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Hydrolysis of plasmid DNA and RNA by amino alkyl naphthalimide as metal-free artificial nuclease

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Abstract—A strategy of dimethylamino alkyldiimide conjugated with an intercalator of naphthalimide for hydrolysis of DNA was suggested and evaluated. **4** can hydrolyze 4 kb plasmid DNA into 2 kb fragments with GC and GG selectivity, which represents a novel example of sequence- or site-selective metal-free DNA artificial nuclease. Results also show it could hydrolyze RNA efficiently.
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Although phosphate diester is far more kinetically stable than other common biological functional groups such as amides or esters,[1] the backbones of nucleic acids are elegantly hydrolyzed by nucleases to facilitate their synthesis, manipulation, and repair. Due to potentials in human medicine and molecular biology, the development of effective chemical nucleases has attracted much attention.[2]

Hydrolysis of RNA is easier than that of DNA because of the existence of 2′-OH. Among reported artificial RNA cleavers, amine-containing compounds are particularly interesting and have been extensively studied.[3–6] Aliphatic diamine and polyamines are proved to accelerate the cleavage of ApA at 50 °C[3] and could hydrolytically degrade poly(A), poly(U), and poly(C) as well.[4] Other efforts revealed that tethering amines to an intercalator enhanced their RNA-cleaving activities.[5] Importantly, Komiyama et al. disclosed that the conjugates of glycine and intercalative anthraquinone efficiently cleaved $tRNA^{Phe}$ with certain site selectivity, indicating a cooperative participation of both carboxylate and amino groups.[6]

Hydrolytic cleavage of DNA by small molecule is far more challenging. Most examples are metal ions or their complexes, involving transition metal ions[7] and their complexes,[8] lanthanide ions[2] and their complexes,[9] as well as actinides.[10] They are proved to rapidly hydrolyze DNA or model phosphodiesters under physiological condition (37 °C) or at a higher temperature (55 °C).[8–11] However, the obligate requirement for metal ions prevented their use in the presence of metal chelators.[11,12] In addition, few examples were found to hydrolyze DNA with sequence selectivity.[12] It is noticed that zinc-containing amino alkylimidoanthraquinone can hydrolyze DNA.[16b] Moreover, amines, diamines, and polyamines could cleave AP-DNA (DNA with apurinic or apyrimidinic sites),[16a,c] and the cleaving efficiency increases with acetoamine conjugated to intercalators or amine through a carbonyl group.[16a] Therefore, we wonder if diamine connected with an intercalator containing a carbonyl moiety could hydrolyze DNA.

Here, we suggested a novel strategy to design and use non-metallic DNA artificial nuclease: dimethylamino alkyldiimide conjugated with a DNA intercalator. Hence, dimethyl alkyldiamine as a side chain connected to the imide moiety of naphthalimide derivative became our target. This is because naphthalimide and its derivatives have two adjacent carbonyl groups (similar to carboxylate) in their structures, and the cooperative participation of carbonyl and amino groups might

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promote DNA and RNA cleavage. In addition, naphthalimide and its derivatives are well-known effective DNA intercalators, and our previous work also revealed that the presence of thio-moiety on heterocyclic conjugation greatly promoted the DNA-intercalation. We thus expected that it might be possible for the designed compounds to damage DNA via hydrolytic mechanism without the presence of metal ions.

We herein report the hydrolysis of both plasmid DNA and RNA by *N*-aminoalkylnaphthalimide **1–4** (Fig. 1). Their structures were synthesized and confirmed by using ^{1}H NMR, MS, IR, and elemental analysis.[13b] The carbonyl groups, DNA-intercalating moiety.[13] and the amino group in the side chain were expected to cooperatively participate in the hydrolysis of DNA and RNA via hydrogen bonding.[6,14,15]

