

Synthesis of a novel linear polymer of a macrocyclic polyamine copper (II) complex and its interaction with plasmid DNA

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Abstract

The novel linear polymer of a macrocyclic polyamine copper (II) complex, which has many cyclen groups linked by epichlorohydrin, has been synthesized as a DNA cleavage agent. The structure of the polymer **3** was identified by ¹HNMR and IR and its molecular weight was measured by GPC. The result of agarose gel electrophoresis assay showed that Cu-(II) complex **4** could act as a powerful catalyst for the cleavage of plasmid DNA under physiological conditions.

Keywords: 1, 4, 7, 10-Tetraazacyclododecane (cyclen), Macrocyclic polyamine, Copper (II) complex, DNA cleavage

Introduction

The remarkable stability of the phosphodiester bond in DNA is one of the essential needs for the survival and maintenance of life, due to the repulsion between the negatively charged phosphodiester backbone and potential nucleophiles [1]. The half-life for spontaneous hydrolysis of the phosphodiester has been estimated as about 10¹¹ years at pH 7 and 30°C [2]. Some natural enzymes, such as restriction endonucleases and topoisomerases, can catalyze phosphoryl cleavage efficiently under physiological conditions, but the enzymatic reaction is difficult to be performed in the laboratory [3]. Thus, great efforts have been made to expand artificial enzyme that work on DNA with similar catalytic mode [4]. Chemical nucleases could recognize a specific DNA sequence and enhance the hydrolysis of the phosphate diester bond, which would rapidly be an invaluable research tool of biology, bioorganic chemistry and molecular biology [5–7]. Most of the known chemical nucleases for DNA hydrolysis are metal complexes [8–12].

Many complexes have been designed and studied as artificial enzyme models. In particular, 1, 4, 7, 10-tetraazacyclododecane (cyclen) has a special structure and can coordinate with most metal cations (transition metal ions and lanthanide ions) [13–16]. At present, many cyclen metal cations complexes have been used in DNA recognition and cleavage [15,17–20]. Our group also reported several new metal complexes of cyclen derivatives, which are used as efficient chemical enzyme to enhance the cleavage ability of DNA [21–26]. However, almost all of those reported complexes of cyclen are mononuclear or dinuclear complexes. Yet to now, to best of our knowledge, a few complex of polymer of cyclen was reported. In most cases, synergistic effects exist between the metal centers when complex as chemical enzyme interact with DNA [27]. Hence, it is anticipated that complexes of polymer of cyclen is an efficient artificial enzyme to cleavage DNA under physical conditions. On the other hand, copper (II) complex which can accelerate hydrolysis cleavage DNA is one of the most

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promising metals for artificial nucleases [26]. Moreover, more than twelve enzymes depend on copper for their activity. Therefore, the synthesis and activity studies with DNA of copper complexes have attracted more attention from different perspectives [28–29].

In this report, we described the synthesis and characterization of a novel linear polymer of cyclen copper (II) complex, which was linked by epichlorohydrin, and its applications on DNA cleavage was also studied. The result showed that it can catalyze the cleavage of supercoiled DNA (pUC 19, plasmid DNA) under physiological conditions to produce selectively nicked DNA (Form II).

Experimental

General

The ^1H NMR spectra were measured on a Varian INOVA-400 spectrometer and the δ scales in ppm were referenced to residual solvent peaks or internal tetramethylsilane (TMS). IR spectra were recorded on a Shimadzu FTIR-4200 spectrometer as KBr pellets or thin films on KBr plates. Inductively Coupled Plasma Atomic Emission Spectrometers (ICP-AES) was conducted on IRIS Advantage. Gel permeation chromatogram (GPC) was using a waters instrument incorporating Shodex columns (OHPAK KB-803) and polyglycol standard was used for calibration. Electrophoresis apparatus was a biomeans stack II-electrophoresis system, PPSV-010. Bands were visualized by UV light and photographed using a gel documentation system by the estimation of the intensity of the DNA bands, recorded on an Olympus Grab-IT 2.0 annotating image computer system. Cyclen were prepared according to the literature [31]. All other chemicals and reagents were obtained commercially and were used without further purification.

