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Short communication

Hydrolytic cleavage of DNA by urea-bridged macrocyclic polyamines

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ABSTRACT

Novel bis-cyclen derivative **A** with urea, mono-cyclen derivative **B** with urea and amino acid urea **C** were synthesized as DNA cleavage agents. The structures of these new compounds were identified by MS and ^{1}H NMR spectroscopy. The catalytic activities on DNA cleavage of these compounds were subsequently studied, and results show that **A** is a much better catalyst in DNA cleavage process than that of **B** and **C**. The effects of reaction time and catalyst concentration were also investigated. The results indicate that **A** can catalyze the cleavage of supercoiled DNA (pUC 19 plasmid DNA) to nicked DNA under physiological conditions with high yields (nearly 100%) via a hydrolytic mechanism.

1. Introduction

For the especially high abilities in recognizing specific DNA sequence and catalyzing the hydrolysis of phosphate diester bonds, chemical nucleases have recently received considerable interests due to their potential applications in the fields of biology, bio-organic chemistry, therapy, and molecular biology [1–6]. Because of its efficient coordination abilities towards a wide range of cations, 1,4,7,10-tetraazacyclododecane (cyclen) has been well known and widely studied. A series of cyclen complexes were investigated and found to be excellent artificial nucleases [7–17]. Comparing with the successful results involving artificial metallonucleases, studies focused on metal-free organic molecules as nucleic acid cleavage agents are not as frequent [18–22]. Actually, the cleavage of DNA by metal-free compounds can be considered safer in the development of biotechnology and gene therapy, including the use of photo-induced processes for treatment of cancer. A series of metal-free compounds have been explored as phosphodiester cleavage agents [23–30]. However, no study upon introducing urea to cyclen compound as DNA cleavage agent has been reported so far.

Very recent reports showed that a wide range of urea-containing compounds have attracted extensive attention for a number of their intriguing properties [31–33]. Some urea-ethidium compounds were confirmed to be stronger DNA binders as compared to ethidium bromide [34]. A previous study by König and co-workers showed the capacity of urea–cyclen derivatives to bind DNA by ethidium bromide displacement assay [35]. To improve the ability of urea to form multiple hydrogen bonds in nucleobase recognition, many urea-substituted compounds were designed and studied in organic solvents as model systems [36,37]. As Zimmerman and co-workers recently described, urea-substituted compounds could show effective recognition towards C–G base pairs on the major groove side through formation of three hydrogen bonds in organic solvent [38]. Furthermore, some urea-containing molecules also showed intriguing anti-HIV activity [39].

In this communication, we report for the first time the design and synthesis of a novel compound **A** amino acid urea-bridged-bis-cyclen, and the studies of its DNA cleavage activity. As for comparison, the amino acid urea-bridged-mono-cyclen **B** and amino acid urea **C** without cyclen moiety were also studied. The result shows that the compound **A** can catalyze the DNA cleavage efficiently.

2. Results and discussion

2.1. Preparation of ligands

The synthetic route to the compounds of **A**–**C** is shown in Scheme 1. Boc_3-cyclen reacted with *N*-benzyloxycarbonyl (Cbz)

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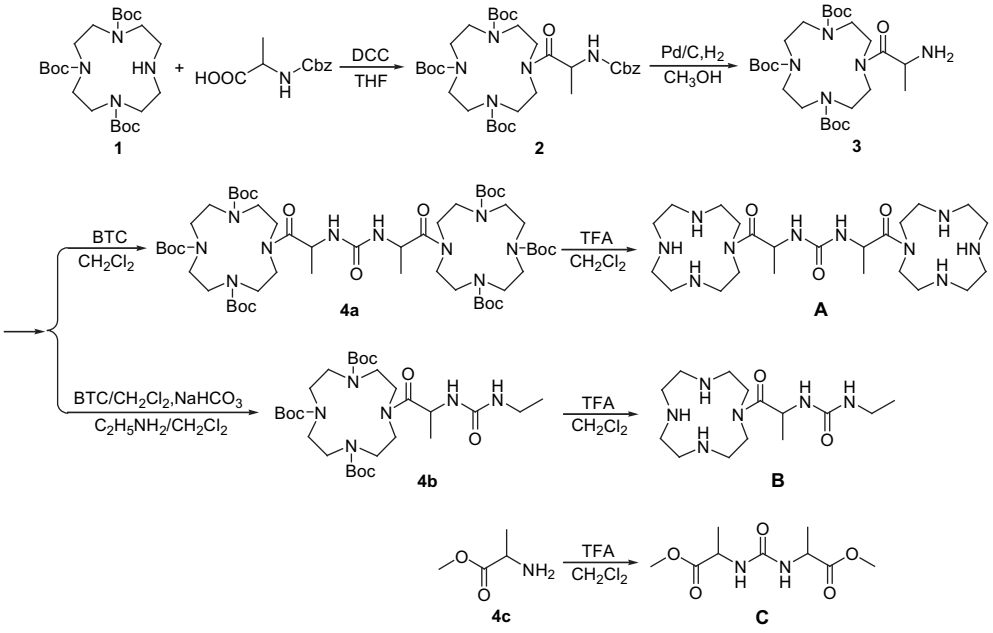