To avoid oxidation, the reaction mixture was degassed before reaction initiation. The cleaving abilities of **1–3**[13b] (0.1 mM) and **4**[13b] (0.01 mM) were examined by using plasmid pBR322 DNA as substrate at 70 °C for 20 min and 1% agarose gel electrophoresis assay gave the order of efficiency as, **4** ≫ **3** ~**1** > **2** (Fig. 2). The cleavage under 50 °C by compounds was much weaker than that under 70 °C. The superior cleaving efficiency of **4** over the others clearly indicated the importance of the side chain and the distance between dimethylamino and diimide moiety. This was similar to the case of zinc-containing amino alkylimidoanthraquinone,[16b] which were disclosed that three rather than two carbon atoms between adjacent nitrogen atoms in the side chain were more efficient for the cleavage of AP-plasmid DNA.[16a,c]

4 (10 μM) efficiently converted supercoiled plasmid DNA (form I) into a relaxed circular form (form II) and then into linear DNA (form III) at 70 °C for 5 h (Fig. 3). Although the supercoiled plasmid DNA itself slightly changed to form II and even to form III after 5 h incubation at 70 °C (lane 5), the addition of **4** at very low concentration (10 μM) highly speeded up the conversion (lane 2). Compared to 70 °C, the reaction rate under 50 °C slowed down and needed 17 h. Interestingly, plasmid DNA could be degraded into smaller fragments starting from 2.5-h incubation (lane 4). With elongation of the reaction time, the degraded DNA would be smear on the electrophoresis gel.

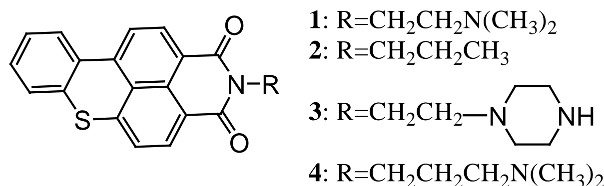

Figure 1. Structures of novel naphthalimides **1–4**.

Figure 2. Plasmid DNA cleaved by compounds **1–4**. Plasmid pBR322 DNA (50 ng/μl) was incubated with **1–3** (0.1 mM) and **4** (0.01 mM) in 20 μL of 20 mM Tris–HCl (pH 7.5) at 70 °C for 20 min in the dark, and analyzed by 1% agarose gel electrophoresis. Lanes 1-4 represented **2**, **1**, **3**, and **4**, respectively; lane 5, pBR322 DNA (50 ng/μl).

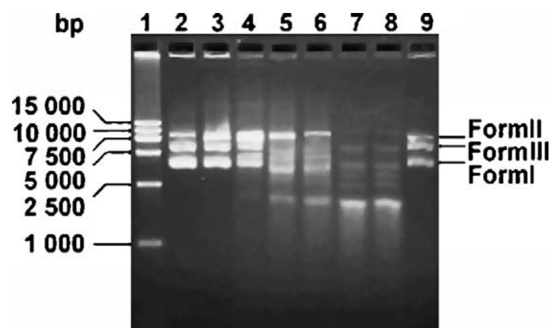

Figure 3. 1% Agarose gel electrophoresis assay of plasmid pBR322 DNA cleaved by compound **4**. Lane 1, DNA marker; lanes 2–8, pBR322 DNA (50 ng/μl) with 10 μM of **4** in 20 μL of 20 mM Tris–HCl (pH 7.5) at 70 °C for 0.5, 1, 2.5, 3.5, 4.0, 4.5, and 5.0, respectively; lane 9, pBR322 DNA (50 ng/μl) in 20 mM Tris–HCl (pH 7.5) at 70 °C for 5 h.

To determine the cleavage site and confirm the hydrolytic mechanism, the DNA fragments around 2 kb were isolated, collected, cloned, and sequenced by following the procedure[17] (see Fig. 4). Since both the blunting enzyme (T4 DNA polymerase) and ligation enzyme (T4 DNA ligase) can only work with 3′-OH and 5′-phosphate ending DNA fragments, the successful ligation between these cleaved DNA fragments and sequencing vectors (pMD-18sT), and the following operations involving cloning and sequencing strongly supported the hydrolytic mechanism. The yield for the ligation between 2 kb products and the sequencing vectors (pMD-18sT) was as high as that for a normal PCR product. Sequencing the cloned DNA fragments gave the cleavage site map, which showed that the cleavage solely occurred at 5′–GC-3′ and 5′–GG-3′ sequences (Fig. 5). The selectivity might be partially attributed to the GC/GG-selective DNA-intercalating ability of the fused planar naphthalimide moiety.[4,13,18,19] We did not observe any selectivity and similar phenomena in the photocleavage of DNA.[13b,d]

