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DNA hydrolysis promoted by 1,7-dimethyl-1,4,7,10-tetraazacyclododecane

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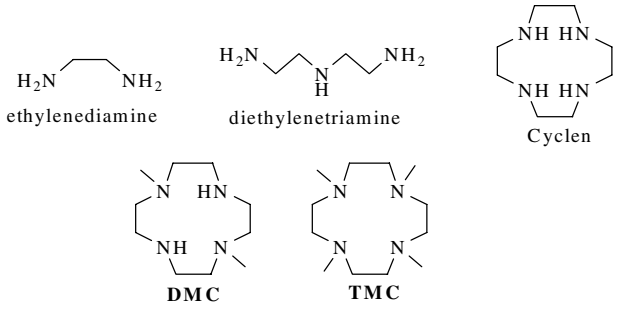
Abstract—Several acyclic and macrocyclic polyamines were evaluated for their ability to cleave DNA. 1,7-Dimethyl-1,4,7,10-tetraazacyclododecane (DMC) could hydrolyze double-strand DNA at a concentration of 25 μM. pH 7.2 was the optimal condition to cleave DNA in the presence of DMC. Supercoiled DNA hydrolytic cleavage by DMC was supported by the evidence from free radical quenching and T4 ligase ligation.

Small molecules that interact with DNA through recognition, binding, modifying, cleaving or crosslinking have attracted great interest and been a challenging project in the research fields of chemistry, biology, and medicine.[1] Like natural enzymes, artificial nucleases can hydrolyze DNA, and therefore these nucleases have been developed for application in DNA manipulations and as potential chemotherapeutic agents. It was reported that interaction of certain synthetic DNA hydrolytic agents with metal ions promotes the hydrolysis of DNA.[2] Macrocyclic polyamines were found to be potential artificial nucleases and have been widely investigated.[3–6] Furthermore, certain complexes with transition metals such as Co(III), Zn(II), and Cu(II) actually exhibited the ability of hydrolytically cleaving DNA, while the free ligands were reported not to have similar activity.[3,5]

Saturated tetraazamacrocycles (e.g., 1,4,7,10-tetraazacyclododecane, cyclen, and 1,4,8,11-tetraazacyclotetradecane, cyclam) and their derivatives have been studied as carrier of metal ions in anti-tumor[4,7] and imaging applications[8] and as anti-HIV agents.[9] Recently, Corey's group found that the introduction of lipophilic side chains to the macrocyclic polyamines could result in the increase of activity because the lipophilicity should facilitate the transport of the macrocycles into the cell.[10] Our group found that metal complexes of macrocyclic polyamines could cleave DNA and introduce apoptosis of cancer cells.[4b,4c] It turns out that the mechanism of interaction between macrocyclic polyamines and DNA or RNA should be studied in detail. Komiyama's group has investigated the efficacy of oligoamines, especially ethylenediamine, as simple catalyst for RNA hydrolysis.[11] Recently, interaction of macrocyclic polyamines and acyclic polyamines with DNA was studied in our group. To our surprise, we found that the free macrocyclic polyamine, 1,7-dimethyl-1,4,7,10-tetraazacyclododecane (DMC, Scheme 1), can hydrolyze double-strand DNA at physiological conditions (37 °C, pH 7.2). Herein, we report our preliminary results of this investigation.

Cyclen, DMC, and TMC (tetramethylcyclen) were synthesized according to the literature[12] and stored as



Scheme 1. Structures of polyamines.

