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DNA cleavage promoted by trigonal-bipyramidal zinc(II) and copper(II) complexes formed by asymmetric tripodal tetradendate 2-[bis(2-aminoethyl)amino]ethanol

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ABSTRACT

Asymmetric trigonal-bipyramidal Zn(II) complex **1** formed by 2-[bis(2-aminoethyl)amino]ethanol (**L**) was found to be able to promote the cleavage of supercoiled plasmid DNA pBR322 to the nicked and linear DNA *via* a hydrolytic manner but only in neutral Tris–HCl buffer, no cleavage was observed in HEPES or NaH_2PO_4/Na_2HPO_4 buffer. However, the copper complex **2** of **L**, possessing the similar coordination geometry, can only promote DNA cleavage *via* an oxidative mechanism in the presence of ascorbic acid. ESI-MS study implies that complex **1** exist mainly as $[Zn(\mathbf{L})]^{2+}/[Zn(\mathbf{L}–H)]^{+}$ in neutral Tris–HCl buffer. Moreover, there is no discriminable species for complex **1** in HEPES or NaH_2PO_4/Na_2HPO_4 buffer. A phosphate activation mechanism *via* phosphate coordinating to Zn(II) center of $[Zn(\mathbf{L})]^{2+}/[Zn(\mathbf{L}–H)]^{+}$ to form the stable trigonal-bipyramidal structure is proposed for the hydrolytic cleavage promote by complex **1**. For complex **2**, the abundance of $[Cu(\mathbf{L})Cl]^{+}$ is higher than that of $[Cu(\mathbf{L})]^{2+}/[Cu(\mathbf{L}–H)]^{+}$ in Tris–HCl buffer. The lower phosphate binding/activating ability of Cu(II) in complex **2** may be the origin for its incapability to promote the hydrolytic DNA cleavage. However, the readily accessible redox potential of Cu(II) makes complex **2** promote the oxidative DNA cleavage. Although the DNA cleavage promoted by complex **1** has no specificity, trigonal-bipyramidal Zn(II) complexes formed by asymmetric tripodal polyamine with ethoxyl pendent should be a novel potential model for practical artificial nuclease.

1. Introduction

DNA cleavage is of great significance due to its potential application in gene therapy, gene engineering and nucleic acid structure detection, etc. [1–3]. Artificial nucleases of high efficiency and selectivity are demanded by both chemists and biochemists. The essential roles of transition metal in natural nucleases makes the transition metal complexes be frequently studied in this field [4–8]. The hydrolytic cleaving promoters are especially appreciated, since the resulting DNA fragments can be re-ligated by ligases facilitating gene engineering [9–15]. Metal complexes of macrocyclic polyamines or analogues bearing ethoxyl pendent were frequently adopted to mimic the function of Zn(II)–serine alkoxide motif in enzymes such as alkaline phosphatase, and most of them have displayed effective promotion to the hydrolysis of different DNA model compounds, and the additional pendant may favor the hydrolysis by providing the synergetic catalytic sites [16–22].

Trigonal-bipyramidal metal complexes formed by asymmetric tripodal tetradendate with hydroxyl pendent were developed recently as promoter of carboxylic ester hydrolysis. The tetradendate occupies four apexes of the trigonal-bipyramid, leaving one apex for activating water or substrate molecule *via* coordinating to metal center [23–25]. For example, complex **1** and complex **2**, formed by 2-[bis(2-aminoethyl)amino]ethanol (**L**), displayed distinct promotion to the ester cleavage of 4-nitrophenyl acetate in weakly alkaline condition (Scheme 1) [24,25]. Complex **1** promotes the acetate cleavage in a catalytic hydrolysis manner *via* Zn(II)-activated water molecule attacking transesterification intermediate, while complex **2**, through the metal alkoxide-promoted transesterification path and 1:1 reaction ratio is demanded. Moreover, the hydroxyl pendent of **L** plays essential roles in transesterification process in both mechanisms, and the asymmetrical trigonal-bipyramidal metal center favors to activate the bound water molecule. All these may be helpful also in promoting the hydrolysis of phosphate ester, favoring the cleavage of DNA. Therefore, in this work, the DNA cleaving behaviors of complexes **1** and **2** was investigated in neutral condition to explore whether the trigonal-bipyramidal transition metal complex formed by asymmetric tripodal tetradendate

