experimental

FAB-MS spectra data were recorded on VG Autospec 3000 mass spectrometer. ^{1}HNMR spectra were recorded at 90.0 MHz and chemical shifts in ppm are reported relative to internal Me_4Si. Elemental analysis was performed by using a Carlo Elba 1106 elemental analytical instrument. Melting points were determined by using a micro-melting point apparatus without any corrections. All other chemicals and reagents were obtained commercially and used without further purification. Electrophoresis grade agarose was purchased from TaKaRa Biotechnology Company. Plasmid DNA (pUC_{18}) purchased from Sino-American Biotechnology Company.



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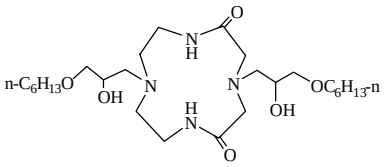

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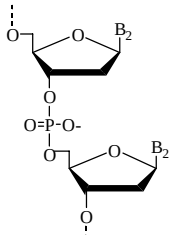


DNA

Ligand

2, 6-Dioxo-1, 4, 7, 10-tetraazacyclododecane **1** (0.40 g, 2.0 mmol)[7] and hexyl glycidyl ether (0.98 g, 6.0 mmol) were refluxed in EtOH (20 mL) for 24 h under nitrogen atmosphere. The solvent was evaporated and the residue was purified by silica gel column chromatography (eluent $CHCl_3$: MeOH = 16:1) to yield **2** as a pale yellow liquid (0.52 g, 50.4% yield). MS (*m/z*, rel. intensity): 516 (M+, 80). ^{1}HNMR (90 MH_Z, $CDCl_3$, δppm): 0.88 (t, 6H, J = 6.90, CH_3), 1.26 (d, 16H, J = 7.10, CH_2), 2.29 - 3.37 (m, 26H, NCH_2+OCH_2), 3.87 (br, s, 2H, CONH). Anal. Calcd. (Found) for $C_{26}H_{52}N_4O_6$: C, 60.09 (60.43), H, 10.52 (10.14), N, 11.01 (10.84).

*Co (II) complex of ligand **2***

To 4,10-bis(1-hexyloxy-2-hydroxypropyl)-2, 6-dioxo-1,4,7, 10-tetraazacyclododecane **2** (0.34 g, 0.66 mmol) in 10 mL of EtOH, the $Co(CH_3COO)_2 \cdot 4H_2O$(0.16 g, 0.66 mmol) dissolved in 10 mL EtOH was added dropwise under nitrogen atmosphere. The reaction mixture was kept at 60°C for 24 h, and then the solvent was evaporated slowly to about 15 mL under reduced pressure, the solid was filtered and washed with cool

EtOH, dried in vacuum to obtain Co(II) complex of **2** as red purple solid (0.30 g, yield 65.1%), mp 160-162°C. FAB-MS (*m/z*, rel. intensity): 573 (M+1-2CH_3COO, 100). Anal. Calcd. (Found) for $C_{30}H_{58}N_4O_{10}Co$: C, 52.01 (51.94), H, 8.40 (8.43), N, 8.06 (8.08), Co, 9.38 (8.50).

General procedure for DNA cleavage

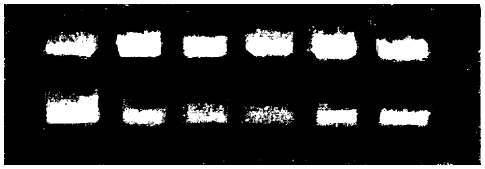
The cleavage of pUC_{18} by **2**·Co(II) complex was accomplished by mixing (in order) 0.04 mol/L. Hepes buffer solution (pH 7.02), **2**·Co(II) complex (containing 2.0 mmol/L surfactant Brij 35), and 0.05 mg/mL of pUC_{18}. After mixed, the DNA solutions were incubated at room temperature. At the appropriate times, the reaction was quenched by addition of Na_2EDTA (0.025 mol/L, EDTA = ethylenediamine tetraacetic acid) and bromphenol blue. Agarose gel electrophoresis was performed by using 0.8% agarose gels under standard conditions (100 mmol/L Tris, 100 mmol/L borate). Electrophoresis was continued typically at 4 v/cm, gels were stained by ethidium bromide, destained and then photographed under UV light.

Results and Discussion

*Effect of concentrations of **2**·Co(II) complex on DNA cleavage*

Cleavage reactions of plasmid DNA (pUC_{18}) by **2**·Co(II) complex were investigated in different concentrations of complex (**Figure 1**). The results showed that **2**·Co(II) complex could cleave pUC_{18} from super strand to nicked strand. When concentration of the complex as 2.0×10^{-6} mol/L, the catalytic activity of **2**·Co(II) complex is still in existence. There is a linear relationship between the efficiency of scission of DNA and the concentration of **2**·Co(II) complex. That means the conversion of DNA increases with the increase of the concentration of complex.

Figure 1 Agarose gel electrophoresis of cleavage reaction of pUC_{18} DNA by different concentrations of **2**·Co(II) complex (ethidium bromide staining)



1 2 3 4 5 6

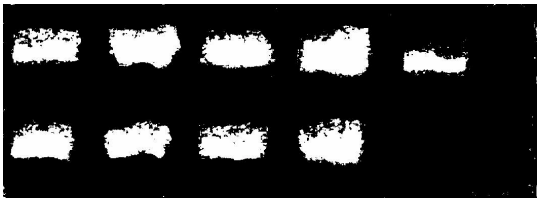
Scission conditions: 0.05 mg/mL DNA, 190 mmol/L ascorbic acid in 0.04 mol/L Hepes buffer, pH 7.02. Lane 1: DNA control. **2**·Co(II) complex concentration, Lane 2-6: 0.012 mol/L, 2.0×10^{-4}mol/L, 2.0×10^{-5}mol/L, 2.0×10^{-6}mol/L, 2.0×10^{-7}mol/L.

Effect of times of agarose gel electrophoresis on DNA cleavage

Cleavge reaction of plasmid DNA (pUC_{18}) by **2**·Co(II) complex were also examined in different time. It has been concluded that the cleavage efficiency of DNA (pUC_{18}) by

that complex also corresponds relationship with reaction time (**Figure 2**). This result is consistent with Dixon's report [8].

Figure 2 Agarose gel electrophresis of cleavage reaction of pUC_{18} DNA by **2**·Co(II) complex (ethidium bromide staining)with different time



1 2 3 4 5

Scission conditions: 0.05 mg/mL DNA; 2.0×10^{-4} mol/L complex, 190 mmol/L ascorbic acid in 0.04 mol/L Hepes buffer, pH 7.02, at room temperature. Lane 1-5: reaction after 30 min, 60 min, 90 min, 120 min, 150 min of reaction.

Conclusion

The nucleophilic activity of two side chain hydroxyl groups on ligand **2** was promoted after the formation of the stable **2**·Co(II) complex. When the interaction between complex and DNA was occurred and the phosphodiester linkage bond was attacked by them, then DNA was cleaved. Because the side chain of the complex is lipophilic, it forms the comicellar system with Brij 35 that could offer the good hydrophobic microenviornment to promote DNA cleavage. The Co(II) complex as chemical nuclease only cleaved plasmid DNA (pUC_{18}) from supercoiled DNA (form I) to nicked DNA (form II), but the latter (form II) was not further cleaved. All above results demonstrate that lipophilic bifuctional arm Co(II) complex is efficient for the cleavage of plasmid DNA in comicellar system at physiological conditions.

Acknowledgments

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