Keywords: Macrocyclic polyamine; DNA cleavage; Ligation.
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hydrobromide salts. These salts were dissolved in deionized water when required for biological assay. Co, Cu, Fe, and Zn concentrations in deionized water and buffer solutions were measured by an Agilent 7500a inductively coupled plasma mass spectrometer (Agilent, Japan) system, and their concentrations all were lower than 1 μg/L.[13] The DNA cleavage chemistry of DMC was evaluated under physiological conditions (37 °C, pH 7.2) as compared with ethylenediamine, diethylene triamine, cyclen, and TMC controls (Fig. 1). The results indicated that DNA could be degraded to form linear DNA in the presence of DMC and TMC. Even by decreasing the concentration of DMC to 25 μM, DNA was still cleaved (Fig. 2). In order to avoid any effect due to residual metal contamination in sample, metal chelator (1 mM EDTA) was introduced to the system. No evidence indicated that inhibition of DNA cleavage was observed in the presence of EDTA (Fig. 3). Notably, catalytic cleavage of DNA was successful only by *N*-alkylated macrocycles (DMC and TMC), whereas oligoamines and cyclen were inert. This could be due to the binding affinity between macrocycles and DNA, either through electrostatic or hydrophobic binding modes.[14] The results also indicated that DMC was the most potent cleaving agent for DNA and that two kinds of amino residues might play a dominant role in phosphodiester degradation.[2,11]

The pH-dependence and kinetic rate constants by the time-dependence of DMC cleaving DNA in the pH range 6.1–9.1 at 37 °C (Figs. 4a and b) were studied.

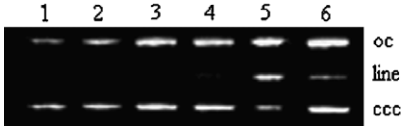

Figure 1. Agarose gel (1%) of pBR322 (0.12 μg) incubated for 2 h at pH 7.2 (10 mM Tris–HCl) and 37 °C with different samples: lane 1, control; lane 2, ethylenediamine 4 mM; lane 3, diethylenetriamine 4 mM; lane 4, cyclen 0.4 mM; lane 5, DMC 0.4 mM; lane 6, TMC 0.4 mM.

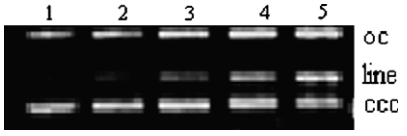

Figure 2. Agarose gel (1%) of pBR322 (0.12 μg) incubated for 2 h at 37 °C and pH 7.2 (10 mM Tris–HCl) with increasing DMC concentrations: lane 1, control; lane 2, 0.025 mM; lane 3, 0.05 mM; lane 4, 0.2 mM; lane 5, 0.4 mM.

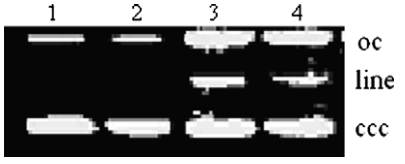

Figure 3. Agarose gel (0.8%) of pBR322 (0.12 μg) incubated for 2 h at 37 °C and pH 7.2 (10 mM Tris–HCl): lane 1, control; lane 2, 1 mM EDTA; lane 3, 0.4 mM DMC + 1 mM EDTA; lane 4, 0.4 mM DMC.

