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Short communication

"Self-activating" chemical nuclease: Ferrocenyl cyclen Cu(II) complexes act as efficient DNA cleavage reagents in the absence of reductant

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Abstract

The interactions of cyclen Cu(II) complexes functionalized by ferrocenyl group with plasmid DNA indicated that these complexes have high cleavage efficiency via an oxidative mechanism in the absence of any reductant or oxidant.

Keywords: Chemical nucleases; DNA cleavage; Cyclen; Complexes

1. Introduction

Chemical nucleases, which have potential applications in the fields of biotechnology and therapeutic reagents [1–3], have been proved to be efficient tools for DNA cleavage. A large number of transition metal complexes have been explored with good to excellent DNA cleavage activities through either hydrolytic or oxidative pathways [4,5]. However, most oxidative cleavage reagents that exhibited outstanding DNA cleavage activity require the addition of external oxidative (e.g. dihydrogen peroxide, molecular oxygen) or reductive reagents (e.g. ascorbic acid, 3-mercaptopropionic acid). In some cases, photo-induction was also needed to initiate the cleavage process. For the above reasons, the in vivo applications of these reagents were limited [5,6]. Therefore, seeking "self-activating" chemical nucleases have become a challenge for chemists, and only few examples were reported [7].

Recent efforts in our laboratory focused on the copper complexes of cyclen derivatives for their excellent DNA cleavage activity [8]. For further studies and development of highly efficient chemical nuclease, we tried to find an electron receptor to transfer electron to DNA strands. Ferrocene, as a characteristic structure of metal atom sandwiched between planar cyclopentadiene rings, exhibits good redox properties and has already shown to replace phenyl moieties advantageously in biologically active compounds [9]. Herein, we introduced a ferrocenyl group to the cyclen ring and expected that this kind of new ligands may display better biological activity [10].

Three cyclen Cu(II) complexs containing ferrocene were designed and synthesized. According to the route shown in Scheme 1 [8e,11], 3Boc-cyclen reacted with *N*-Cbz-protected

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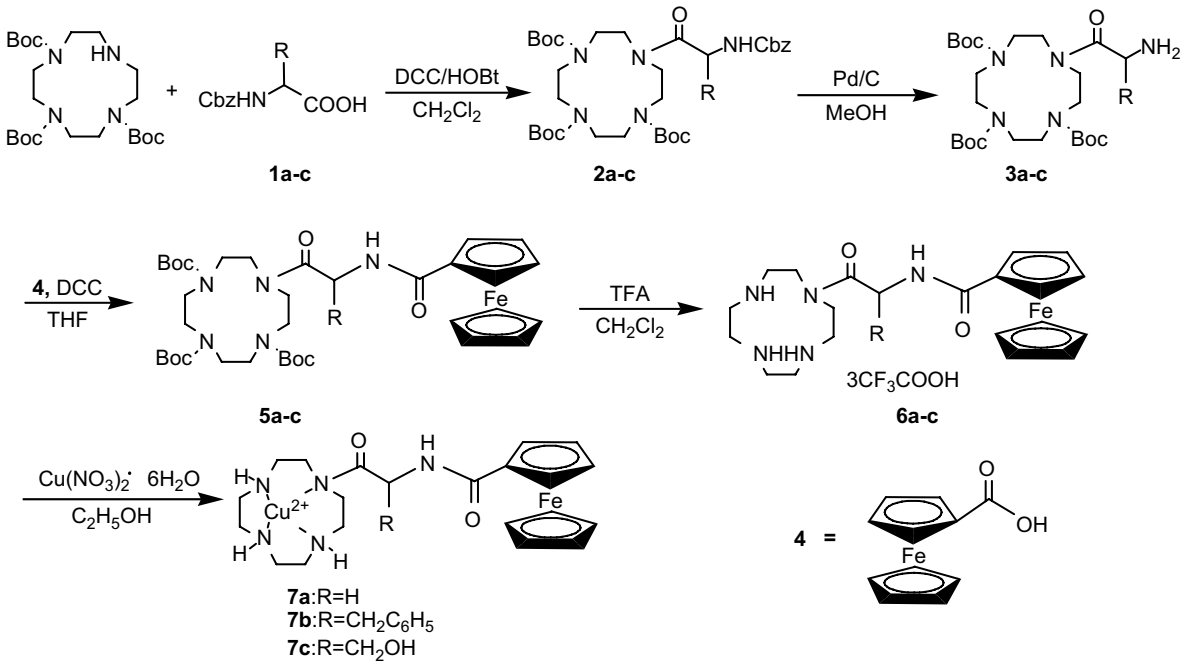