Synthesis of polymer 2

1, 7-bis (diethoxyphosphoryl)-1, 4, 7, 10-tetraazacyclododecane (130 mg, 0.293 mmol) were dissolved in $\text{C}_2\text{H}_5\text{OH}$, then H_2O and epichlorohydrins (27.1 mg, 0.293 mmol) were added. Under the protection of N_2 , the reaction was stirring at 80°C for 24 h. After adding NaOH (26.4 mg, 0.659 mmol), the system was stirred at 80°C for another 24 h. The solvent was removed and the alkaline solution was extracted with hot CHCl_3 . The organic layer was dried over anhydrous Na_2SO_4 , and then the solvent was evaporated to 5 mL. After dropping petrol ether to the solution, polymer 2 was obtained. IR (CH_2Cl_2 , cm^{-1}): 3329, 2968, 2928, 2843, 1458, 1373, 1249, 1216, 1159, 1090, 1045, 755. ^1H NMR (400 MHz, CDCl_3): δ (ppm) = 4.16–3.84 (m, 8H, OCH_2CH_3), 3.90–3.84 [m, 1H, $\text{CH}(\text{OH})$], 3.65–3.45 [m, 4H,

$\text{CH}(\text{OH})(\text{CH}_2)_2$], 3.40–3.20 [m, 8H, $\text{N}(\text{CH}_2)_2$], $\text{PO}(\text{OCH}_2\text{CH}_3)_2$], 2.95–2.70 [m, 8H, $\text{N}(\text{CH}_2)_2$], 2.00 (bres, 1H, OH), 1.43–1.30 (m, 12H, OCH_2CH_3).

Synthesis of polymer 3

10 mL $\text{C}_2\text{H}_5\text{OH}$ and 10 mL HCl (37%) were added to the complex 2 (330 mg) and the system was stirring at 50°C for 24 h under the protection of N_2 . After the solvent had been removed, the pH was adjusted to pH 12 with 5 N NaOH aqueous solution. The alkaline solution was extracted with hot CHCl_3 . The organic layer was dried over anhydrous Na_2SO_4 , and then the solvent was evaporated to 5 mL. After dropping petrol ether to the solution, polymer 3 was obtained. The molecular weight of polymer 3 was measured by GPC ($M_w = 1077$). IR (CH_2Cl_2 , cm^{-1}): 3395, 2924, 2825, 1342, 1161, 1091, 1032, 713. ^1H NMR (400 MHz, DMSO): δ (ppm) = 5.7 (m, 1H, OH), 4.10–3.86 [m, 2H, $\text{NH}(\text{CH}_2)_2$], 3.65–3.45 [m, 4H, $\text{CH}(\text{OH})(\text{CH}_2)$], 3.40–3.20 [m, 8H, $\text{N}(\text{CH}_2)_2$], 2.95–2.70 [m, 8H, $\text{N}(\text{CH}_2)_2$]. Elemental analysis (HCl salt): C, 37.27; N, 15.20; H, 7.30.

Synthesis of complex 4

To a solution of polymer 3 (12 mg) and $\text{C}_2\text{H}_5\text{OH}$ (2 mL), a solution of $\text{CuCl}_2\cdot\text{H}_2\text{O}$ (15 mg) and $\text{C}_2\text{H}_5\text{OH}$ (1.0 mL) were added slowly. After the reaction mixture stirring overnight at room temperature, a pink blue solid (complex 4) was obtained after filtering. IR (KBr, cm^{-1}): 3438, 2961, 2875, 1458, 1229, 1079, 1025, 805. ICP-AES [Cu^{2+}]: 3.200 $\mu\text{g}/\text{mL}$. Found: 2.975 $\mu\text{g}/\text{mL}$.