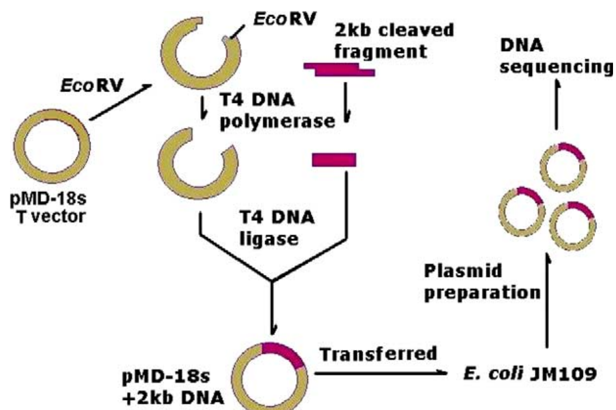

Figure 4. Molecular biological methodology for identifying DNA cleavage sites by **4**.

(250)	5′	GCAATTTCT⋯⋯ACCGCATC	3′	(2347)
(262)	5′	CGCACCCGT⋯⋯CGCTCTTC	3′	(2358)
(611)	5′	CTGGGCTGC⋯⋯GGGAAGCG	3′	(2717)
(704)	5′	CGGGGCATG⋯⋯ACCCCCCG	3′	(2804)
(778)	5′	CTCTGGTCA⋯⋯TTATCGCC	3′	(2877)
(870)	5′	GCTCAAGCC⋯⋯TACACTAG	3′	(2973)
(1187)	5′	CGGGTTGG⋯⋯ TTGGTCTG	3′	(3209)
(1798)	5′	CACAACGTT⋯⋯GCTCTTGC	3′	(3901)

Figure 5. DNA sequences of cleaved fragments derived from plasmid pBR322 by **4**. The number in the bracket represents the location of the terminal nucleic acid of ≈2 kb cleaved fragments in plasmid pBR322.

The reason for the selective formation at about 2 kb band might be due to many possibilities. One of them is exclusion among intercalators and the nature of plasmid DNA. Neighbor-exclusion principle[20] with a gap (e.g., 7–15 Å about 2–4 bp) for DNA intercalators has been only observed for double-stranded linear DNA, but the gap for plasmid DNA may probably be longer (corresponding to much more bp) as it was supercoiled and highly compact. Second, a possible case is, when the duplex of plasmid DNA at 50 or 70 °C would be partially melted and a site relaxed for the first nick, it gave a special impact on the opposite site to the first nick on the ring of DNA[16e] to facilitate the second nick, as the plasmid DNA was also strained. Once these nicks for single strand were developed to cleavages of double strands at the original nick site, the ring would be totally opened and 2 kb linear fragments (near to 1/2 of plasmid size) formed during the hydrolysis in the utmost probability (in fact, we already observed 3 kb, 4 kb fragments from the hydrolysis of the 6 kb, 8 kb DNA, respectively). In fact, it was reported that the highest cleaving efficiency by compounds was about 1.2–1.4 AP-site nick/plasmid,[16b,c] and pBR322 plasmid DNA at pH 5 at 70 °C for 8 min could generate 2 AP-sites nick,[16d] which is very close to our observation of about 2-site cleavages. In addition, natural endogenous nuclease of sugar beet cells was found to work efficiently at 70 °C.[21]

We checked **4**'s binding affinities to DNA again[13b] with the fluorescence quenching method by comparing with similar analogues,[13c] and believed that **4** might bind DNA via an electrostatic attraction between the ammonium (from the protonation of the *N*,*N*-dimethyl amino group at the end of the side chain) and DNA, and intercalation exerted by the naphthalimides.