Scheme 1. Synthetic route of cyclen–ferrocene Cu(II) complexes.

amino acids **1** to give **2** by using coupling reagent DCC. Cbz group was removed by using 10% Pd/C in methanol under hydrogen atmosphere. The coupling compounds **5** were obtained by the reactions of **3** and ferrocenecarboxylic acid **4** in the presence of DCC in THF. After deprotection by TFA and subsequent metalation, the cyclen–ferrocene Cu(II) complexes **7** were obtained in moderate yields (45%, 38% and 25% for **7a**, **b** and **c**, respectively). All ligands were characterized by IR,

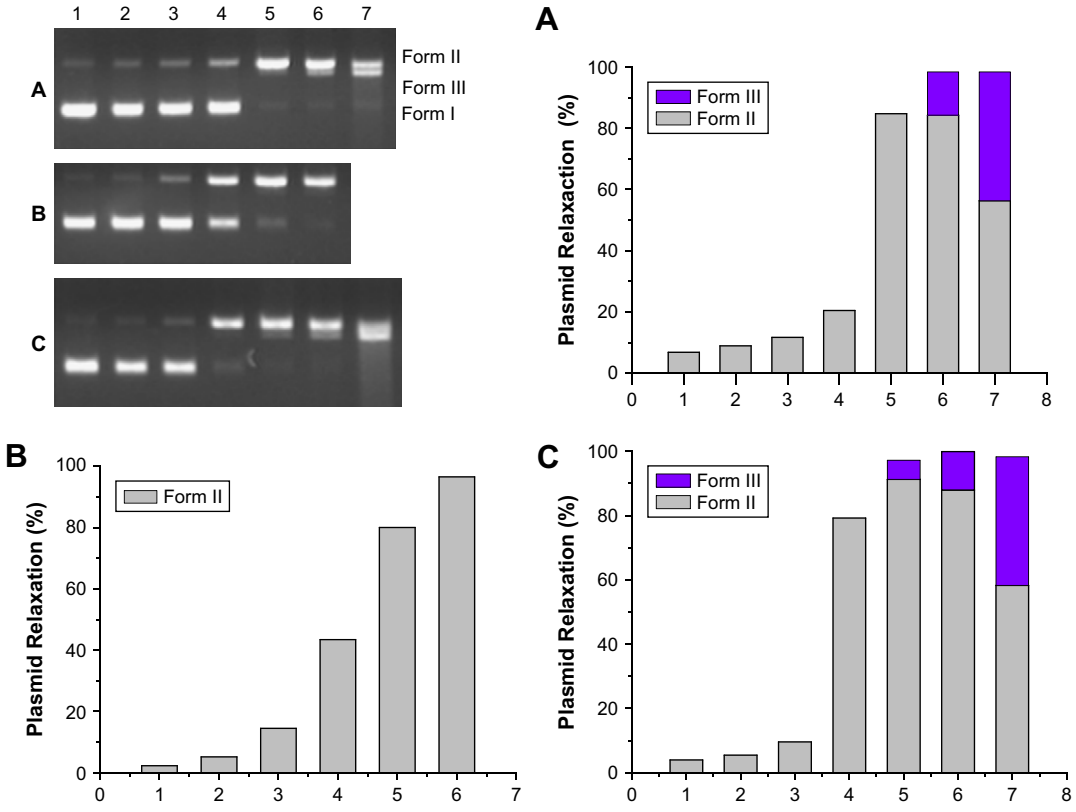

Fig. 1. Agarose gel electrophoresis patterns for the cleavage of pUC19 plasmid DNA (5 μg/mL) by various concentrations of complexes **7a–c**, and the quantities of percentage plasmid relaxation (Form II or Form III) relative to plasmid DNA per lane. Reaction was done in an NaH_2PO_4/Na_2HPO_4 buffer (100 mM, pH 7.4) at 37 °C for 5 h. (A) Lane 1: DNA control; lane 2–7: DNA + complex **7a** of 16.9, 28.3, 56.6, 106, 170, 424 μM. (B) Lane 1: DNA control; lane 2–6: DNA + complex **7b** of 14.4, 24.0, 48.0, 90.0, 125 μM. (C) Lane 1: DNA control; lane 2–7: DNA + complex **7c** of 10.7, 17.8, 35.7, 68.0, 107, 267 μM.