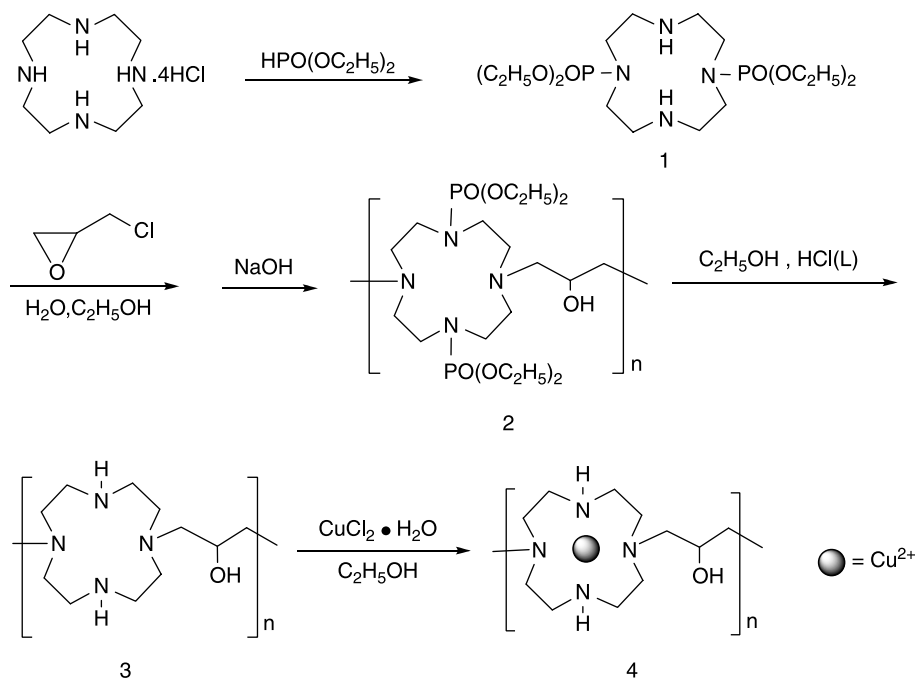
Plasmid DNA cleavage

Plasmid DNA (pUC 19) cleavage activity of complex 4 was monitored by using agarose gel electrophoresis. In a typical experiment, supercoiled pUC 19 DNA (5 μL , 0.025 $\mu\text{g}/\mu\text{L}$) in Tris-HCl buffer (100 mM) was treated with different concentration of complex 4, followed by dilution with the Tris-HCl buffer to a total volume of 17.5 μL . The samples were then incubated at 37°C for 4 – 72 h, and loaded on a 1% agarose gel containing 1.0 mg/mL ethidium bromide. Electrophoresis was carried out at 40 V for 30 min in TAE buffer. Bands were visualized by UV light and photographed followed by the estimation of the intensity of the DNA bands using a Gel Documentation System.

Result and discussion

Preparation of polymer of cyclen Cu (II) complex

Scheme 1 shows the synthetic route for the novel linear polymer of cyclen Cu (II) complex (complex 4) studied in this work. The 1, 7-bis (diethoxyphosphoryl)-1, 4, 7,



Scheme 1. The synthetic route of the linear polymer of cyclen copper (II) complex.

10-tetraazacyclododecane (**1**) [30] has been found to be an effective protecting group for the synthesis of 1, 7-disubstituent cyclen. Hence, **1** was used as the intermediate, which was linked with epichlorohydrin to produce the polymer **2**. The Dep protective groups were cleaved completely from polymer framework by dense hydrochloric acid, which was indicated by ^1H NMR. Polymer **3** was allowed to react with $\text{CuCl}_2 \cdot \text{H}_2\text{O}$ in EtOH overnight to give complex **4**. ICP-AES indicated that almost all cyclen were coordinated with copper (II). However, the polymer **3** is a liquid and bibulous substance so that it is difficult to analyze its element content at room temperature. Therefore, we convert polymer **3** to its HCl salt to analyze its element content.

