Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry 16 (2008) 1103–1110
Bioorganic & Medicinal Chemistry

DNA cleavage by novel copper (II) complex and the role of β-cyclodextrin in promoting cleavage

Yu Huang,[a] Qiao-Sen Lu,[a] Ji Zhang,[a] Zhong-Wei Zhang,[b] Yu Zhang,[a] Shan-Yong Chen,[a] Kun Li,[a] Xin-Yu Tan,[a] Hong-Hui Lin[b,*] and Xiao-Qi Yu[a,c,*]

[a]*Department of Chemistry, Key Laboratory of Green Chemistry and Technology (Ministry of Education), Sichuan University, Chengdu 610064, PR China*

[b]*Key Laboratory of Bio-resources and Eco-environment (Ministry of Education), College of Life Sciences, Sichuan University, Chengdu, Sichuan 610064, China*

[c]*State Key Laboratory of Biotherapy, West China Hospital, West China Medical School, Sichuan University, Chengdu, Sichuan 610041, PR China*

Received 16 September 2007; revised 26 October 2007; accepted 28 October 2007
Available online 1 November 2007

Abstract—A new cyclen derivative *N*-1-naphthyl-[4-amino-5-oxo-5-(1,4,7,10-tetraazacyclododecan-1-yl)]valeramide and the copper (II) complex were synthesized and characterized. The copper (II) complex showed DNA cleavage ability without the existence of other additives. The pUC19 plasmid DNA was cleaved to linear form by 0.71 μM of complex under physiological conditions. β-Cyclodextrin was used to investigate the relationship of nuclease activity and DNA binding ability. The addition of β-cyclodextrin exhibited an unexpected ability to promote the cleavage of DNA. The role of the β-cyclodextrin in DNA cleavage process was studied by ^{1}H NMR and fluorescence spectrum. According to the data of viscosity measurement, it was confirmed that the binding of complex with DNA should be a groove binding model. All the results suggested that the increasing of the DNA cleavage ability was attributed to the interaction between β-cyclodextrin and the naphthyl moieties. β-Cyclodextrin could include the naphthyl moieties and keep it from the minor/major groove of DNA and decreased the DNA binding ability, therefore, the copper (II) center was activated to generate more reactive oxygen species, which was responsible for DNA cleavage.

1. Introduction

For the especial stability under physiological conditions, DNA that contains the genetic instructions is one of the most important biomacromolecules in all living organisms. The half-life of DNA by spontaneous hydrolysis will be thousands to billions of years.[1–4] However, it could be hydrolyzed with the help of artificial nucleases[5–7] or be damaged by reactive oxygen species (ROS).[8] The ROS such as single oxygen ($^1O_2$), superoxide anion (O_2^-), and hydroxyl radical (·OH) usually were produced under aerobic environment with the help of some oxidants or ultraviolet light.[9–11] The damage of DNA by ROS might have potential therapeutic applications that could eradicate tumor cells.[12,13] And the knowledge about the DNA damage by ROS can be used to avoid the harm of ROS and to investigate the process of repairing of the damaged DNA in living organism.[14,15] Many transition metal complexes were used as DNA cleavage agents. Copper (II) ion is one of the most important ions in human body. Moreover, in the presence of H_2O_2, the DNA damage effected by copper ions is more significant than those of any other transition metal ions.[16] Copper ions were employed in many artificial metal nucleases and attracted an increasing attention.[17–19]

Small aromatic hydrocarbons and their derivatives showed an excellent DNA binding ability. Intercalation and groove binding are the most likely binding modes in the interaction between aromatic hydrocarbons and DNA.[20,21] Agents binding to DNA were explored as probes of DNA structure or as chemotherapeutic agents.[22–25] Cyclodextrins, water soluble cyclic oligosaccharides, have been recognized as ideal molecular receptors. Their rigid, bucket-shaped

Keywords: 1,4,7,10-Tetraazacyclododecane (cyclen); Copper complexes; DNA cleavage; β-Cyclodextrin.
* Corresponding authors. Fax: +86 28 85415886; e-mail: xqyu@tfol.com

0968-0896/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2007.10.088

hydrophobic cavities are good container for small molecules.[26,27] This kind of commercially available natural products was widely used in chemical and biochemical processes, especially in enzyme mimic. Cyclodextrins were also used in the cleavage of DNA, it was reported that some cyclodextrins-C_{60} conjugates were synthesized and used as photodriven DNA-cleavage reagents.[28–30] In this process, cyclodextrins were used just to improve the water solubility, the photodriven DNA cleavage ability was attributed to the fullerene moieties.[31,32]

In this work, a copper (II) complex Cu(CGN) (Scheme 1) was synthesized and characterized (CGN = *N*-1-naphthyl-[4-amino-5-oxo-5-(1,4,7,10-tetraazacyclododecan-1-yl)]valeramide). In the ligand structure, naphthyl ring was linked with the cyclen moiety, which could coordinate to copper (II) ions, through a glutamic acid bridge. Cu(CGN) showed DNA cleavage ability under physiological conditions. In the cleavage process, the copper center of the complex could bind to the phosphate oxygen atoms of DNA by electrostatic interaction,[33] meanwhile, the naphthylamine moiety could bind to DNA by partial intercalation from the minor/major grooves.[34] In order to elucidate the role of naphthylamine moiety in DNA cleavage, β-cyclodextrin was used to form an inclusion with the naphthylamine moiety. The nuclease activity was improved with the addition of β-cyclodextrin. To the best of our knowledge, this is the first example that the addition of cyclodextrins could promote the cleavage of DNA.

