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Hydrolysis of DNA by a Dipeptides Containing Histidine

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Abstract Three ligands which contain histidine and conjugated by a flexible linker, have been characterized and evaluated as DNA cleavage agents. The cleavage activity of metal complexes were evaluated by monitoring the conversion of supercoiled plasmid DNA (pUC19) (Form I) to nicked circular DNA (Form II) by agarose gel electrophoresis. The results showed that the cleavage activity of Cu(II) complexes was enhanced compared with histidine. Specially, at a high reaction concentration (0.2 mM), Cu(II) complexes can cleave the plasmid DNA with some selectivity.

Keywords DNA cleavage · Artificial nuclease · Cu(II) complexes

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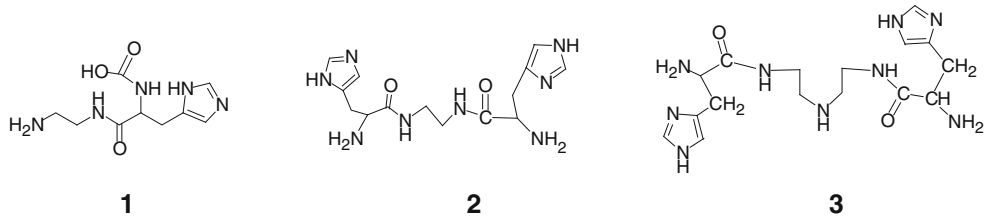
Introduction

With the high abilities in recognizing specific DNA sequence and catalyzing the hydrolysis of phosphate diester bonds, chemical nucleases have rapidly become an invaluable research tool in the fields of biology, bioorganic chemistry, therapy, and molecular biology (Westheimer 1987; Schroeder et al. 2006). Many groups reported different complexes, which could accelerate the cleavage of DNA. However, the catalytic efficiency of currently available chemical nucleases cannot be mentioned in the same breath as that of the natural enzyme. Recently, there is a growing interest in the field of histidine due to its ability to form chelates in aqueous solutions and to coordinate with most metal cations. Metal complexes of histidine are known to be effective catalysts for the cleavage of DNA (Wang et al. 2009). Researchers have shown substantial interest in the rational design of novel transition metal complexes, which could bind and cleave duplex DNA with high sequence and structure selectivity (Williams and Maxwell 1999; Shao et al. 2008; Sheng et al. 2008; Komiyama et al. 2002; Li et al. 2006; Yang et al. 2006).

We have previously reported the synthesis of several mimic polypeptides containing histidine, and their interaction with DNA. We found they can efficiently hydrolyze DNA at high concentrations (Ye et al. 2006a, b). In this paper, we describe the synthesis of three novel histidine-containing ligands (their structure shown in Scheme 1) and their applications in the DNA cleavage. The result shows that the complexes can catalyze DNA cleavage efficiently. As higher DNA affinity always leads to higher DNA cleavage efficiency, we hope the bis-His ligand, which has potentially stronger affinity with DNA than that of mono-histidine ligand to be a promising DNA cleavage reagent.

Scheme 1 The structure of title compound



Materials and Methods

Materials

The pUC19 plasmid DNA was purchased from TaKaRa Biotechnology (Dalian). Ethidium bromide (EB) was purchased from Sigma. The electrophoresis apparatus was a Biomeans stack II-electrophoresis system, PPSV-010. In the preparation of 1 and 2 (Scheme 1), histidine acid was firstly protected by Boc_2O then reacts with ethylenediamine to afford the middle products. 1 and 2 were obtained by deprotection of the middle products with CF_3COOH in CH_2Cl_2. Compound 3 was synthesized by a similar method.

Compound **1**: 93% yield, Mp: 118–120°C. ^{1}H NMR (400 MHz, D_2O, ppm): 7.58 (s, 1H), 6.84 (t, $J = 10.4$ Hz, 1H), 3.53 (t, 1H), 3.20–3.47 (m, 2H), 2.75 (d, $J = 16.4$ Hz, 2H). 2.71 (t, $J = 7.6$ Hz, 2H); ^{13}C NMR (100 MHz, D_2O, ppm): 181.05,177.07,135.98,117.08,54.95,39.60,39.48, 32.02; IR (cm^{-1}, KBr): 3092,2136,1687,1552,1439,1264, 1195,1133,995,940,834,771,722,622; ESI–MS $[M + H]^+$: 198. $[M + Na]^+$: 220.

Compound **2**: yield 95%. Mp: 200–202°C. ^{1}H NMR (400 MHz, D_2O, ppm): 7.55 (s, 2H), 6.79 (s, 2H), 3.49 (t, $J = 6.4$ Hz, 2H), 3.07 (t, $J = 4.8$ Hz, 4H), 2.69–2.80 (m, 4H); ^{13}C NMR (100 MHz, D_2O, ppm): 176.39, 135.90, 133.01, 117.18, 54.79, 38.80, 31.74; IR (cm^{-1}, KBr): 3369, 3101, 2898, 2624, 1616, 1539, 1454, 1317, 1276, 1247, 1100, 983, 933, 890, 845, 765, 621; ESI–MS $[M + H]^+$: 335, $[M + Na]^+$: 357.

