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Linear disulfide-containing low polymer as efficient DNA cleavage reagent

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ABSTRACT

A novel linear poly[1,7-bis(mercaptoacetyl)-1,4,7,10-tetraazacyclododecane] (PBMAC) containing macrocyclic polyamine was synthesized through oxidation of 1,7-bis(mercaptoacetyl)-1,4,7,10-tetraazacyclododecane (BMAC) and characterized. Gel electrophoresis experiments showed that PBMAC can promote the DNA cleavage more efficiently than its monomer under physiological conditions without any thiol additives. Fluorescence quenching and DNA melting experiments demonstrated that PBMAC has stronger binding ability with DNA than that of monomer.

Phosphodiester bonds in DNA are very difficult to be hydrolyzed under physiological conditions.[1] The half-life of DNA by spontaneous hydrolysis will be thousands to billions of years.[1,2] As widely used in the fields of molecular biology and therapy, the cleavage of DNA with high selectivity has become an invaluable tool.[3–5] Many natural enzymes such as restriction endonucleases, recombinases, and topoisomerases, can catalyze phosphoryl cleavage efficiently.[6] For the difficulties of mimicing enzymatic reaction in the lab,[7] design and synthesis of high efficient chemical nucleases have emerged to be an important research field. Varies of metal complexes,[8–12] which could accelerate the cleavage of DNA, were reported.[11] As a widely studied type of compounds, the metal complexes of 1,4,7,10-tetraazacyclododecane (cyclen) have been used as artificial nucleases in DNA recognition and cleavage.[13–17] Recently, we also reported a copper complex of a novel linear polymeric macrocyclic polyamine as an effective artificial nuclease.[18] However, free ligands without transition metals always showed much decreased DNA cleavage abilities.[19,20]

Free small molecules have been employed widely in the interaction with DNA through molecular recognition, groove binding, functional group modification, cleavage or crosslinking.[13,21,22] Wu and co-workers found that 1,7-dimethylcyclen can hydrolyze double stranded DNA under physiological conditions.[23] Some multisulfide compounds were also reported as effective DNA cleavage agents by adding necessary sulfur-containing additives.[24,25] Our group reported three nitrogen–sulfur ligands as good artificial catalysts for the cleavage of DNA without thiol additives.[26] Gates speculated that the thiol-dependent formation of oxygen radicals mediated by polysulfides hinges upon the unusual reactivity of intermediates generated in the reaction of thiols with polysulfides.[27] To the best our knowledge, no synthesized polymer of thiol-containing ligand has been reported as chemical nuclease for DNA cleavage.

In this Letter, we prepared a novel nitrogen-sulfur-containing ligand named 1,7-bis(mercaptoacetyl)-1,4,7,10-tetraazacyclododecane (BMAC) and its low polymer (PBMAC), which has both thiol ending and disulfide bonds in the polymeric chain. In the preparation of PBMAC (Scheme 1), 2-mercaptoacetic acid was firstly protected by Boc_2O to give compound **1**. The reaction between **1** and 1,7-bis(tert-butyloxycarbonyl)-1,4,7,10-tetraazacyclododecane[28] afforded the desired product **2**. BMAC was obtained by deprotection of **2** with HCl (g) in CH_2Cl_2. Subsequent oxidation with O_2 could give polymeric product PBMAC. The molecular weight of PBMAC was determined as 1597.29 ($n = 5$) by MALDI-TOF/TOF mass spectrometry (Fig. S1 in Supplementary data). The final product PBMAC was prepared in several batches with invariable molecular weight distributions. Elemental analysis was also applied to confirm the structure of PBMAC (Data are shown in Supplementary data). As higher DNA affinity always leads to higher DNA cleavage efficiency,[9,29,30] we hope the polymer PBMAC, which has potentially stronger affinity with DNA than that of monomer BMAC, to be a promising DNA cleavage reagent.

The cleavage activities of BMAC and PBMAC towards pUC 19 supercoiled DNA were then studied. Supercoiled plasmid DNA

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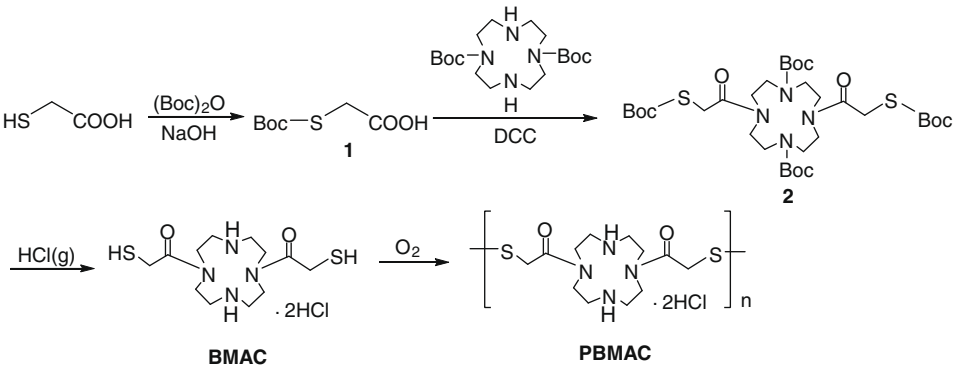

Scheme 1. Synthetic route of **BMAC** and **PBMAC**.