Cleavage of plasmid DNA

The activities of complex **4** with pUC 19 supercoiled DNA were studied. The cleavage of the supercoiled plasmid DNA (Form I) was processed to produce selectively open-circular (Form II). The amounts of strand scission were assessed by agarose gel electrophoresis. Typical gels showed that the highest activity was observed at pH 7.4 and the results for these experiments were shown in Figures 1–3.

Firstly, we compared the DNA cleavage abilities of complex **4** under different pH buffer. As shown in Figure 1, with the pH decrease from 7.4 to 6.6, complex **4** has almost the same catalytic effect for DNA cleavage, and no nicked DNA (Form II) were found when the buffer pH = 6.2 and 5.8. On the

other hand, the quantity of nicked DNA increased from 7.3 to 20% when the pH of buffer reduced from 7.4 to 6.6. From the above analysis, eliminating the nicked DNA by buffer, complex **4** has the best catalytic ability for DNA cleavage at pH = 7.4. So, complex **4** has the best catalytic effect under physical condition.

Figure 2A and 2B showed the effect of cleavage time of complex **4**. The decrease of supercoiled Form I was accompanied the appearance of opening circular Form II. It was found that the longer time reacted, the more product of DNA cleavage was detected. When the reaction time reached 72 h, 37.3% nicked DNA (Form II) was observed (Figure 2B).

The cleavage of DNA by different concentration of complex **4** was also studied (Figure 3). The amount of nicked DNA (Form II) observed in agarose gel electrophoresis diagram increased with increase of concentration of complex **4** under the reaction system (Figure 3A). The concentration of complex **4** increased in the order of 0.2, 0.4, 0.6 and 0.8 mg/mL, resulting in 37.9, 47.1, 47.3 and 50% nicked DNA, respectively (Figure 3B). When the concentration of complex **4** reach to 1.0 mg/mL, there was more DNA cleavage (52.3% nicked DNA).

In conclusions, we first designed and synthesized a novel linear polymer of cyclen copper (II) complex. The agarose gel electrophoresis assay indicated that complex **4** as an artificial enzyme can quicken up the plasmid DNA cleavage to nicked DNA (Form II) under physical conditions.

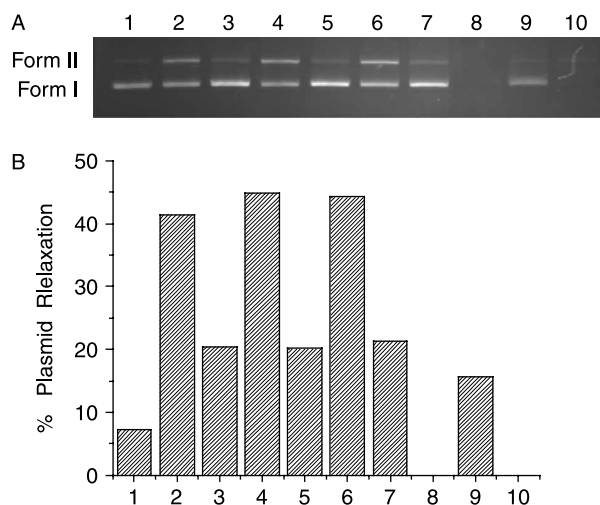


Figure 1. Effect of pH on the cleavage reaction of pUC 19 DNA ($7 \mu\text{g/mL}$) with complex 4 (1 mg/mL) in a Tris-HCl buffer (100 mM) at 37°C for 72 h. (A) Agarose gel electrophoresis diagram: lane 1, DNA control, pH 7.4; lane 2, pH 7.4; lane 3, DNA control, pH 7.0; lane 4, pH 7.0; lane 5, DNA control, pH 6.6; lane 6, pH 6.6; lane 7, DNA control, pH 6.2; lane 8, pH 6.2; lane 9, DNA control, pH 5.8; lane 10, pH 5.8. (B) quantitation of % plasmid relaxation relative to plasmid DNA per lane.