2. Results and discussion

2.1. General synthesis

The synthetic route is shown in Scheme 1. Compound **3** was prepared by the coupling reaction between α-naphthylamine (**1**) and 4-(*tert*-butoxycarbonylamino)-5-oxo-5-(4′,7′,10′-tris(*tert*-butoxycarbonyl)-1′,4′,7′,10′-tetraazacyclododecan-1′-yl)valeric acid (**2**) in the presence of isobutylchloroformate. Then **3** was deprotected by TFA and then basified with NaOH to give the free ligand **4**. The copper (II) complex **5** was obtained by the coordination reaction of **4** and $Cu(NO_3)_2$.

2.2. ^{1}H NMR spectrum studies

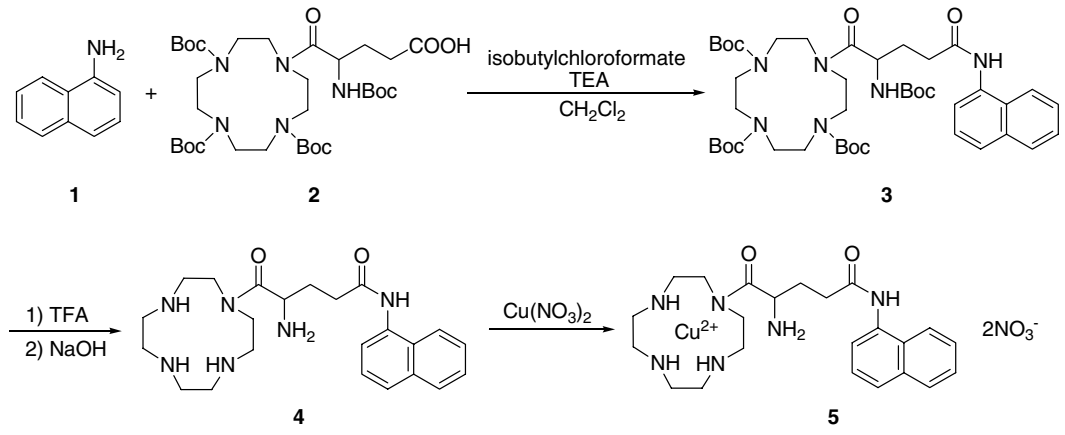
^{1}H NMR spectrum is one of the useful methods in studying the formation of cyclodextrin inclusions.[35,36] The aromatic ^{1}H NMR signals of free ligand CGN indicated the competitive interactions with the naphthyl ring between calf thymus DNA (CT DNA) and β-cyclodextrins (Fig. 1). The aromatic ^{1}H NMR signals shifted to higher field after the addition of CT DNA or β-cyclodextrin (Fig. 1b and c). This shift indicated the interactions between α-naphthylamine moieties and DNA, and it also implied the occupation of CD cavities by α-naphthylamine. The upfield shift was only 0.1–0.2 ppm, therefore the binding mode of CGN to CT DNA should not be the intercalation between naphthyl ring and the base pair stack[37] (Fig. 1b). For the poor solubility of β-cyclodextrin in water, only a small amount of β-cyclodextrin was added to the D_2O solution of 20 mM CGN and 5.2 mM CT DNA, the concentration of β-cyclodextrin was 9.3 mM and the ratio of β-cyclodextrin and CGN was only 1:2. The ^{1}H NMR signals slightly and obviously shifted after the addition of β-cyclodextrin (Fig. 1d), this result indicated that part of the α-naphthylamine moieties entered into the CD cavity from the binding position of CT DNA.

2.3. Fluorescence titration

Fluorescence titration is a useful method in the studies of binding properties of small molecules to DNA[38,39] and the studies of the host–guest interaction in cyclodextrin chemistry.[40–42] Considering the strong fluorescence quench effect of copper (II) ion, only free ligand (CGN) was used in this study. The addition of CT DNA could quench the fluorescence of CGN. The fluorescence quench results were quite consistent with the classical Stern–Volmer equation.[43]

$$F_0/F = 1 + K_{sv}[Q]$$

where F_0 and F are the fluorescence intensities of CGN in the absence and presence of CT DNA, respectively.



Scheme 1. Preparation of Cu(CGN).

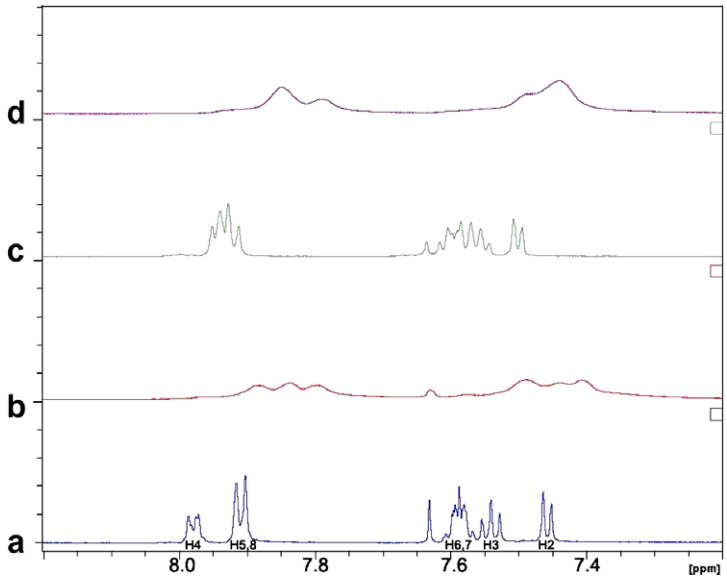


Figure 1. ^{1}H NMR spectra of the naphthyl moieties in 0.5 mL of D_2O. (a) CGN only; (b) CGN + CT DNA; (c) CGN + β-cyclodextrin; (d) CGN + CT DNA + β-cyclodextrin.