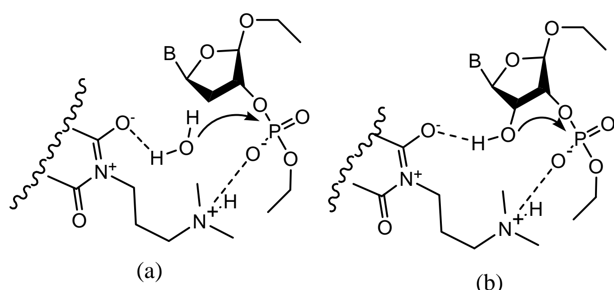

Figure 6. Proposed mechanism for the cleavage of plasmid DNA (a) or RNA (b) by **4**.

A possible mechanism was proposed. The naphthalimide might first intercalate to double-stranded DNA with 5′–GC-3′ or 5′–GG-3′ sequence selectivity, binding would occur between ammonium and phosphate anion, meanwhile hydrogen bonding[14,15] took place between the nitrogen or oxygen atoms at the imide moiety and water (drawing as resonance structure with positive nitrogen and negative oxygen atoms, that is N^+–C-O^-, and the oxygen coordinating to H_2O), which catalyzed the removal of proton from water to give a hydroxide anion similar to a role of the 2′-hydroxyl group in RNA and then that attacked the phosphorus atom leading to the break down of the phosphate ester bond (Fig. 6a). Of course, because of the strain and mechanics for plasmid DNA, the second nick would happen to the opposite site of the first nick on the ring of DNA in the utmost possibility, and finally these nicks were developed to cleavages.

Furthermore, the cleavage efficiency of **4** by using total RNA extracts consisting of 28S and 18S as substrate was evaluated and performed in the dark to avoid the photo-activation of naphthalimide and only Millipore water was applied to ensure a metal-free solution environment. RNA sample together with **4** (0.03 mM) was incubated at 37 °C, pH 7.5. 1% agarose gel electrophoresis assay revealed that the compound dramatically catalyzed the time-dependent degradation of total RNA to leave 54%, 43%, and 31% intact RNA after 2, 3, and 4 h, respectively, whereas the RNA control remained intact under the identical conditions (Fig. 7).

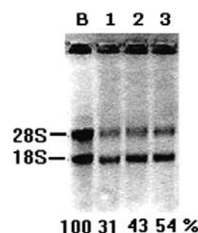

Figure 7. 1% Agarose gel electrophoresis assay for the degradation of total RNA (2 μg) by **4** (0.03 mM) at 37 °C, pH 7.5, in the dark. Lanes 1–3 represent the degradation that was allowed for 2, 3, and 4 h, respectively; B, total RNA at 37 °C for 4 h.

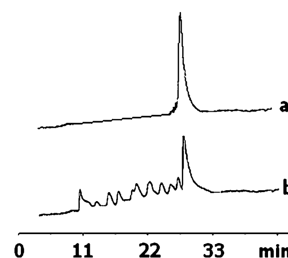

Figure 8. Ion-exchange HPLC chromatograms showing the hydrolysis of poly$(A)_{10}$ by **4**. (a) 0.05 mM poly $(A)_{10}$; (b) 0.05 mM poly$(A)_{10}$ was hydrolyzed by **4** (0.05 mM) in 20 mM Tris–HCl, pH 7.5, at 37 °C for 30 h. The sample was eluted by a linear gradient from 0 to 1.0 M NaCl in 10 mM NaH_2PO_4 aqueous solution for 60 min.

To elucidate the mechanism of RNA hydrolysis by **4**, the conversion of poly$(A)_{10}$ to hydrolytic products was monitored by HPLC. At 37 °C in the dark, 0.04 mM of **4** dissolved in DMSO could efficiently hydrolyze 0.1 mM poly$(A)_{10}$ buffered in 20 mM Tris–HCl, pH 7.5 (Fig. 8). As shown in Figure 8, the poly$(A)_{10}$ was degraded into several oligonucleotides varying in length.