Scheme 1. Synthetic route of compounds **A**–**C**.

alanine to give compound **2** using *N*,*N*′-dicyclohexylcarbodiimide (DCC) as coupling agent. Deprotection of Cbz group was subsequently carried out by treating the methanol solution of **2** with 10% Pd/C. Then product **3** was coupled by bis(trichloromethyl) carbonate (BTC) to give bicyclen compound **4a**. Alternatively, **3** could be coupled with BTC, and followed by the treatment with $C_2H_5NH_2$ to give compound **4b**. The trifluoroacetic acid (TFA) salts of **A** and **B** were obtained by deprotection of **4a** and **4b** respectively with TFA in CH_2Cl_2 at room temperature. Subsequent basification with NaOH gave the free compounds **A** and **B**. Similar to **4a**, compound **C** could be obtained by the coupling **4c** with BTC. The compounds were characterized by ^{1}H NMR, ESI-MS, and HRMS.

2.2. Cleavage of plasmid DNA

The cleavage activities of **A**–**C** with pUC 19 supercoiled DNA were studied under physiological conditions. Supercoiled plasmid DNA (Form I) was cleaved to produce open-circular form (Form II). The amounts of strand scission were assessed by agarose gel electrophoresis. Firstly, we compared the DNA cleavage abilities of the compounds under physiological conditions (Fig. 1). The results show the catalytic efficiency in the order of **A** ≫ **B** > **C** in the cleavage of plasmid DNA (pUC 19). It was obvious that bis-cyclen derivative **A** with the urea bridge exhibited an unexpected ability to promote the cleavage of DNA. Therefore, subsequent studies focused on the optimization of catalytic ability of **A** under physiological conditions. The cleavage of DNA with different concentrations of **A** and different reaction times were investigated. Nearly all of supercoiled DNA could be cleaved to open-circular form in 32 h in the presence of 357 μM of **A** (Fig. S1). Meanwhile, the time course plot indicates that the extension of supercoiled DNA cleavage varied exponentially with reaction time, giving pseudo-first-order kinetics with an apparent first-order rate constant of $0.046 \pm 0.001\ h^{-1}$ (Fig. S2B). It also shows a 10^7-fold rate acceleration over the cleavage reaction in the absence of any catalyst [40–45]. These results reveal that **A** as chemical nuclease is capable to accelerate the cleavage of plasmid DNA dramatically, which may be due to the electrostatic interaction between the electrophilic

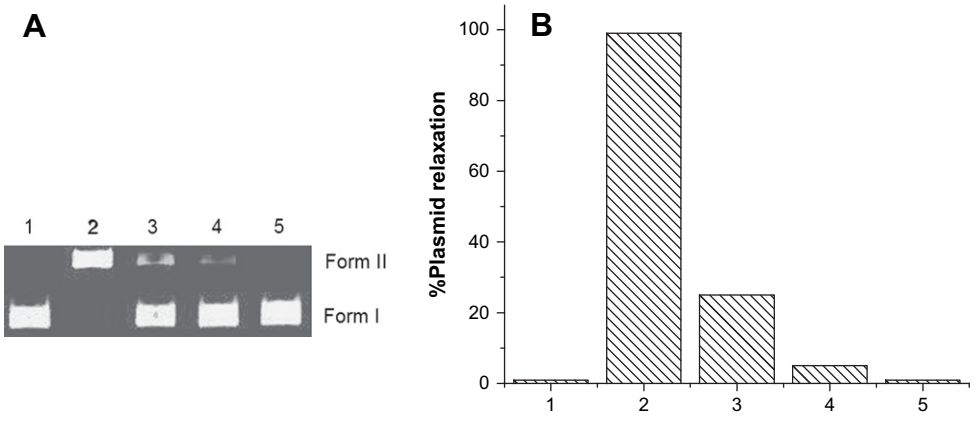

Fig. 1. Effect of different compounds **A**–**C** (357 μM) and cyclen (1.43 mM) on the cleavage reactions of pUC 19 DNA (7 μg/mL) in a NaH_2PO_4/Na_2HPO_4 buffer (100 mM, pH 7.4) at 37 °C for 36 h. (A) Agarose gel electrophoresis diagram: Lane 1, DNA control; Lanes 2–4, **A**–**C**; Lane 5, cyclen; (B) Quantitation of % plasmid relaxation relative to plasmid DNA per lane.

