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DNA cleavage promoted by 2,9-dimethyl-4,7-diazadecane-2,9-dithiol (DDD) derivatives

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Abstract—Three piperidine derivatives of 2,9-dimethyl-4,7-diazadecane-2,9-dithiol (DDD), NEPDDD, NEMPDDD, and NEMMPDDD, were synthesized and used as catalysts in DNA cleavage. Under physiological conditions, a series of experiments have been done. The effects of DNA cleavage with three ligands were studied under different concentrations, cleavage time, and pH values. The results strongly suggested that the plasmid DNA (pUC 19) can be cleaved efficiently by these ligands. For the cleavage reaction catalyzed by NEMPDDD, Form I DNA could convert to Form II completely, and the DNA-cleavage mechanism involved an oxidative pathway.

As a kind of basic chemical bonds in DNA or RNA, the phosphodiester bond is too stable in physiological conditions to be hydrolyzed. The half-life of phosphodiester bond is about 130,000 years.[1] Because DNA and RNA cleavage agents have the ability to accelerate the hydrolysis of these bonds, they are widely used in the fields of molecular biology and therapy.[2–6] Series of natural nucleases and ribonucleases, including restriction endonucleases, recombinases, and topoisomerases,[7] have been found and used to cleave DNA or RNA. Some of the natural metal-containing enzymes have the capability to hydrolyze DNA in a second. Artificial mimicked nucleases, such as macrocyclic polyamine complexes with metal ions, have been found to be the catalyst in DNA cleavage.[8] Furthermore, certain artificial complexes with transition metal such as Co(III), Zn(II), and Cu(II) exhibit high activity in the hydrolytic DNA cleavage process.[9] However, some of the free ligands in the absence of transition metal could not cleave DNA.[10]

Free small molecules can interact with DNA through recognition, binding, modifying, cleaving or crosslinking. These molecules have been employed widely.[11] Recently, Wu and co-workers found that 1,7-dimethyl-1,4,7,10-tetraazacyclododecane could hydrolyze double stranded DNA under physiological conditions (37 °C, pH 7.2).[12] Li and co-workers reported that the polythio-amine, varacin, could be used as DNA cleavage agent.[13] This reagent could cleave single stranded DNA (pBR322) effectively at pH 5.5 (81%) in the presence of 2-mercaptoethanol. The amount of cleaved DNA increased associated with the increase of the concentration of 2-mercaptoethanol. Gates and co-workers reported that polysulfides could also cleave DNA.[14]

Ligands with N_2S_2 structures, especially 2,9-dimethyl-4,7-diazadecane-2,9-dithiol (DDD), have been studied in recent years.[15] DDD is a strong chelating agent and can form stable lipophilic chelate complex with technetium or rhenium in high yields.[16] The ^{188}Re lableled compounds of DDD derivatives exhibit excellent lipophilicity, and these compounds can be used to treat liver cancer.[17] Lever and co-workers reported the preparation and biodistribution of a ^{99}Tc-triaminedithiol complex, which was used to estimate the regional cerebral blood flow of mice.[18] In this paper, three DDD derivatives were synthesized and used as DNA cleavage reagents. It was found that these free ligands, especially NEMPDDD, have highly efficient activity in the cleavage of DNA.

NEPDDD [2,9-dimethyl-4,7-diaza-4-(β-piperidinylethyl)-decane-2,9-dithiol], NEMPDDD [2,9-tetramethyl-4,7-diaza-4-(4′-methyl-β-piperidinylethyl)-decane-2,9-dithiol], and NEMMPDDD [2,9-tetramethyl-4,7-diaza-4-(3′,5′-dimethyl-β-piperidinylethyl)-decane-2,9-dithiol] were prepared according to the reported procedures[19]

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and stored as hydrochloride salts. These salts were dissolved in deionized water for biological assay. The structures of three DDD derivatives are shown as follows (Fig. 1).

The DNA cleavage reactions catalyzed by NEPDDD, NEMPDDD, and NEMMPDDD were studied under physiological condition at pH 7.0. Under such conditions, the three ligands and plasmid DNA pUC 19 were incubated at 37 °C. Supercoiled plasmid DNA (Form I) was cleaved to give open-circular form (Form II) selectively. The amounts of strand scission were assessed by agarose gel electrophoresis.