(Form I) was cleaved under physiological conditions to give open-circular form (Form II). The amount of strand scission was assessed by agarose gel electrophoresis. The cleavage abilities of BMAC and PBMAC were firstly compared under different concentrations. As shown in Figure 1, when the concentration of sulfur compounds increased from 7 mg/L to 56 mg/L, the amounts of nicked DNA (Form II) promoted by PBMAC were 31%, 66%, 92% and 100%, respectively. While the cleavages in the presence of BMAC only gave Form II with the amounts of 20%, 24%, 44% and 63%, respectively. These results indicated that the polymer can cleave DNA more efficiently than monomer.

To further compare the cleavage abilities of BMAC and PBMAC, the DNA cleavage reactions proceeding with different reaction times with the same reagent concentration were performed. As shown in Figure 2, increasing the reaction time resulted in the increase of Form II. Supercoiled DNA could be completely cleaved in 8 h in the presence of PBMAC. On the other hand, BMAC cleaved only 35% of supercoiled DNA in the same time span, and 92% of nicked DNA was obtained even by extending reaction time to 24 h. It is obvious that PBMAC promoted the cleavage of plasmid DNA (pUC 19) much more quickly than BMAC under physiological conditions. Moreover, PBMAC is also a better reagent for DNA cleavage than some metal–cyclen complexes and nitrogen–sulfur ligands we reported previously.[26,31–33]

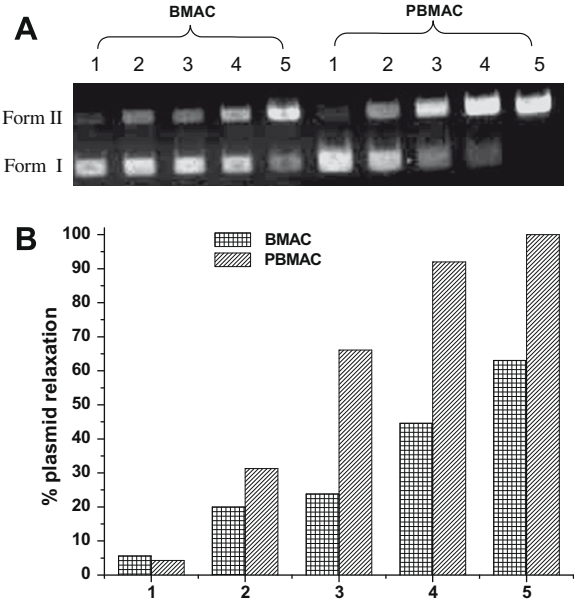

Figure 1. Effect of concentration of BMAC and PBMAC on the cleavage reactions of pUC 19 DNA (7 μg/mL) in a pbs (100 mM, pH 7.4) at 37 °C for 24 h. (A) Agarose gel electrophoresis diagram: lane 1, DNA control 24 h; lane 2, 7 mg/L; lane 3, 14 mg/L; lane 4, 28 mg/L; lane 5, 56 mg/L of sulfur compounds. (B) quantitation of % plasmid relaxation relative to plasmid DNA per lane.

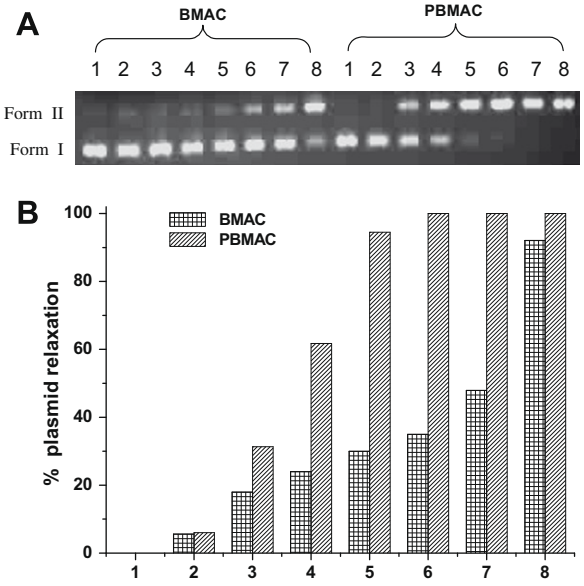

Figure 2. Effect of reaction time on the cleavage reaction of pUC 19 DNA (7 μg/mL) with BMAC and PBMAC (56 mg/L) in a pbs (100 mM, pH 7.4) at 37 °C. (A) Agarose gel electrophoresis diagram: lane 1, DNA control, 0 h; lane 2, DNA control, 24 h; lane 3, 1 h; lane 4, 2 h; lane 5, 4 h; lane 6, 8 h; lane 7, 12 h; lane 8, 24 h. (B) quantitation of % plasmid relaxation relative plasmid DNA per lane.