A possible mechanism for the hydrolysis of RNA was proposed. First, a binding occurred between ammonium and phosphate anion, meanwhile hydrogen bonding[14,15] formed between the nitrogen or oxygen atoms at imide moiety and 2′-hydroxyl group in RNA. Then, phosphorus atom was attacked leading to the break down of phosphate ester bond (Fig. 6b).

In conclusion, a strategy of dimethylamino alkyldiimide conjugated with a DNA intercalator of naphthalimide for hydrolysis of DNA was suggested and evaluated. Compound **4** determined to be the most efficient DNA cleaver suggested that the side chain had dramatic effects on potency. Experimental results indicated **4** as a novel example of sequence- or site-selective metal-free DNA artificial nuclease.

Acknowledgments

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References and notes

1. (a) Corey, D. R.; Schultz, P. G. *Science* **1987**, *238*, 1401(b) Williams, N. H. In *Nucleic acids and molecular biology*, Zenkova, M. A., Ed.; Springer-Verlag: Berlin Heidelberg, 2004; Vol. 13, pp 3–7.
2. (a) Chin, J. *Curr. Opin. Chem. Biol.* **1997**, *1*, 514; (b) Komiyama, M.; Sumaoka, J. *Curr. Opin. Chem. Biol.* **1998**, *2*, 751; (c) Franklin, S. J. *Curr. Opin. Chem. Biol.* **2001**, *5*, 201.
3. (a) Yoshinari, K.; Komiyama, M. *Chem. Lett.* **1990**, 519; (b) Komiyama, M.; Yoshinari, K. *J. Org. Chem.* **1997**, *62*, 2155.
4. Yoshinari, K.; Yamazaki, K.; Komiyama, M. *J. Am. Chem. Soc.* **1991**, *113*, 5899.
5. Shinozuka, K.; Shimizu, K.; Nakashima, Y.; Sawai, H. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1979.
6. Endo, M.; Hirata, K.; Ihara, T.; Sueda, S.; Takagi, M.; Komiyama, M. *J. Am. Chem. Soc.* **1996**, *118*, 5478.
7. (a) Moss, R. A.; Zhang, J.; Ragunathan, K. G. *Tetrahedron Lett.* **1998**, *39*, 1529; (b) Ott, R.; Kramer, R. *Appl. Microbiol. Biotechnol.* **1999**, *52*, 761.
8. (a) Deck, K. M.; Tseng, T. A.; Burstyn, J. N. *Inorg. Chem.* **2002**, *41*, 669; (b) Roelfes, G.; Branum, M. E.; Wang, L.; Que, L. J.; Feringa, B. L. *J. Am. Chem. Soc.* **2000**, *122*, 11517; (c) Neves, A.; Terenzi, H.; Horner, R.; Horn, A. J.; Szpoganicz, B.; Sugai, J. *Inorg. Chem. Commun.* **2001**, *4*, 388.
9. (a) Branum, M. E.; Tipton, A. K.; Zhu, S.; Que, L. J. *J. Am. Chem. Soc.* **2001**, *123*, 1898; (b) Branum, M.; Que, L. *J. Biol. Inorg. Chem.* **1999**, *4*, 593; (c) Vijayalakshmi, R.; Kanthimathi, M.; Subramanian, V.; Nair, B. U. *Biochem. Biophys. Res. Commun.* **2000**, *271*, 731.
10. Moss, R. A.; Bracken, K.; Zhang, J. *Chem. Commun.* **1997**, *6*, 563.
11. Rangarajan, S.; Shankar, V. *Biochim. Biophys. Acta* **1999**, *1473*, 293.