First, we compared the DNA cleavage abilities between NEPDDD, NEMPDDD, and NEMMPDDD under pH 7.0 and at 37 °C for 24 h. The results are shown in Figure 2. Lanes 3–5 represent the DNA cleavage catalyzed by NEPDDD, NEMPDDD, and NEMMPDDD, respectively. Electrophoresis and densitometry indicated that single cleavage of the supercoiled form (Form I) yielded 74.8%, 100%, and 48.2% nicked form (Form II), respectively. These results indicated that NEMPDDD showed much better catalytic activity than NEPDDD and NEMMPDDD.

According to Figure 2, NEMPDDD was chosen as the key ligand in the subsequent experiments. Figure 3 shows the results of the DNA cleavage catalyzed by NEMPDDD proceeding with different reaction times. The cleavage effect was improved with the extending of incubating time. After incubating for 1 h, the percentage of nicked form rose to 30.9% (lane 3). All the plasmid DNA was converted into nicked form within 24 h.

The results of DNA cleavage reactions with different concentrations of NEMPDDD are shown in Figure 4. Increasing the concentration of NEMPDDD resulted in the increase of nicked form of DNA (lanes 3–8). The plasmid DNA could be cleaved to nicked form completely within 24 h by using 0.286 mM NEMPDDD (lane 7).

The effect of pH value on the catalytic activity of NEMPDDD was also studied. The results are shown in Figure 5. We were pleased to find that under physiological condition (pH 7.0), NEMPDDD could be most effective for DNA cleavage, in which 40.4% nicked DNA was produced after 12 h (lane 8).

To determine the mechanism of DNA cleavage promoted by NEMPDDD, singlet oxygen scavenger and

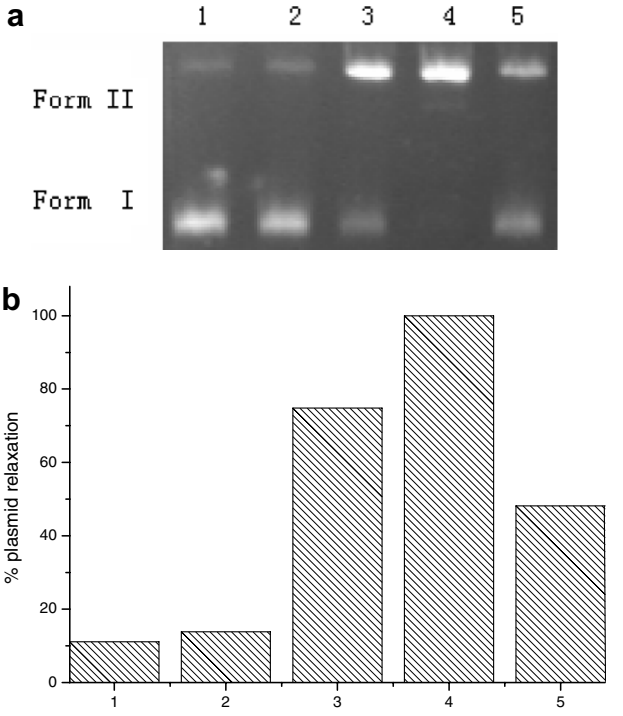

Figure 2. Effect of different ligands NEPDDD, NEMPDDD, NEMMPDDD (0.571 mM) on the cleavage reactions of pUC 19 DNA (7 μg/mL) in a NaH_2PO_4/Na_2HPO_4 buffer (100 mM, pH 7.0) at 37 °C for 24 h. (a) Agarose gel electrophoresis diagram. Lane 1: DNA control; lane 2: DNA control, 0 h; lanes 3–5: DNA cleavage catalyzed by NEPDDD, NEMPDDD, NEMMPDDD. (b) Quantitation of % plasmid relaxation relative to plasmid DNA per lane.

radical scavenger were added to the system. The singlet oxygen scavenger NaN_3 (lane 3) and NaI (lane 6) effectively inhibit the DNA cleavage by NEMPDDD, and the hydroxyl radical scavengers DMSO (lane 4) and *tert*-butyl acohol (lane 5) also show the inhibition ability. The cleavage of DNA is promoted when added the H_2O_2 (lane 7). All the results suggested that hydroxyl radical or singlet oxygen oxidative cleavage occurs in the reaction, and singlet oxygen may be the primary role. Therefore, DNA cleavage promoted by NEMPDDD might occur by an oxidative pathway (Fig. 6).