Compound **3**: 51% yield. Mp: 185–187°C; ^{1}H NMR (400 MHz, D_2O, ppm): δ 7.94 (t, 2H), 6.06 (s, 2H), 4.03 (t, $J = 2.0$ Hz, 2H), 3.46 (t, $J = 8.0$ Hz, 4H), 3.04–3.14 (m, 8H); ^{13}C NMR (100 MHz, D_2O, ppm): 170.74, 135.66, 129.81, 117.10, 53.07, 46.64, 35.78, 28.17; IR (cm^{-1}, KBr): 3136, 2802, 2010, 1661, 1564, 1404, 1265, 1199, 1135, 835, 800, 721, 622; ESI–MS $[M + H]^+$: 378, $[M + Na]^+$: 400.

Methods

The preparation of Cu(II) complexes **1**a as follows: To the water solutions of the compound 1 was added **1** equiv of $Cu(NO_3)_2$ as water solution, and the pH was controlled at 8.0 by addition of NaOH solution. DNA cleavage experiments were performed as follows: supercoiled pUC 19 DNA in 50 mM Tris–HCl buffer was treated with complexes 1a, followed by dilution with the Tris–HCl buffer to a total volume of 35 µl. The samples were then incubated at 37°C and loaded on a 1% agarose gel containing ethidium bromide. Electrophoresis was carried out at 40 V for 30 min in TAE buffer.

Results and Discussion

Cleavage of Plasmid DNA

First, we compared the DNA cleavage abilities between compounds **1**, **2**, and **3** under pH 7.0 and at 37°C for 24 h.. The results are shown in Fig. 1. Lanes 2–4 represent the DNA cleavage catalyzed by 1, 3 and 2, respectively. Electrophoresis and densitometry indicated that single cleavage of the supercoiled form (Form I) yielded 34.2, 65, and 88.2% nicked form (Form II), respectively. These results indicated that 3 showed much better catalytic activity than 1 and 2.

Interaction Between Zn(II), Cu(II), and Co(II) Complexes and Plasmid pUC19 DNA

According to Fig. 1, compound **3** was chosen as the key ligand in the subsequent experiments. Then, we studied the

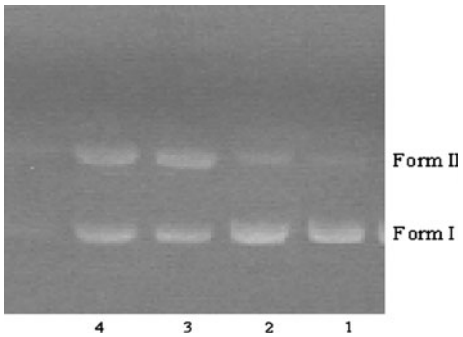


Fig. 1 Effect of different ligands 1, 2, 3 (0.3 mM) on the cleavage reactions of pUC19 DNA (0.5 µg/µL) Tris–HCl buffer (0.05 M, pH 7.5), at 37°C for 24 h. *Lane 1* DNA control, *lanes 2–4* DNA cleavage catalyzed by 1 + Cu, 3 + Cu, 2 + Cu

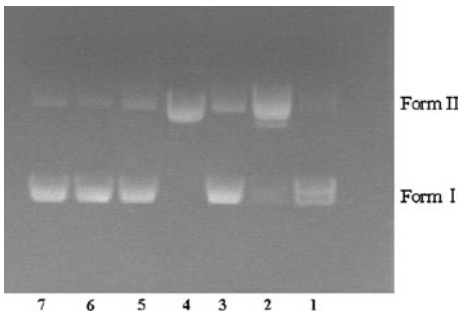


Fig. 2 Effect of metal ion on the cleavage activity of pUC19 DNA by complex 3-M: pUC19 DNA: 0.5 μg/μL, 37°C. Tris–HCl buffer (0.05 M, 0.1 M NaCl), pH 7.0, *Lane 1* DNA control, *lanes 2–4* (0.2 mm): 3 + Cu, 3 + Zn, 3 + Co, *lanes 5–8* (0.1 mm): 3 + Cu, 3 + Zn, 3 + Co