K_{sv} is the Stern–Volmer quenching constant and [Q] is the concentration of DNA. According to the quench curve (Fig. 2), the value of K_{sv} is 3.0×10^4 L/mol ($R = 0.997$). It is a quite high value compared with that of the similar compound.[20]

Contrasting to that of CT DNA, the addition of β-cyclodextrins to CGN induced the enhancement of fluorescence intensity (Fig. 3a), which indicated the formation of β-cyclodextrin inclusion. This result could be used to prove the competition binding with CGN between DNA and β-cyclodextrin. Therefore, we add β-cyclodextrin to a CGN–DNA solution and test the fluorescence intensity of the solutions. If the fluorescence intensity were enhanced, we would conclude that the α-naphthylamine moieties entered into the cavities of β-cyclodextrin from the DNA groove. As shown in Figure 3b, the titration results accorded with our suppo-

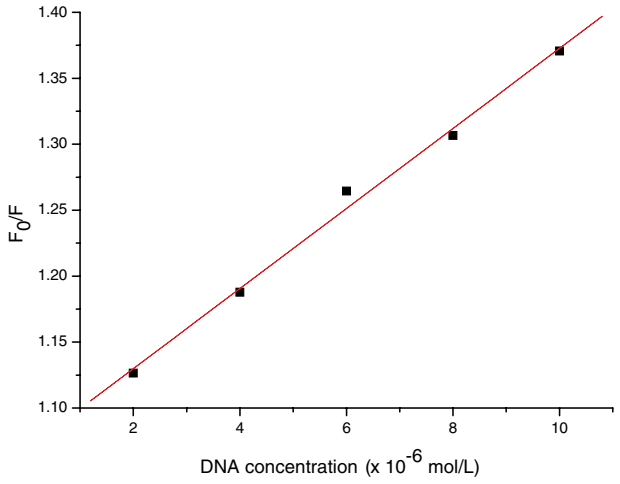


Figure 2. Fluorescence quench curve of CGN by CT DNA. [CGN] = 0.2 mM, λ_{ex} = 274 nm. Phosphate buffer (0.1 M), pH 7.4.

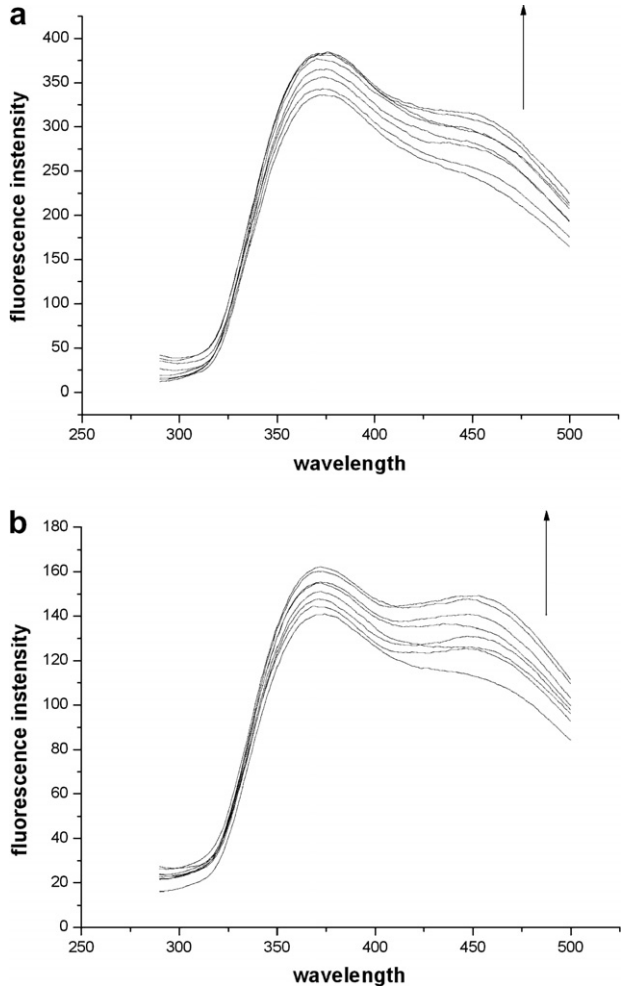


Figure 3. Fluorescence titration of β-cyclodextrin to (a) CGN and (b) DNA–CGN. [DNA] = 1.2×10^{-5} mol/L, [CGN] = 2.0×10^{-4} mol/L, λ_{ex} = 274 nm. Phosphate buffer (0.1 M), pH 7.4.

sition. In such a system, in which CGN fully interacted with CT DNA, the fluorescence intensities increased associated with the addition of β-cyclodextrins. The experiment results were consistent with the previous results of ^{1}H NMR.

2.4. Absorption spectrum studies

The absorption spectrum of CT DNA in the presence and absence of Cu(CGN) or CGN was studied. When Cu(II) complex or the free ligand was added to DNA solution, the absorption intensity increased. However, the spectra only added up the absorption of DNA and CGN (Cu(CGN)). This result indicated that the binding mode between CGN (Cu(CGN)) and DNA was not intercalation.

2.5. Viscosity measurements

The viscosity measurement of DNA is regarded as the most effective method to confirm the DNA binding mode.[44–46] Classical intercalative mode needs the reagent to intercalate into the base pairs of DNA and to lengthen the DNA helix, so the viscosity of DNA will increase. By contrast, if the partial or non-classical intercalation in DNA helix occurred, the changes of viscosity will be less or no changes will occur. The results of the effect of Cu(CGN) on the viscosity of CT DNA solution are shown in Figure 4. The relative viscosity of DNA was very slightly decreased associated with the addition of Cu(CGN). Considering the ^{1}H NMR and absorption spectrum results, the binding mode of Cu(CGN) with DNA was partial intercalation between the α-naphthylamine moieties and the minor/major groove.