All results revealed that NEPDDD, NEMPDDD, NEMMPDDD were good artificial catalysts for the cleavage of DNA. For NEMPDDD, plasmid DNA could be cleaved from Form I to Form II completely under mild conditions (pH 7.0) in a short time. We have discovered that the cleavage mechanism involved an oxidative pathway.

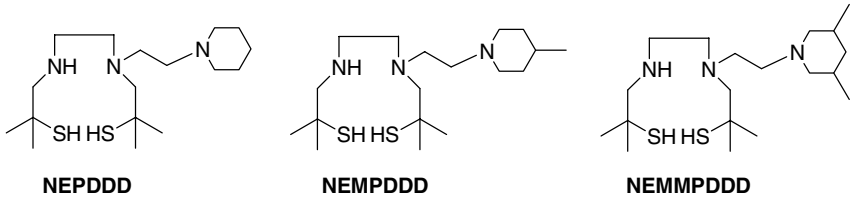

Figure 1. Structures of DDD derivatives.

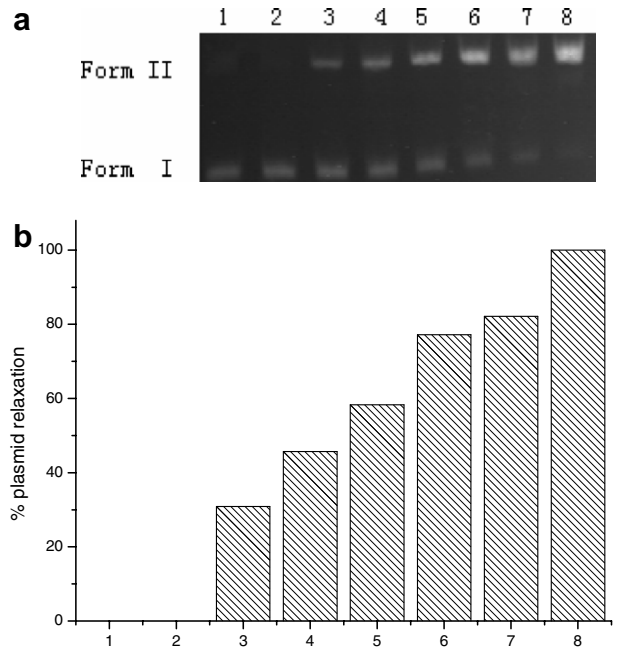

Figure 3. Effect of reaction time on the cleavage reaction of pUC 19 DNA (7 μg/mL) with ligand NEMPDDD (0.571 mM) in NaH_2PO_4/Na_2HPO_4 buffer (100 mM, pH 7.0) at 37 °C. (a) Agarose gel electrophoresis diagram. Lane 1: DNA control, 24 h; lane 2: DNA control, 0 h; lanes 3–8: 1, 2, 4, 8, 12h, and 24 h. (b) Quantitation of % plasmid relaxation relative to plasmid DNA per lane.