complexes 3-M. A solution containing plasmid DNA was incubated in a 0.5 ml tube with catalyst 3-Zn, 3-Cu and 3-Co (0.2 mM or 0.1 mM) in a Tris–HCl buffer (50 mM, pH 7.0) under 37°C for 48 h. The results are shown in Fig. 2. The equivalent molar Zn^{2+}, Co^{2+}, Cu^{2+} complexes **3a–c** exhibited comparable cleavage reactivity toward DNA. It can be seen that the complex 3a-Cu^{2+} is more reactive than 3b-Co^{2+} and complex 3b-Co^{2+} is more reactive than complex 3c-Zn^{2+}. It may suggest that the kind of metal ion and the steric effect of the ligands play an important role in the DNA cleavage ability. We can see that cobalt and zinc complex cleaves super-coiled DNA (form I) to relaxed (double-stranded cut, form II) DNA, and copper complexes can yield linear (single-strand broken, form III) DNA under the same conditions in the lanes 2–4 of Fig. 2. Thus, the order of cleavage activity is Cu (II) complex > Co(II) complex > Zn(II) complex for metal ions. The results showed that 0.2 mM of complex 3-M was an excellent chemical nuclease and the amount of nicked DNA (Form II) was more than the reaction catalyzed in 0.1 mM. It can be seen that the more concentration, the more DNA cleavage ability.

Interaction Between Cu(II) Complexes and Plasmid pUC19 DNA

The cleavage of DNA by different concentrations of complex 3a was investigated (Fig. 3a). Increasing the concentration of 3a resulted in increasing yield of nicked form DNA (lanes 2–6). When the concentration of 3a was increased to 0.20 mM, the plasmid DNA could be cleaved to the nicked form in 48 h (lane 5). Figure 3a indicated that the best concentration of complex 3a-Cu is 0.2 mM. Further decrease of concentration led to the decrease of Form II. The reason might be that the DNA could not be incised to pieces in low concentration of catalyst. The results revealed that bis-histidine complexes as chemical nucleases are capable to accelerate the plasmid DNA dramatically.

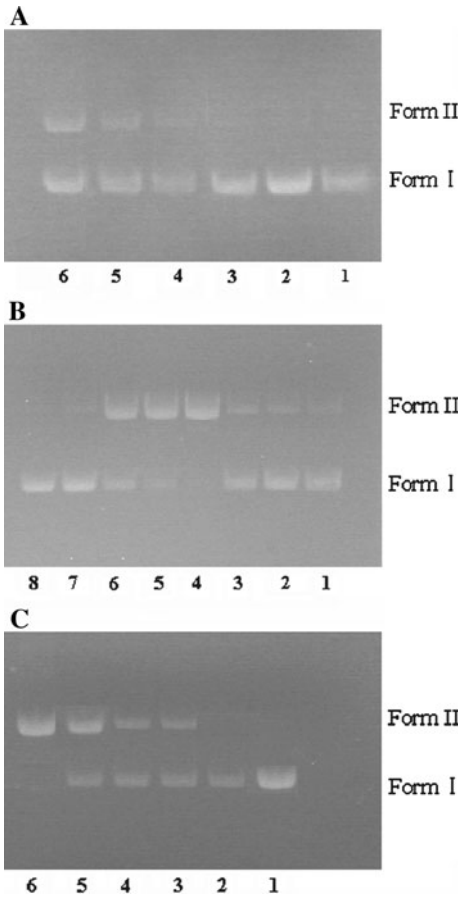


Fig. 3 Agarose gel electrophoresis of cleavage reaction of pUC 19 (DNA) (0.5 μg/μL) in Tris–HCl buffer (50 mM, pH 7.0) at 37°C. **a** By different *concentration* of complex [3 + Cu]. Scission condition: time = 48 h, [NaCl] = 0.1 M. *Lane 1* DNA control, *lanes 2–6* [3 + Cu] = 0.05, 0.1, 0.15, 0.2, and 0.3 mM. **b** By different *pH value*. Scission condition: [3 + Cu] = 0.2 mM, time = 48 h, *lane 1* DNA control (pH 6.0), *lanes 2–7* pH 6.0, 6.5, 7.0, 7.5, 8.0, and 8.5, *lane 8* DNA control (pH 8.5). **c** By different *cleavage time*. Scission condition: [3 + Cu] = 0.2 mM, *lane 1* DNA control, *lanes 2–6* time = 1, 12, 24, 48 and 60 h

The effect of pH value on the catalytic activity of 3 was also studied. The results are shown in Fig. 3b. We were pleased to find that under physiological condition (pH 7.0), 3 could be most effective for DNA cleavage, in which 90% nicked DNA was produced after 48 h (lane 4).

The effect of reaction time on the cleavage reaction is shown in Fig. 3c. The catalytic effect was improved by extending the incubation time. After incubating for 12 h, the percentage of nicked form rose to 42.4% (lane 3). All the plasmid DNA was converted into nicked form within 60 h.

Conclusion

In conclusion, we have successfully synthesized three histidine derivatives, and investigated their metal

complexes cleavage abilities. The results revealed that they were good artificial catalysts for the cleavage of DNA. For 3, plasmid DNA could be cleaved from Form I to Form II completely under mild conditions (pH 7.0) in a short time.

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