2.6. Nuclease activity of complex

2.6.1. Cleavage of pUC19 DNA by copper (II) complex. The DNA cleavage ability of Cu(CGN) was studied under physiological conditions (37 °C and pH 7.4, in 0.1 M phosphate buffer without additional reagent). The cleavage of the substrate, supercoiled pUC19 plasmid DNA, was monitored by gel electrophoresis on agarose. And the DNA cleavage ability was obtained from the conversed amount of supercoiled DNA (SC, Form I) to nicked circular form (NC, Form II) and linearized DNA (LC, Form III).

Incubating DNA with the complex, increase of complex concentration led to the increase of conversion ratio of SC DNA (Fig. 5). At a higher concentration of complex (0.714 mM), SC DNA was almost converted to nicked DNA completely (Fig. 5, lane 7), and, meanwhile, small amount of LC DNA was produced.

The chemistry of DNA cleavage by Cu(CGN) was kinetically studied by quantifying the amount of SC and NC forms of DNA.[47] These data were used to perform simple kinetics. As shown in Figure 6, a time course plot described the decrease of SC form and the increase of NC form during the reaction. Both of two curves fitted well to a pseudo-single exponential curve. According to the curve fits, the DNA cleavage rate constant at 37 °C with the complex concentration of 0.714 mM was estimated to be 0.428 h^{-1} (R^2 = 0.961).

2.6.2. Mechanistic studies. The cleavage mechanism was studied by using a series of scavengers that could inhibit the reactive oxygen species. For example, sodium azide was used as singlet oxygen scavenger, SOD was used

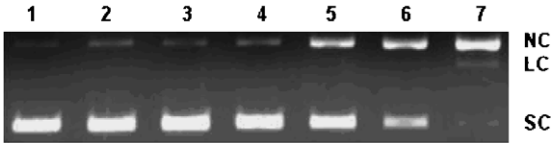


Figure 5. Agarose-gel-electrophoresis patterns for the cleavage of plasmid pUC19 DNA (7 μg/mL) in NaH_2PO_4/Na_2HPO_4 buffer (pH 7.4) at 37 °C for 12 h. Lane 1, DNA control; lanes 2–7, DNA with [Cu(CGN)] = 17.9 μM, 35.7 μM, 0.072 mM, 0.143 mM, 0.357 mM, 0.714 mM.

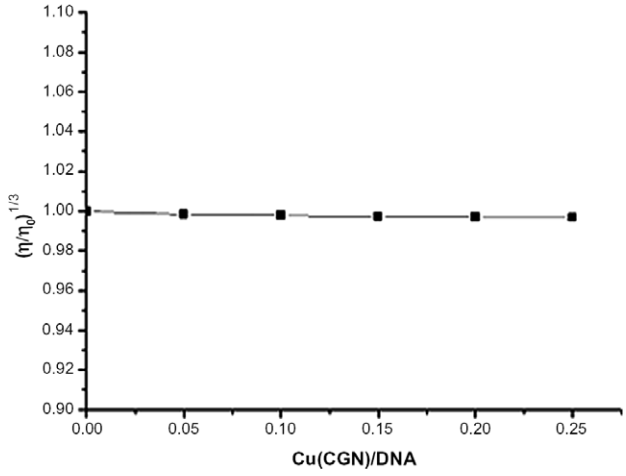


Figure 4. Effects of increasing amounts of Cu(CGN) on the relative viscosity of CT DNA. [DNA] = 0.2 mM.

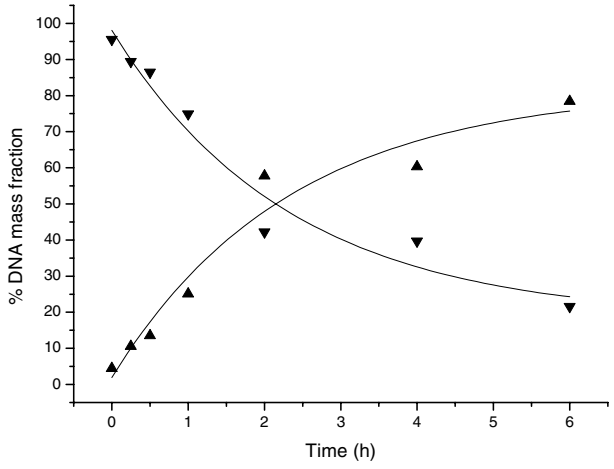


Figure 6. Time course of DNA cleavage (7 μg/mL) by Cu(CGN) (0.714 mM) at 37 °C in NaH_2PO_4/Na_2HPO_4 buffer (pH 7.4). ▼, SC DNA; ▲, NC DNA.

as superoxide scavenger, dimethylsulfoxide (DMSO) and *tert*-butylalcohol were used as scavenger of hydroxyl radical. Plasmid pUC19 DNA was incubated with Cu(CCN) in the presence of sodium azide, SOD, DMSO, and *tert*-butylalcohol, respectively, and the results are shown in Figure 7. It is obvious that sodium azide completely inhibited the cleavage process, and SOD partially inhibited the cleavage. However, DMSO and *tert*-butylalcohol did not show any inhibition ability against DNA cleavage. That is to say, singlet oxygen and superoxide were the most possible reactive oxygen species, and the possibility of hydroxyl radical in the mechanism could be ruled out. This result was different from many other DNA cleavage reactions, in which hydroxyl radical acted as reactive oxygen species, and singlet oxygen scavenger could not inhibit the cleavage process.

To be sure of the nuclease active point in Cu(CGN), the cleavage reaction catalyzed by Cu(CGN) was compared with those reactions catalyzed by equimolar of free ligand, copper (II) cyclen complex, and $Cu(NO_3)_2$, respectively. The results are listed in Table 1. The nuclease activity of Cu(CGN) was not the simple summation of the activities of free ligand and copper (II) salt. Cu(CGN) showed much higher cleavage ability than CGN, that is to say, the coordination of copper (II) ions should be the core unit in cleavage.[48] The copper complexes have the ability to bind O_2 molecule and to activate the molecular oxygen to form ROS.[49] A proposed mechanism of DNA cleavage is shown in Scheme 2.