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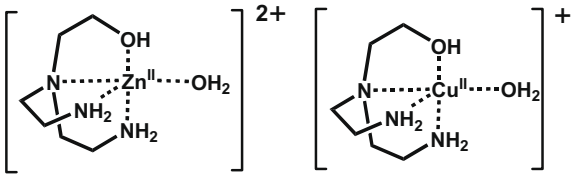

(A) (B)

Scheme 1. Hydrates of complexes **1** (A) and **2** (B).

with hydroxyl pendent is a suitable candidate as artificial nucleases.

2. Experimental

2.1. Materials

Plasmid pBR322 DNA was obtained from MBI Fermentas. Ethidium bromide (EB), agarose and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) were purchase from Ameresco, Biowest and Sigma, respectively. *Ex Taq* DNA polymerase and restriction enzymes *Eco*R I were the product of TaKaRa. Oligonucleotide primer (5′-CGGAAGGAGCTGACTGGGTTG-3′) was designed and synthesized by Invitrogen based on the sequence of pBR322 DNA. QIAquick Gel Extraction Kit was purchased from Qiagen. All other chemicals and solvents were of analytical grade and used without further purification.

2-[Bis(2-aminoethyl)amino]ethanol (**L**), its Zn(II) complex $[ZnH_{-0.25}\mathbf{L}(H_2O)](ClO_4)_{1.75}$ (**1**) and Cu(II) complex $[Cu\mathbf{L}(Cl)](ClO_4)$ (**2**) were synthesized according to the reported procedures [24,25]. Doubly distilled water was used in the DNA cleaving experiments. Stock solution of complex **1** (**I**, 1 mM) for DNA cleaving experiments was prepared directly by dissolving complex **1** in Tris–HCl buffer (50 mM, pH 7.4). The Cu(II) complex stock solution (**II**, 1 mM) was prepared by mixing aqueous solution of complex **2** (2 mM) with one equivalent of $AgBF_4$ aqueous solution (2 mM) followed by stirring overnight in dark and removing the precipitate *via* centrifugation.

2.2. Methods and instrumentations

An LCQ electrospray ionization mass spectrometer (ESI-MS, Finnigan MAT) was employed to determine the complex species in aqueous methanol or buffers, and the calculated isotope distribution patterns were calculated by IsoPro 3.0 program. A pHS-3C instrument with Phoenix Ag-AgCl reference electrode was adopted to determine the pH values. Electrophoresis experiments were carried out with a Bio-Rad electrophoretic system. The image acquisition and analysis software was DigiDoc-It™ system (version 1.1.23, UVP, Inc. Unpland, CA). DNA amplification was performed with an ABI PCR amplifier.

2.3. DNA cleavage promoted by complexes 1 and 2

The cleavage of supercoiled pBR322 DNA promoted by Zn(II) complex was proceeded by mixing 1.0 μl of pBR322 DNA (0.25 μg/μl, 50 mM Tris–HCl, pH 7.4) with different volume of stock solution **I** (0.5–12 μl), followed by adding 50 mM Tris–HCl (pH 7.4) buffer to the total volume of 18 μl. Then the mixed solutions were incubated at 37 °C for 4 h. The DNA cleaving experiments promoted by complex **2** were studied by two methods. The hydrolytic cleaving experiments were carried out in a similar procedure for complex **1** with stock solution **II** replacing stock solution **I**. The oxidative DNA cleaving behavior of complex **2** was studied in the presence of ascorbic acid. Therefore, the cleavage was carried out by mixing 1.0 μl of pBR322 DNA (0.25 μg/μl; 50 mM Tris–HCl, pH 7.4) with stock solution **II** (1 mM, 0.5–3.5 μl) and ascorbic acid (5 mM, 0.5–3.5 μl), followed by adding Tris–HCl buffer (pH 7.4) to a total volume of 18 μl. The incubation temperature was also 37 °C.