Table 1
Time-dependent cleavage of pUC19 plasmid DNA by complex **7a** (170 μM) and **7b** (144 μM)

Complex	Time [h]	Supercoiled [%]	Nicked [%]	Linear [%]
DNA control	12.0	100	0	0
7a	0.5	33	67	0
7a	1.0	19	81	0
7a	2.0	2	90	8
7a	4.0	6	83	11
7a	8.0	0	79	21
7a	12.0	0	57	43
7b	0.5	23	75	2
7b	1.0	13	84	3
7b	2.0	7	87	6
7b	4.0	2	79	19
7b	8.0	0	75	25
7b	12.0	0	68	32

^{1}H NMR, and HRMS-ESI. The metal complexes **7a**–**c** were characterized by HRMS, IR and element analysis.

The DNA cleavage activities of complexes **7** were studied under physiological conditions. Firstly, we compared the catalytic abilities of **7a**–**c** for DNA cleavage. The results are shown in Fig. 1. The cleavage activity was improved significantly associated with the increase of the concentration of complexes **7**. Complexes **7a** and **7b** could convert the supercoiled DNA (Form I) to nicked DNA (Form II) efficiently at the concentrations of 106 and 122 μM, respectively. For complex **7c**, the yield of Form II increased to nearly 80% when [**7c**] was only 35.7 μM, no linear DNA was formed either. Further increase of the concentration of **7** led to the appearance of Form III.

Then we studied the effect of reaction time on the DNA cleavage process. The contents of each form are listed in Table 1. The results indicated that **7a** and **7b** could catalyze the cleavage of supercoiled DNA (Form I) to the nicked DNA form (Form II) in 2 h at the concentration of 170 and 144 μM, respectively. Increasing the reaction time led to the increase of Form III.

Meanwhile, unlike **7a** and **7b**, complex **7c** could catalyze the cleavage of Form I to Form II in 20 min at the concentration of 107 μM. In order to observe the gradual change of the yields in the DNA cleavage process, we reduced the concentration of complex **7c** to 66.8 μM (Fig. 2). The quantification of decrease of Form I and increase of Form II allowed evaluating the kinetic parameters accordingly, as shown in Fig. 2B. Since no Form III was observed under this reaction condition, it is easy to deduce that the cleavage reaction followed a pseudo-fist-order kinetic mechanism with $k_1 = 7.3 \times 10^{-3}\ \mathrm{min}^{-1}$ and $R^2 = 0.98$ [1a,12], which was in agreement with the mechanism presented by other copper complexes [1a,7f].

From the above results we might know that complex **7c** is superior to **7a** and **7b** as catalyst in the DNA cleavage reaction. This may be due to the hydroxyl group contained in **7c**, as this group could make it easy to form singlet oxygen or metal–oxygen complex [5a,b,13].

To identify the reactive oxygen intermediates, which might be formed in the DNA cleavage process, experiments in the presence of a variety of radical scavengers were also carried out (Fig. 3). DMSO and *tert*-butyl alcohol were found to have little effect on DNA cleavage reaction (lanes 4 and 5), which indicated that hydroxyl radicals are not the active species. However, the DNA cleavage could be inhibited dramatically when NaI or NaN_3 was added to the system (lanes 2 and 6), and T4 ligase ligation indicated that the DNA fragments (Form II and Form III) could not be ligated [14]. These results pointed out that the singlet oxygen as active species might be involved in the DNA