12. (a) Chen, W.; Kitamura, Y.; Zhou, J. M.; Sumaoka, J.; Komiyama, M. *J. Am. Chem. Soc.* **2004**, *126*, 10285; (b) Komiyama, M.; Shiiba, T.; Takahashi, Y.; Takeda, N.; Matsumura, K.; Kodama, T. *Supramol. Chem.* **1994**, *4*, 31; (c) Komiyama, M. *J. Biochem.* **1995**, *118*, 665; (d) Kovacic, R. T.; Welch, J. T.; Franklin, S. J. *J. Am. Chem. Soc.* **2003**, *125*, 6656; (e) Zelder, F. H.; Mokhir, A. A.; Kramer, R. *Inorg. Chem.* **2003**, *42*, 8618.
13. (a) Xu, Y.; Huang, X.; Qian, X.; Yao, W. *Bioorg. Med. Chem.* **2004**, *12*, 2335; (b) Qian, X.; Li, Y.; Xu, Y.; Liu, Y.; Qu, B. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2665; (c) Xu, Y.; Qian, X.; Yao, W.; Mao, P.; Cui, J. *Bioorg. Med. Chem.* **2003**, *11*, 5427; (d) Yang, Q.; Qian, X.; Xu, J.; Sun, Y.; Li, Y. *Bioorg. Med. Chem.* **2005**, *13*, 1615.
14. (a) Gao, Y. Q.; Marcus, R. A. *J. Phys. Chem. A.* **2002**, *106*, 1956; (b) de Costa, M. D. P.; de Silva, A. P.; Pathirana, S. T. *Can. J. Chem.* **1987**, *69*, 141; (c) de Silva, A. P.; Gunaratne, H. Q. N.; Habib-Jiwan, J.; McCoy, C. P.; Rice, T. E.; Soumillion, J. *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 1728.
15. (a) Piaütek, A. M.; Gray, M.; Anslyn, E. V. *J. Am. Chem. Soc.* **2004**, *126*, 9878; (b) Cui, D.; Qian, X.; Liu, F.; Zhang, R. *Org. Lett.* **2004**, *6*, 2757.
16. (a) Steullet, V.; Edwards-Bennett, S.; Dixon, D. W. *Bioorg. Med. Chem.* **1999**, *7*, 2531; (b) Boseggia, E.; Gatos, M.; Lucatello, L.; Mancin, F.; Moro, S.; Palumbo, M.; Sissi, C.; Tecilla, P.; Tonellato, U.; Zagotto, G. *J. Am. Chem. Soc.* **2004**, *126*, 4543; (c) Pierre, J.; Lavel, J. *J. Biol. Chem.* **1981**, *256*, 10217; (d) Lindahl, T.; Andersson, A. *Biochemistry* **1972**, *11*, 3618; (e) Rippe, K.; Mücke, N.; Langowski, J. *Nucleic Acids Res.* **1997**, *25*, 1736.
17. Sambrook, J.; Russell, D. W. In *Molecular Cloning: A Laboratory Manual*, 3rd ed.; Cold Spring Harbor Laboratory: New York, 2001.
18. Bailly, C.; Brana, M.; Waring, M. J. *Eur. J. Biochem.* **1996**, *240*, 195.
19. (a) Matsugo, S.; Miyahara, T.; Yamaguchi, K.; Mori, T.; Saito, I. *Nucleic Acids Res. Symp. Ser.* **1991**, *21*, 109; (b) Matsugo, S.; Kawanishi, S.; Yamamoto, K.; Sugiyama, H.; Matsuura, T.; Saito, I. *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 1351; (c) Saito, I.; Takayama, M.; Kawanishi, S. *J. Am. Chem. Soc.* **1995**, *117*, 5590; (d) Saito, I.; Takayama, M.; Sugiyama, H.; Nakatani, K.; Tsuchida, A.; Yamamoto, M. *J. Am. Chem. Soc.* **1995**, *117*, 6406.
20. (a) Crothers, D. M. *Biopolymers* **1968**, *6*, 575; (b) Arnott, S.; Bond, P. J.; Chandrasekaran, R. *Nature* **1980**, *287*, 561; (c) Rao, S. N.; Kollman, P. A. *Proc. Natl. Acad. Sci. U. S. A.* **1987**, *84*, 5735; (d) Laib, S.; Seeger, S. *J. Fluorescence.* **2004**, *14*, 187.
21. Klein, J.; Altenbuchner, J.; Mattes, R. *J. Biotech.* **1998**, *60*, 145.