2.6.3. β-Cyclodextrin-promoted DNA cleavage. β-Cyclodextrin did not show any DNA cleavage ability. However, as shown in Figure 8, it was found that the addition of β-cyclodextrin was able to promote the DNA cleavage ability of Cu(CGN). To study the role of β-cyclodextrin in the cleavage process, different equivalents of β-cyclodextrin were added to the cleavage system. The increase of β-cyclodextrin led to the increase of relative amount of LC DNA (Fig. 8). That is to say, the addition of β-cyclodextrin promoted the nuclease activity of Cu(CGN). As shown in Figure 8b, the tendency of the amount of LC DNA demonstrated the competitive interaction with Cu(CGN) between DNA and β-cyclodextrin.

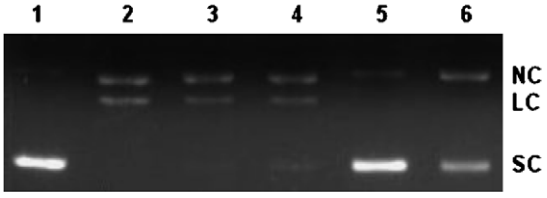


Figure 7. Agarose-gel-electrophoresis patterns for the cleavage of plasmid pUC19 DNA (7 μg/mL) by Cu(CGN) (0.714 mM) in NaH_2PO_4/Na_2HPO_4 buffer (pH 7.4) at 37 °C for 12 h. Lane 1, DNA control; lane 2, Cu(CGN) control; lanes 3–6, with the addition of 1 M DMSO, *tert*-butylalcohol, sodium azide, and 100 U SOD, respectively.

Table 1. Results of pUC19 DNA cleavage catalyzed by 0.714 mM of different cleavage agents for 12 h

Cleavage agent	NC DNA (%)	LC DNA (%)
Control	5.0	
Cu(CGN)	84.7	15.3
CGN	7.4	
β-Cyclodextrins	6.3	

Scheme 2. A proposed mechanism to form ROS.

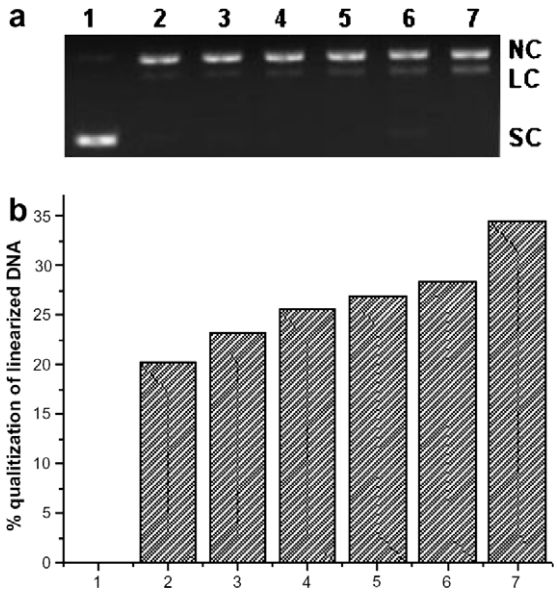


Figure 8. (a) Agarose-gel-electrophoresis patterns for the cleavage of plasmid pUC19 DNA (7 μg/mL) by Cu(CGN) (0.714 mM) in NaH_2PO_4/Na_2HPO_4 buffer (pH 7.4) at 37 °C for 12 h. Lane 1, DNA control; lanes 2–7, DNA + Cu(CGN) + β-cyclodextrin, the concentrations of cyclodextrins were 0, 0.714, 1.428, 3.57, 7.14, 14.28 mM respectively. (b) Quantitation of linearized DNA relative to nicked DNA per lane.

Associating with the results of ^1H NMR spectrum and the fluorescence titration, the promotion of nuclease ability by β-cyclodextrin attributed to the decrease of the DNA binding ability. β-Cyclodextrin could include the naphthyl moieties and keep it from the minor/major groove of DNA, and, as a result, the interaction between copper center and phosphate oxygen atoms of DNA backbone would be weakened.[50] The process is shown below (Fig. 9).

3. Conclusion

The preparation and DNA nuclease activity of a new copper (II) complex was investigated. It could cleave DNA by the generation of $^1O_2$ and O_2^-, which was an oxidative mechanism. β-Cyclodextrin was proved to improve the nuclease activity of Cu(CGN), which included an α-naphthylamine moiety. Addition of β-cyclodextrin could broke the groove binding between Cu(CGN) and CT DNA, as a result, the DNA binding ability of Cu(CGN) was weakened, and $^1O_2$ and O_2^- could be generated more effectively. The fluorescence quench experiments of free ligand CGN induced by CT DNA