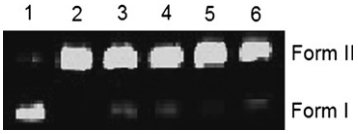

Fig. 2. Inhibition studies on the cleavage of pUC 19 DNA (7 μg/mL) catalyzed by **A** (357 μM). Reactions were carried out for 36 h as described above except that different ROS scavengers were added before the DNA to the system. Lane 1: DNA control; Lane 2: DNA + **A**; Lane 3: DNA + **A** + NaN_3; Lane 4: DNA + **A** + KI; Lane 5: DNA + **A** + *tert*-butyl alcohol; Lane 6: DNA + **A** + DMSO.

cyclen moieties and DNA backbone combined with the potential of hydrogen bond caused by urea. In the cleavage process catalyzed by **A**, urea moiety might form multiple hydrogen bonds that led to strong recognition towards C–G base pairs [36–38], and therefore, the substrate could be oriented to the major groove side of the DNA duplex. As a result, a synergistic electrostatic interaction between the cyclen moieties and DNA was facilitated.

To investigate the mechanism of DNA cleavage promoted by **A** [46], typical scavengers [47] for reactive oxygen species (ROS), including NaN_3 (singlet oxygen scavenger, 10 mM), KI (superoxide scavenger, 10 mM), *t*-BuOH and DMSO (hydroxyl radical scavenger, 1 mM), were introduced to the catalytic cleavage system. Fig. 2 shows that none of these scavengers displays evident inhibition effect, which suggests that ROS is not involved in the cleavage process. Therefore, DNA cleavage promoted by **A** should not occur via an oxidative pathway but by hydrolytic pathway [48]. To further prove the conclusion, we used TBARS-method [49] to detect hydroxyl radical, and no 532 nm absorbance was observed.

DNA melting experiment is an important method to distinguish the binding mode between DNA and small molecule [50,51]. Intercalation of small molecules into the double helix is well known to increase the T_m of DNA, the temperature at which the double helix denatures into single stranded DNA. As shown in Fig. 3, the T_m of CT-DNA alone is 73.6 °C, and the T_m of CT-DNA increased by 1.9, 4, and 3 °C after the introduction of compounds **A**–**C** to achieve a concentration ratio of [compound]/[CT-DNA] = 1:3, respectively. It was reported that for intercalate binding, the T_m of CT-DNA would increase about 13–14 °C [52]. Thus, the slight ΔT_m values suggest that the binding mode of compounds **A**–**C** towards CT-DNA should not be classic intercalation.

The viscosity measurement of DNA is regarded as the most effective method to confirm the DNA binding mode [53,54].

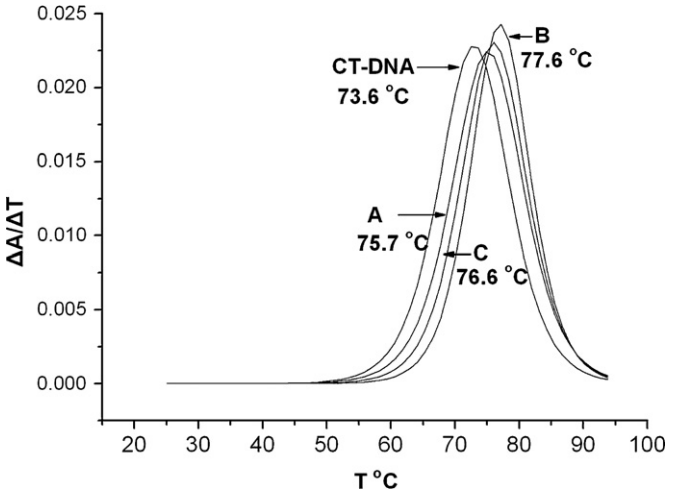

Fig. 3. First derivative plots for the thermal denaturation curves of the DNA duplex.

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 happen. In our experiments, the viscosity of CT-DNA was scarcely changed by the addition of compounds **A**–**C**, even by increasing compounds concentration to 0.5 equiv (CT-DNA, 1.0 equiv). Considering with the DNA melting experiment results, no intercalation happened in the interaction between compounds **A**–**C** and CT-DNA.

3. Conclusion

In summary, we designed and synthesized three urea derivatives with or without cyclen moieties. The DNA cleavage experiments demonstrate that bis-cyclen derivative **A** can catalyze the cleavage of DNA much more efficiently than mono-cyclen derivative **B** and non-cyclen derivative **C** under physiological conditions in the absence of exogenous agents. According to the results of DNA melting experiments and viscosity measurement, it is confirmed that the binding of such compound to DNA should be an externally electrostatic binding model. We also demonstrated that the cleavage of DNA catalyzed by **A** might undergo via a hydrolytic mechanism by ROS inhibition experiments and TBARS-method.

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Appendix. Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.ejmech.2009.06.030.

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