After incubation, all the mixtures were analyzed by agarose gel electrophoresis. Therefore, the reaction was quenched by 2 μl EDTA solution (0.5 M), and 3.5 μl loading buffer containing bromophenol, xylene cyanol and glycerol were added thereafter. The electrophoresis analysis of the digested DNA solution was performed on 1% agarose gel with ethidium bromide (EB 0.5 μg/ml) at 80 V for 1.5 h in a buffer containing 1 × TAE buffer. The electrophoresis patterns were determined *via* densitometric analysis of ethidium bromide-stained agarose gel using the volume quantification method of Glyko BandScan 4.30. Supercoiled plasmid DNA values were corrected with a factor of 1.3 due to the lower EB binding ability [26,27].

2.4. Cleavage specificity determination via DNA amplification

The linear DNA obtained in pBR322 DNA cleavage promoted by zinc(II) complex **1** was isolated and purified using a QIAquick Gel Extraction Kit. Then the linear DNA was used as the template for DNA amplification. DNA amplification system (50 μl) containing the linear DNA (250 ng), the primer (5′-CGGAAGGAGCTGACTGGGTTG-3′, 0.4 μM), four deoxynucleoside triphosphates (ATP, TTP, GTP and CTP, 200 μM each), $MgCl_2$ (2 mM) and 1.25 U of *Ex Taq* DNA polymerase were treated in the following procedure: denaturation at 94 °C for 5 min; 10 cycles of 30 s at 94 °C, 30 s at 64 °C, 3 min at 72 °C. The amplification products were monitored by agarose gel electrophoresis. The supercoiled pBR322 DNA and the linear DNA obtained from the *Eco*R I-promoted cleavage of pBR322 DNA were utilized as the control.

3. Results and discussion

3.1. pBR322 DNA cleavage promoted by complex 1

Fig. 1 shows the agarose gel electrophoresis patterns of pBR322 DNA after incubation with complex **1** for 4 h in Tris–HCl buffer (pH 7.4) at 37 °C. Compared with the DNA control (lane 1), the patterns of lanes 2–8 demonstrate that the DNA cleavage occurs only when incubated with complex **1**. The conversion of the supercoiled pBR322 DNA (SC DNA, form I) to the nicked DNA (NC DNA, form II) and linear DNA (form III) becomes more efficient when increasing the concentration of complex **1**. Form I almost totally turns to form II when the concentration of **1** attains to 166.7 μM (lane 4), and form III appears at 277.8 μM (lane 5). There is no coexistence of all the three DNA forms, linear DNA only can be found after all the supercoiled DNA has been nicked. It suggests that the linear DNA must be formed from SC DNA *via* a NC DNA-intermediated consecutive twice cleaving process and the second break to form linear DNA should occur on the complementary strand within

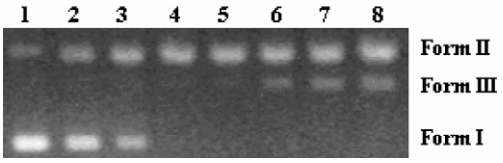

Fig. 1. Agarose gel electrophoresis patterns of pBR322 DNA (23.6 μM DNA base pairs) after incubation with complex **1** in 50 mM Tris–HCl buffer (pH 7.4) at 37 °C for 4 h. Lane 1: DNA control. The concentration of complex **1** from lanes 2 to 8 is 27.8, 55.6, 166.7, 277.8, 388.9, 500 and 666.7 μM, respectively.