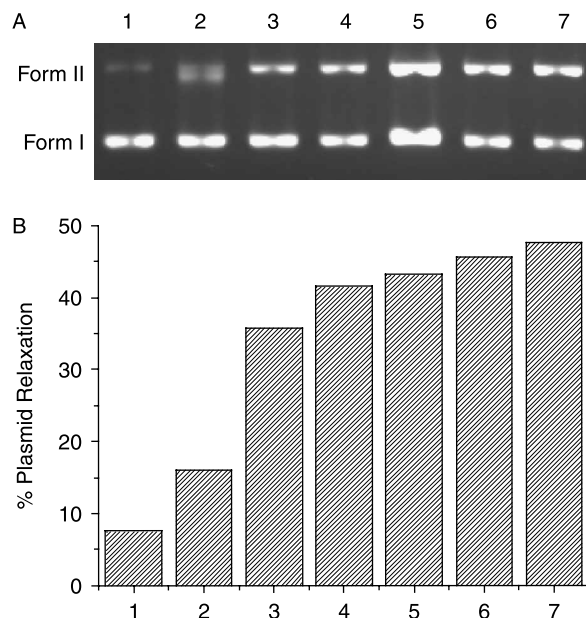


Figure 3. Effect of concentration of complex 4 on the cleavage reaction of pUC 19 DNA ($7 \mu\text{g/mL}$) with complex 4 in a Tris-HCl buffer (100 mM , pH 7.4) at 37°C . (A) Agarose gel electrophoresis diagram: lane 1, DNA control, 0 h; lane 2, DNA control, 72 h; lane 3, 0.2 mg/mL ; lane 4, 0.4 mg/mL ; lane 5, 0.6 mg/mL ; lane 6, 0.8 mg/mL ; lane 7, 1.0 mg/mL . (B) quantitation of % plasmid relaxation relative to plasmid DNA per lane.

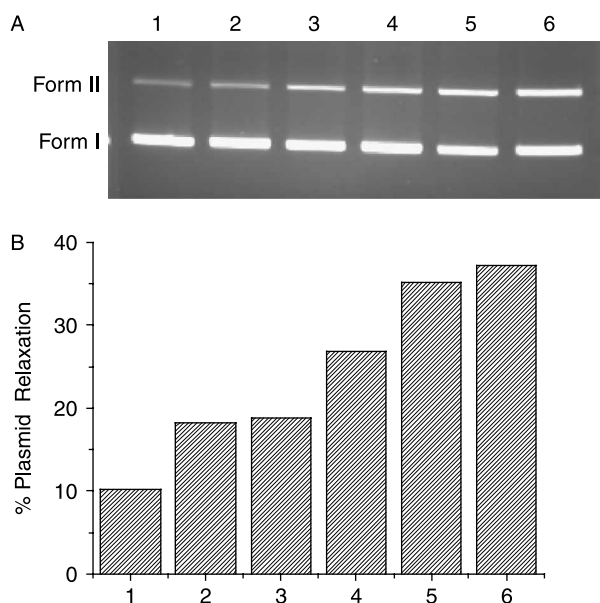


Figure 2. Effect of time on the cleavage reaction of pUC 19 DNA ($7 \mu\text{g/mL}$) with complex 4 (1 mg/mL) in a Tris-HCl buffer (100 mM , pH 7.4) at 37°C . (A) Agarose gel electrophoresis diagram: lane 1, DNA control, 0 h; lane 2, DNA control, 72 h; lane 3, 4 h; lane 4, 8 h; lane 5, 24 h; lane 6, 72 h. (B) quantitation of % plasmid relaxation relative to plasmid DNA per lane.

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