DNA affinity, which is the critical step for DNA cleavage in most cases, was reported to correlate with DNA cleavage efficiency. Helix melting assays were performed to compare the pDNA-binding capability of BMAC and PBMAC. Helix melting was monitored by noting the CT-DNA absorbance at 260 nm as a function of temperature. The absorbance increased substantially when the double helix dissociates into single stranded DNA. The mid point of this transition represents denaturation of the double helix and the corresponding temperature is a parameter as the helix melting temperature (T_m). When a ligand binds with double helix, the helix stability is improved and the T_m is increased, which can indicate the strength of its interaction with DNA. The helix melting results were shown in Figure 3. The T_m of CT DNA alone is 73.5 °C. After addition of BMAC and PBMAC, the ΔT_m of 3.4 °C and 4.0 °C was observed, respectively. Melting studies show that PBMAC has appreciably stronger binding affinity than BMAC.

In order to further compare the binding affinities of BMAC and PBMAC towards DNA, ethidium bromide (EB) exclusion assays

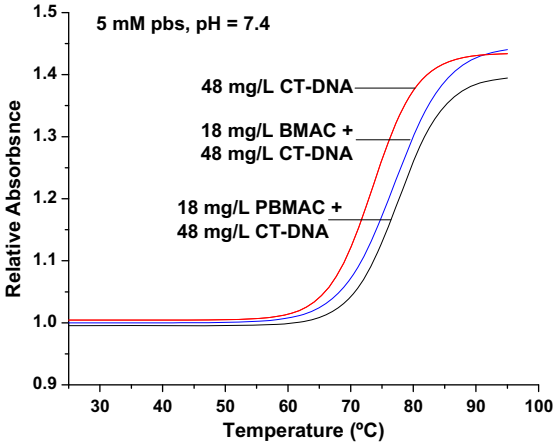

Figure 3. Helix melting curves of CT-DNA (48 mg/L in 5 mM pbs, pH 7.4): (a) alone (red); (b) +18 mg/L BMAC (blue); (c) + 18 mg/L PBMAC (black).

were carried out in vitro under pH 7.4 at room temperature. EB has weak fluorescence, but its emission intensity in the presence of DNA could be greatly enhanced because of its strong intercalation between the adjacent DNA base pairs. It was previously reported that this enhanced fluorescence could be quenched, or at least partly quenched by the addition of a second molecule with higher DNA-binding ability. The percent of decreased fluorescence value was used as a parameter to evaluate the DNA binding affinity of a given molecule. In fluorescence quenching assays, DNA was added to the solution containing EB and allowed to equilibrate for five seconds. Each aliquot of BMAC or PBMAC were then added to the solution and the fluorescence measured after five seconds of equilibration. All the samples were excited at 480 nm and emissions were measured at 600 nm. As shown in Figure 4, the addition of PBMAC to DNA pretreated with EB caused more evident decrease in the emission intensity than that caused by BMAC, indicating that the DNA-bound EB fluorophore can be partially replaced by PBMAC more easily. These results suggested that PBMAC has stronger binding affinity than BMAC, which is accordant with the results in agarose gel electrophoresis. From the differences in ΔT_m and fluorescent experiments between the two compounds (PBMAC > BMAC for both values), we believe that the polymer has higher binding affinity with DNA than monomer. Further studies about the mechanism of oxidative cleavage promoted by this type of polymer are now in progress.

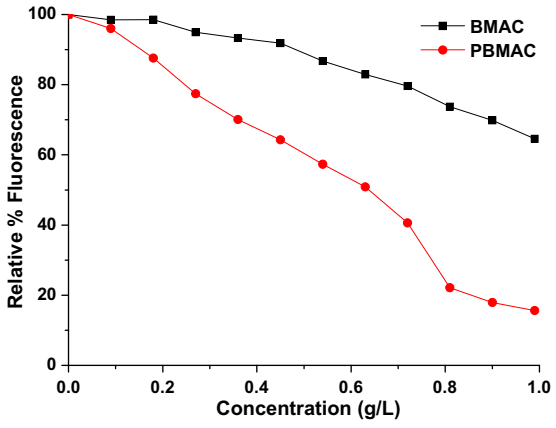

Figure 4. Fluorescence spectra of EB bound to CT-DNA in the presence of BMAC and PBMAC with different concentration. [EB] = 5 μM, [DNA] = 10 μM, [BMAC, PBMAC] = 0, 0.09, 0.18, 0.27, 0.36, 0.45, 0.54, 0.63, 0.72, 0.81, 0.90, 0.99 g/L. The fluorescence spectroscopic were excited at 480 nm and emissions were measured at 600 nm. Fluorescence quenching assay is completed in five minutes.

In conclusion, we first designed and synthesized a novel linear polymer of nitrogen–sulfur ligand (PBMAC). The results of agarose gel electrophoresis show that PBMAC is a more effective chemical nuclease for DNA cleavage than its monomer (BMAC). Helix melting and fluorescent spectroscopic studies indicate that PBMAC has stronger DNA binding affinity than BMAC. As this low polymer can catalyze DNA cleavage quickly at low concentration with better efficiency than those cyclen-based ligands and complexes, we consider that PBMAC can be used as a promising DNA cleavage reagent.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.05.015.

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