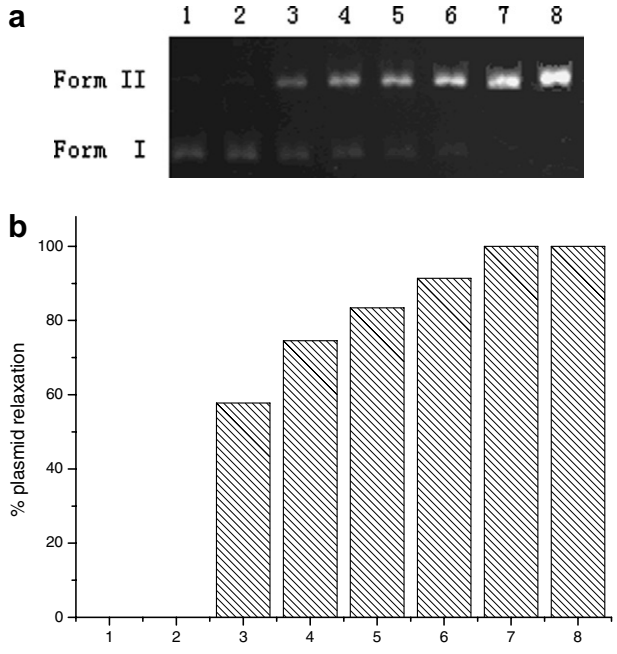

Figure 4. Effect of concentration of ligand NEMPDDD on the cleavage reactions of pUC 19 DNA (7 μg/mL) in a NaH_2PO_4/Na_2HPO_4 buffer (100 mM, pH 7.0) at 37 °C for 24 h. (a) Agarose gel electrophoresis diagram. Lane 1: DNA control, 24 h; lane 2: DNA control 0 h; lanes 3–8: [NEMPDDD] = 14.3 μM, 28.6 μM, 0.072 mM, 0.143 mM, 0.286 mM, 0.571 mM. (b) Quantitation of % plasmid relaxation relative to plasmid DNA per lane.

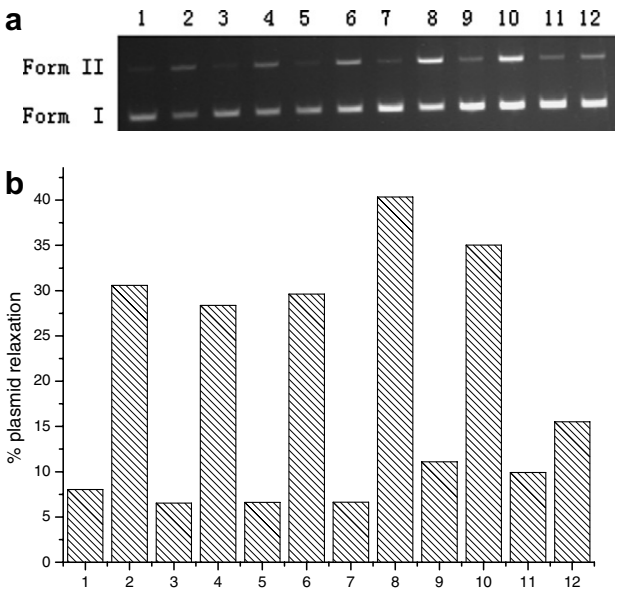

Figure 5. Effect of pH on the cleavage reaction of pUC 19 DNA (7 μg/mL) with ligand NEMPDDD (0.143 mM) in NaH_2PO_4/Na_2HPO_4 buffer (100 mM) at 37 °C for 12 h. (a) Agarose gel electrophoresis diagram. Lanes 1, 3, 5, 7, 9, 11: DNA control, pH 5.8, 6.2, 6.6, 7.0, 7.4, 7.8; lanes 2, 4, 6, 8, 10, 12: NEMPDDD as catalyst, pH 5.8, 6.2, 6.6, 7.0, 7.4, 7.8. (b) Quantitation of % plasmid relaxation relative to plasmid DNA per lane.

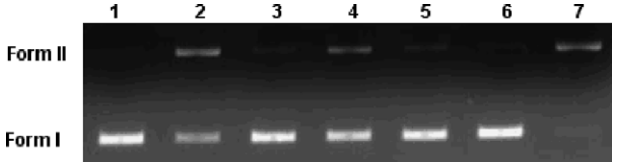

Figure 6. Effect of scavenger on the cleavage reaction of pUC 19 DNA (7 μg/mL) with ligand NEMPDDD (0.143 mM) in NaH_2PO_4/Na_2HPO_4 buffer (100 mM) at 37 °C for 12 h. Lane 1: DNA control; lane 2: NEMPDDD control; lane 3: 10 mM NaN_3; lane 4: 10 mM DMSO; lane 5: 10 mM *tert*-butyl acohol; lane 6: 10 mM NaI; lane 7: 10 mM H_2O_2.

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