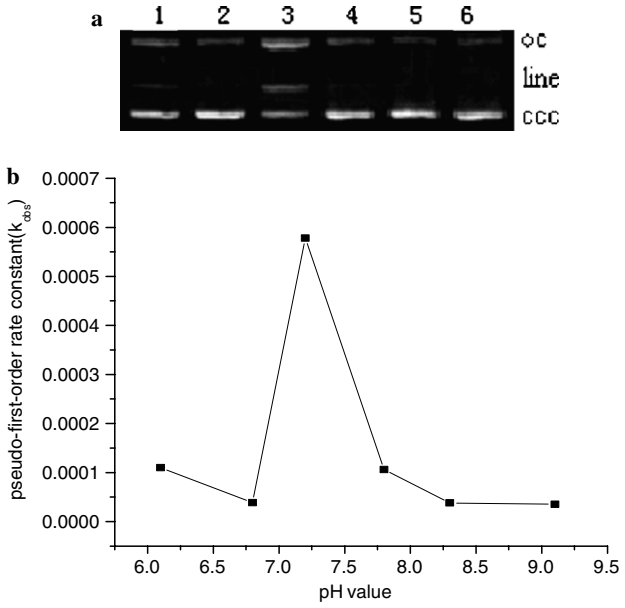

Figure 4. (a) Agarose gel (1%) of pBR322 (0.12 μg) incubated for 2 h at 37 °C with 0.4 mM DMC in different pH: lane 1, pH 6.1 (KH_2PO_4–K_2HPO_4 10 mM); lane 2, pH 6.8 (KH_2PO_4–K_2HPO_4 10 mM); lane 3, pH 7.2 (Tris–HCl 10 mM); lane 4, pH 7.8 (Tris–HCl 10 mM); lane 5, pH 8.3 (Tris–HCl 10 mM); lane 6, pH 9.1 (Tris–HCl 10 mM); (b) pH-dependent kinetic profile for DNA cleavage promoted by DMC where [DMC] = 0.2 mM.

pH 7.2 was the optimal condition to cleave DNA in the presence of DMC. Potentiometric equilibrium measurements suggested that $[HDMC]^+$ and $[H_2DMC]^{2+}$ were the most important species in the wide pH range 1.5–10,[12a] and the reaction of DNA cleavage perhaps was related to H_2O molecules activated by protonated DMC.

To investigate the mechanism of DNA cleavage promoted by DMC, hydroxyl radical scavengers (10 mM glycerol and DMSO) and singlet oxygen scavenger (10 mM NaN_3) were introduced to the system. No evident inhibition of DNA cleavage was observed in the presence of scavengers (Fig. 5), which suggested that hydroxyl radical or singlet oxygen oxidative cleavage might not occur in the reaction. Therefore, DNA cleavage promoted by DMC might not occur by an oxidative pathway but takes place probably by a hydrolytic pathway.

Further experiments support this assumption. It is well known that in DNA hydrolytic cleavage 3′-OH and 5′-OPO_3 (5′-OH and 3′-OPO_3) fragments remain intact

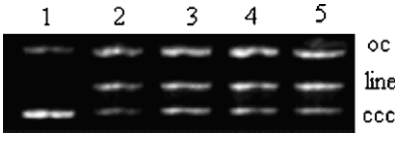

Figure 5. Agarose gel (1%) of pBR 322 (0.12 μg) incubated for 2 h at 37 °C and pH 7.2 (10 mM Tris–HCl) with 0.4 mM DMC and different scavengers: lane 1, DNA control; lane 2, DMC control; lane 3, 10 mM NaN_3; lane 4, 10 mM glycerol; lane 5, 10 mM DMSO.

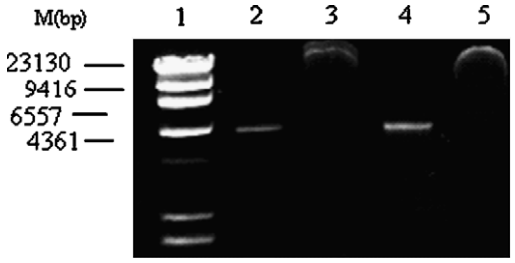

Figure 6. Agarose gel (1%) for ligation of pBR322 DNA linearized by DMC: lane 1, λ*Hin*dIII DNA markers; lanes 2 and 3 pBR322 DNA linearized by *Eco*RI without and with T4 DNA ligase; lanes 4 and 5 pBR322 DNA linearized by DMC without and with T4 DNA ligase.

and that these fragments can be enzymatically ligated and end-labeled.[15] We tried to recover the linear DNA from an agarose low melting point gel by cutting off the gel fragment and subjecting it to the DNA recovering system, the linear DNA recovered was then subjected to overnight ligation reaction with T4 DNA ligase. The result after electrophoresis (Fig. 6) indicated that the pBR322 DNA that was linearized by DMC could be ligated nearly 100% just like the linear DNA mediated by *Eco*RI. This suggests that the cleavage of DNA by DMC could be a hydrolysis that takes place by a reaction similar to that of the natural enzyme *Eco*RI.

In conclusion, we have discovered that the free macrocyclic polyamine DMC can cleave DNA without metal ions and the cleavage mechanism involved hydrolysis. Further confirmation and the detailed mechanism are currently under investigation.

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