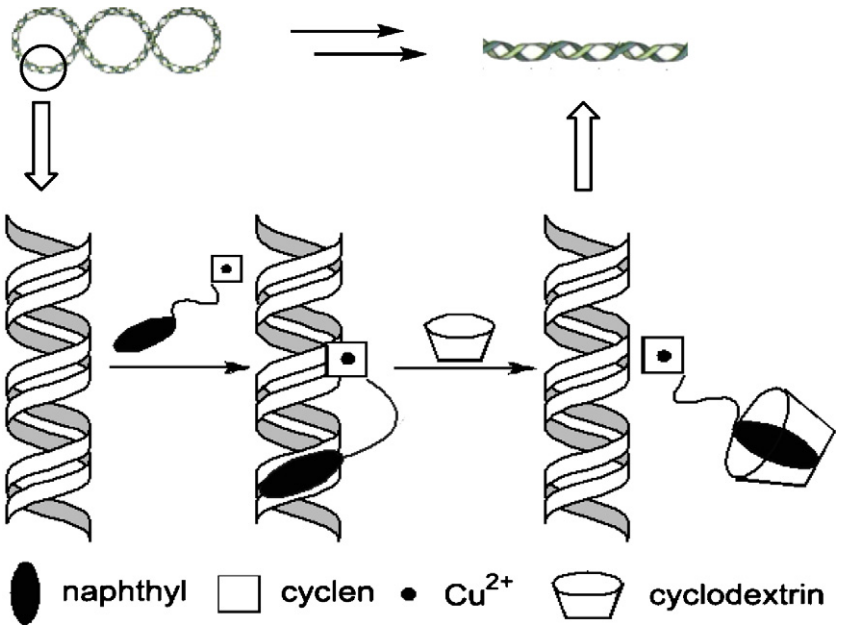


Figure 9. DNA cleavage process promoted by β-cyclodextrins.

or β-cyclodextrin were studied. The competitive interaction with CGN between DNA and β-cyclodextrin was confirmed by ^{1}H NMR and fluorescence titration. The binding mode of Cu(CGN) with CT DNA was partial intercalation from the minor/major groove according to the results of absorption spectrum and viscosity measurement. It was for the first time reported that β-cyclodextrin could promote the cleavage of DNA.

4. Experimental

4.1. Materials and characterizations

High Resolution Mass Spectrometry (HR-MS) data were recorded on a Bruker Daltonics Bio TOF mass spectrometer. The ^{1}H NMR spectra were measured on Bruker AV II-400 MHz and Bruker AV II-600 MHz spectrometer and the δ scale in ppm referenced to residual solvent peaks or internal tetramethylsilane (TMS). Fluorescence spectra were measured at room temperature in air by a Hitachi FL-4500 fluorescence spectrometer and corrected for the system response. Absorption spectra were recorded in aqueous solution on a TU-1901 spectrophotometer. All other chemicals and reagents were obtained commercially and used without further purification. CT DNA was directly dissolved in water at a concentration of 1 mg/mL and stored at 4 °C. Phosphate buffer (0.1 M) was prepared by mixing 1 M of aq sodium dihydrogen phosphate and 1 M of aq disodium hydrogen phosphate, then diluted to 100 mL. CGN, Cu(CGN), and β-cyclodextrin solutions were prepared by dissolving an amount of relative compound in water and stored in dark.

4.2. Synthesis of Cu(II) complex

4.2.1. Preparation of 3. A mixture of 4-(*tert*-butoxycarbonylamino)-5-oxo-5-(4,7,10-tris(*tert*-butoxycarbonyl)-1,4,7,10-tetraazacyclododecan-1-yl)valeric acid **2** (0.685 mmol, 0.48 g) and TEA (0.105 mL) in 80 mL of CH_2Cl_2 under an N_2 atmosphere was cooled to −15 °C, isobutylchloroformate (0.105 mL) was then added. After stirring for 2 min, α-naphthylamine **1** (0.1 g, 0.685 mmol) was added and the mixture was stirred at −15 °C for 0.5 h, then stirred at room temperature overnight. The solvent was removed under reduced pressure, and the residue was purified by column chromatography (silica gel, ethyl acetate–petrol ether, 2:1, v/v) to yield the product as a white solid **3**. Yield: 55%. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.39–1.46 (36H, m, Boc-CH_3), 1.95–2.05 (2H, m, CH_2–CH), 2.60–2.67 (2H, m, CH_2–CO), 3.20–3.67 (16H, m, cyclen-CH_2), 4.60 (1H, m, CH–CO), 5.68 (1H, s, NH-Boc), 7.45–7.49 (3H, m, naphthalene-H), 7.68–7.70 (1H, d, J = 8.0 Hz, naphthalene-H), 7.85–7.87 (1H, t, J = 8.0 Hz, naphthalene-H), 7.99–8.00 (1H, d, J = 5.6 Hz, naphthalene-H), 8.13 (1H, s, naphthalene-H), 8.81 (1H, s, NH–CO); IR (KBr) v: 3426, 2975, 2929, 1695, 1464, 1407, 1366, 1249, 1165, 970, 858, 776, 673, 559 cm^{-1}; HR-MS (ESI): Calcd for $C_{43}H_{66}N_6O_{10}$ $[M+H]^+$: m/z = 827.4919. Found: 827.4601.

4.2.2. Preparation of 4. To a solution of **3** (0.17 mmol, 140 mg) in 30 mL of CH_2Cl_2 was added TFA (1 mL). After stirring at room temperature for 4 h, the solvent was removed under reduced pressure to give an oil. To the oil was added 50 mL of ether, and a white solid was formed by trituration. The solid was washed for three times with ether and dried under vacuum to give the product salt. The salt was dissolved in water, and 10 mL of 2 N aqueous NaOH solution was added, the mixture was then extracted with CH_2Cl_2 (3× 30 mL). The organic phase was washed with brine and then dried over anhydrous Na_2SO_4. After removing the solvent under reduced pressure, the product was obtained as a white solid. Yield: 70%. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.88–1.91 (2H, m, CH_2–CH), 2.25–2.31 (2H, m,

CH_2–CO), 2.60–2.90 (12H, m, CH_2–NH), 3.57–3.63 (4H, m, CH_2–N), 3.97–3.98 (1H, m, CH–CO), 7.45–7.54 (3H, m, naphthalene-H), 7.69–7.71 (1H, d, $J = 8$ Hz, naphthalene-H), 7.85–7.87 (2H, d, $J = 6.8$ Hz, naphthalene-H), 7.95–7.97 (1H, d, $J = 7.6$ Hz, naphthalene-H), 9.04 (1H, s, NH–CO); IR (KBr) *v*: 3428, 2925, 2852, 1631, 1539, 1501, 1456, 1400, 1261, 1127, 799, 674 cm^{-1}; HR-MS (ESI): Calcd for $C_{23}H_{34}N_6O_2$ $[M+H]^+$: $m/z = 427.2816$. Found: 427.2188.