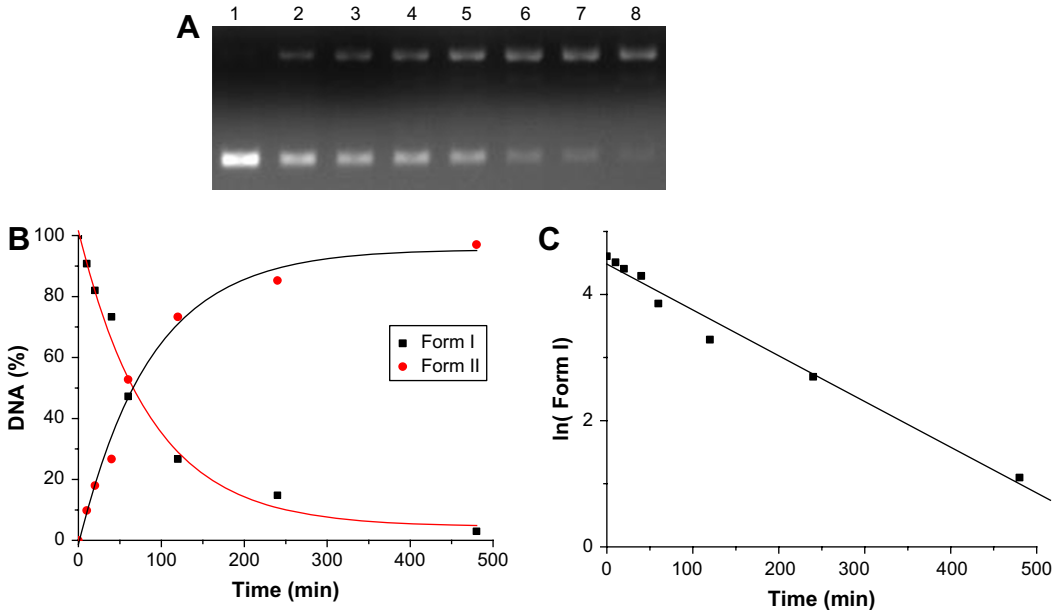

Fig. 2. Effect of reaction time on the cleavage of pUC19 DNA (5 μg/mL) with **7c** (66.8 μM) in an Na_2HPO_4/NaH_2PO_4 buffer (100 mM, pH 7.4) at 37 °C. (A) Agarose gel electrophoresis diagrams: lane 1: DNA control; lanes 2–8: complex **7c**, 10, 20, 40, 60, 120, 240, 480 min. (B) Quantity of pUC19 plasmid forms. (C) Linear simulation of pseudo-first-order reaction according to the quantity of supercoiled DNA (Form I).

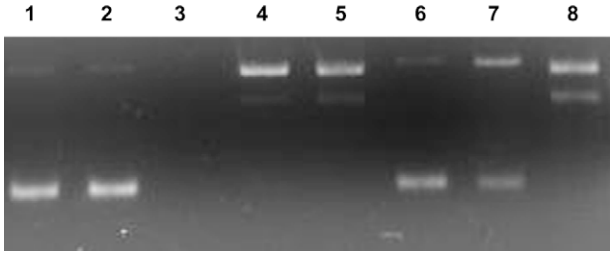

Fig. 3. Inhibition studies on the cleavage of pUC19 DNA (5 μg/mL) by complex **7c** (0.107 mM). Reactions were carried out for 6 h. Lane 1: DNA control; lane 2: DNA +**7c** + 143 mM of NaI; lane 3: DNA + **7c** + 143 mM of H_2O_2; lane 4: DNA + **7c** + 143 mM of DMSO; lane 5: DNA + **7c** + 143 mM of *tert*-butyl alcohol; lane 6: DNA + **7c** + 143 mM of NaN_3; lane 7: DNA + 143 mM of H_2O_2 + 0.2 mM of $CuSO_4$ [15]; lane 8: DNA + **7c**.

cleavage process. Effect of H_2O_2 on the reaction catalyzed by **7c** was also examined (lane 3), and the result showed that H_2O_2 could enhance the cleavage activity dramatically, which further supported the oxidative mechanism. Under the same cleavage conditions, complex **7c**–H_2O_2 system could convert supercoiled DNA to small fragments, which could not be detected by gel electrophoresis.

In conclusion, we designed and synthesized three novel ferrocenyl Cu(II)–cyclen complexes and applied them to catalyze the cleavage of supercoiled pUC19 DNA. The results demonstrated that the complexes **7a–c** are excellent chemical nucleases. The cleavage might be carried out via an oxidative pathway, and no reductant or oxidant was needed. Complex **7c** was found to be the most efficient reagent for DNA cleavage at low concentration and in short time.

Acknowledgements

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Appendix. Supporting information

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.ejmech.2008.03.029.

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[12] The k_1 value was calculated according to the equation $\ln[\text{Form I}] = -k_1 t + A$.

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[14] *Procedure for DNA ligation experiments*. After incubation of pUC19 plasmid DNA with complex **7c** (0.107 mM) for 6 h at 37 °C, the cleavage fragments (Form II and Form III) were purified on a G-25 column, then were incubated with 10 U of T4 ligase at 15 °C for 28 h in the buffer provided by the supplier. The reaction products were resolved on an agarose gel adopting the same method described above.

[15] We examined the Cu(II)–H_2O_2 system for the control experiment, and results indicated that part of supercoiled DNA (48%) could be damaged in higher Cu(II) concentration (0.2 mM) in the presence of H_2O_2 (143 mM).