4.2.3. Preparation of 5. To a solution of **4** (0.12 mmol, 50 mg) in ethanol was added $Cu(NO_3)_2{\cdot}3H_2O$ (0.24 mmol, 58 mg). After stirring for 5 h, a blue powder appeared. The blue powder was filtered and then washed with ethanol. Yield: 90%. IR (KBr) *v*: 3435, 3243, 2924, 1630, 1592, 1384, 1128, 1012, 801, 572 cm^{-1}; HR-MS (ESI): Calcd for $C_{23}H_{34}CuN_8O_8$ $[M-NO_3+Na]^{2+}$: $m/z = 287.0908$. Found: 287.1226.

4.3. ^{1}H NMR study

Six hundred megahertz ^{1}H NMR spectra were gained on Bruker AV II-600 MHz spectrometer. CGN, CGN/DNA, CGN/β-cyclodextrins, and CGN/DNA/β-cyclodextrins were directly dissolved in D_2O, respectively.

4.4. Fluorescence spectrum study

4.4.1. Fluorescence quench of CGN by CT DNA. Hundred microliters of 5 mM CGN was added to 2.5 mL of phosphate buffer (pH 7.4). A series of 1.0 mg/mL DNA solution (5–25 μL) were added and the fluorescence spectrum in the absence (F_0) or presence (F) of CT DNA was measured at $\lambda_{ex} = 274$ nm and $\lambda_{em} = 373$ nm.

4.4.2. Fluorescence titration of β-cyclodextrin. Hundred microliters of 5 mM CGN was added to 2.5 mL of phosphate buffer (pH 7.4). The range of 10 mM β-cyclodextrin (20–100 μL) were added in the absence or presence of 1.0 mg/mL CT DNA (30 μL), respectively. Fluorescence intensities of the blank (F_0) and the mixed solution (F) were measured at $\lambda_{ex} = 274$ nm and $\lambda_{em} = 373$ nm.

4.5. Viscometric studies

Viscosity experiments were carried out with an Ubbelodhe viscometer in a water bath and maintained the constant temperature at 37 °C. A range of 30–150 μL of 5 mM Cu(CGN) solution were added in 15 mL of 0.2 mM CT DNA solution. The flow time was measured by a digital stop watch.

4.6. DNA cleavage experiment

Plasmid DNA (pUC19) cleavage activity of Cu(CGN) was monitored by agarose gel electrophoresis. In a typical experiment, supercoiled pUC19 DNA (5 μL, 0.025 g/L) in NaH_2PO_4/Na_2HPO_4 (100 mM, pH 7.4) was treated with different concentrations of complex, followed by dilution with the NaH_2PO_4/Na_2HPO_4 buffer to a total volume of 35 μL. The samples were then incubated at 37 °C for different time and loaded on a 1% agarose gel containing 1.0 g/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.

Acknowledgments

This work was financially supported by the National Science Foundation of China (Nos. 20725206, 20732004, and 20572075), Program for New Century Excellent Talents in University, Specialized Research Fund for the Doctoral Program of Higher Education, and Scientific Fund of Sichuan Province for Outstanding Young Scientist, and Y.Z. also thanks the sustentation of Student Innovation Fund of Sichuan University.

References and notes

1. Schroeder, G. K.; Lad, C.; Wyman, P.; Williams, N. H.; Wolfenden, R. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 4052–4055.
2. Corey, D. R.; Schultz, P. G. *Science* **1987**, *238*, 1401.
3. Westheiner, F. H. *Science* **1987**, *235*, 1173–1178.
4. Williams, N. H.; Takasaki, B.; Chin, J. *Acc. Chem. Res.* **1999**, *32*, 485–493.
5. Hegg, E. L.; Burstyn, J. N. *Coord. Chem. Rev.* **1998**, *173*, 133–165.
6. Liu, C.; Wang, M.; Zang, T.; Sun, H. *Coord. Chem. Rev.* **2004**, *248*, 147–168.
7. Mancin, F.; Scrimin, P.; Tecilla, P.; Tonellato, U. *Chem. Commun.* **2005**, 2540–2548.
8. Jiang, Q.; Xiao, N.; Shi, P. F.; Zhu, Y. G.; Guo, Z. J. *Coord. Chem. Rev.* **2007**. doi:10.1016/j.ccr.2007.02.013.
9. Jin, Y.; Cowan, J. A. *J. Am. Chem. Soc.* **2005**, *127*, 8408–8415.
10. Maheswari, P. U.; Barends, S.; Ozalp-Yaman, S.; de Hoog, P.; Casellas, H.; Teat, S. J.; Massera, C.; Lutz, M.; Spek, A. L.; van Wezel, G. P.; Gamez, P.; Reedijk, J. *Chem. Eur. J.* **2007**. doi:10.1002/chem.200601525.
11. Dhar, S.; ethaji, M.; Chakravarty, A. R. *Inorg. Chem.* **2006**, *45*, 11043–11050.
12. Burger, R. M. *Chem. Rev.* **1998**, *98*, 1153–1169.
13. Chifotides, H. T.; Dunbar, K. R. *Acc. Chem. Res.* **2005**, *38*, 146–156.
14. Kimura, S.; Sakaguchi, K. *Chem. Rev.* **2006**, *106*, 753–766.
15. Schlacher, K.; Pham, P.; Cox, M. M.; Goodman, M. F. *Chem. Rev.* **2006**, *106*, 406–419.
16. Borras, J.; Alzuet, G.; Gonzalez-Alvarez, M.; Garcia-Gimenez, J. L.; Macias, B.; Liu-Gonzalez, M. *Eur. J. Inorg. Chem.* **2007**, 822–834.
17. Ferrer, S.; Ballesteros, R.; Sambartolome, A.; Gonzalez, M.; Alzuet, G.; Borras, J.; Liu, M. V. *J. Inorg. Biochem.* **2004**, *98*, 1436–1446.
18. Macias, B.; Villa, M. V.; Sanz, F.; Borras, J.; Gonzalez-Alvarez, M.; Alzuet, G. *J. Inorg. Biochem.* **2005**, *99*, 1441–1448.
19. Gonzalez-Alvarez, M.; Alzuet, G.; Borras, J.; Pitie, M.; Meunier, B. *J. Biol. Inorg. Chem.* **2003**, *8*, 644–652.
20. Long, E. C.; Barton, J. K. *Acc. Chem. Res.* **1990**, *23*, 271–273.
21. Suh, D.; Chaires, J. B. *Bioorg. Med. Chem.* **1995**, *3*, 723–728.
22. Kumar, C. V.; Asuncion, E. H. *J. Am. Chem. Soc.* **1993**, *115*, 8547–8553.

23. Dervan, P. B.; Poulin-Kerstien, A. T.; Fechter, E. J.; Edelson, B. S. *Top. Curr. Chem.* **2005**, *253*, 1–31.
24. Dias, N.; Vezin, H.; Lansiaux, A.; Bailly, C. *Top. Curr. Chem.* **2005**, *253*, 89–108.
25. Daneshtalab, M. *Top. Heterocycl. Chem.* **2006**, *2*, 153–206.
26. Szejtli, J. *Chem. Rev.* **1998**, *98*, 1743–1753.
27. Rekharsky, M. V.; Inoue, Y. *Chem. Rev.* **1998**, *98*, 1875–1917.
28. Liu, Y.; Liang, P.; Chen, Y.; Zhao, Y. L.; Ding, F.; Yu, A. *J. Phys. Chem. B* **2005**, *109*, 23739–23744.
29. Liu, Y.; Zhao, Y. L.; Chen, Y.; Liang, P.; Li, L. *Tetrahedron Lett.* **2005**, *46*, 2507–2511.
30. Nakanishi, I.; Fukuzumi, S.; Konishi, T.; Ohkubo, K.; Fujitsuka, M.; Ito, O.; Miyata, N. *J. Phys. Chem. B* **2002**, *106*, 2372–2380.
31. Nakamura, E.; Isobe, H.; Tomita, N.; Sawamura, M.; Jinno, S.; Okayama, H. *Angew. Chem. Int. Ed.* **2000**, *39*, 4254–4257.
32. Bernstein, R.; Prat, F.; Foote, C. S. *J. Am. Chem. Soc.* **1999**, *121*, 464–465.
33. Armitage, B. A. *Top. Curr. Chem.* **2005**, *253*, 55–76.
34. Yamaguchi, K.; Ebitani, K.; Yoshida, T.; Yoshida, H.; Kaneda, K. *J. Am. Chem. Soc.* **1999**, *121*, 5426–5436.
35. Schneider, H. J.; Hacket, F.; Rudiger, V.; Ikeda, H. *Chem. Rev.* **1998**, *98*, 1755–1786.
36. Schneider, H. J.; Blatter, T.; Simova, S. *J. Am. Chem. Soc.* **1991**, *113*, 1996–2000.
37. Ikeda, T.; Yoshida, K.; Schneider, H. *J. Am. Chem. Soc.* **1995**, *117*, 1453–1454.
38. Valis, L.; Mayer-Enthart, E.; Wagenknecht, H. A. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3184–3187.
39. Baguley, B. C.; Lebret, M. *Biochemistry* **1984**, *23*, 937–943.
40. Takakusa, H.; Kikuchi, K.; Urano, Y.; Higuchi, T.; Nagano, T. *Anal. Chem.* **2001**, *73*, 939–942.
41. Tong, A. J.; Yamauchi, A.; Hayashita, T.; Zhang, Z.-Y.; Smith, B. D.; Teramae, N. *Anal. Chem.* **2001**, *73*, 1530–1536.
42. McAlpine, S. R.; Garcia-Garibay, M. A. *J. Am. Chem. Soc.* **1996**, *118*, 2750–2751.
43. Lakowicz, J. R.; Weber, G. *Biochemistry* **1973**, *12*, 4161–4170.
44. Tan, L. F.; Chao, H. *Inorg. Chim. Acta* **2007**, *360*, 2016–2022.
45. Satyanarayana, S.; Dabrowiak, J. C.; Chaires, J. B. *Biochemistry* **1992**, *31*, 9319–9324.
46. Peng, B.; Chao, H.; Sun, B.; Li, H.; Gao, F.; Ji, L. N. *J. Inorg. Biochem.* **2007**, *101*, 404–411.
47. Ren, R.; Yang, P.; Zheng, W.; Hua, Z. *Inorg. Chem.* **2000**, *39*, 5454–5463.
48. Chand, D. K.; Schneider, H. J.; Bencini, A.; Bianchi, A.; Giorgi, C.; Ciattini, S.; Valtancoli, B. *Chem. Eur. J.* **2000**, *6*, 4001–4008.
49. Zhao, Y. M.; Zhu, J. H.; He, W. J.; Yang, Z.; Zhu, Y. G.; Li, Y. Z.; Zhang, J. F.; Guo, Z. J. *Chem. Eur. J.* **2006**, *12*, 6621–6629.
50. König, B.; Pelka, M.; Subat, M.; Dix, I.; Jones, P. G. *Eur. J. Org. Chem.* **2001**, 1943–1949.