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Water-soluble 2-Amino-1,8-naphthyridine as a Novel DNA Binding Photosensitizer for One-Electron Oxidation of DNA

Kazuhiko Nakatani*, Shinsuke Sando, Kohzo Yoshida, and Isao Saito*

Department of Synthetic Chemistry and Biological Chemistry, Faculty of Engineering, Kyoto University, CREST, Kyoto 606-8501, Japan

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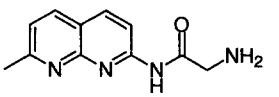
Abstract: DNA binding and photoinduced DNA cleaving properties of water-soluble naphthyridine derivative **1** were investigated. The photoinduced cleavage of DNA was studied by both PAGE and product analysis, indicating that the DNA cleavage proceeds via one-electron oxidation pathway.

Studies on the interaction of small molecules with DNA provide an important insight into the molecular design of DNA binders[1] and potent anticancer drugs.[2] We previously demonstrated several different types of small molecules that can alkylate guanine in a sequence specific fashion[3,4] or oxidize[5] DNA at guanine residues. As a part of our research to develop new ligands that are useful for DNA modification, we have investigated binding interaction and photoreactions of water-soluble 1,8-naphthyridines with DNA. We herein report that 1,8-naphthyridine **1** is a novel chromophore for photoinduced one-electron oxidation of DNA.[6]



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Synthesis of **1** was outlined in Scheme 1. Cyclization of 2,6-diaminopyridine (**2**) with dimethylacetal of 3-ketobutanal in phosphoric acid produced 2-amino-7-methyl-1,8-naphthyridine (**3**) in 79% yield.[7] Coupling of **3** with *N*-(*tert*-butoxycarbonyl)glycine *N*-hydroxysuccinimidyl ester (Boc-Gly-OSu) followed by deprotection of the terminal amino group furnished the synthesis of **1**.[8]

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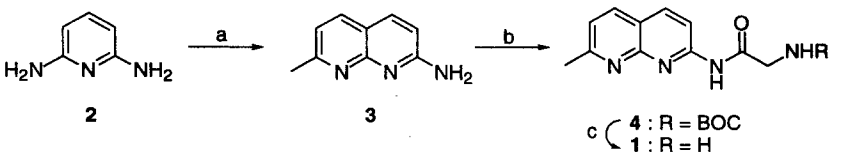
Scheme 1[a]



[a] *Reagents*: (a) 3-ketobutanal dimethylacetal, H_3PO_4, 79%; (b) Boc-Gly-OSu, $CHCl_3$, 95%; (c) HCl, AcOEt, $CHCl_3$, quant.

The binding of **1** to DNA duplex was examined by means of BIAcore technique.[9] 5'-Biothinylated 33-mer ODN 5'-d(CGT TAT CAT TGG TTA TCA TTG GGT TAT CAT TCG)-3' (bio-ODN1) was immobilized on a sensor chip coated with streptavidine and hybridized with a complementary strand (Figure 1a). Binding of **1** to the immobilized DNA duplex was examined in HEPES buffer (10 mM, pH 7.4) containing EDTA (1 mM) at 25 °C. The flow rate of the solution containing **1** was 7 μl / min. The formation of complex **1**-DNA was directly monitored by the change of surface plasmon resonance (Figure 1b). The association constant of **1** to the immobilized duplex was determined as 4.3×10^3 M^{-1} (l =1.6) by fitting a Scatchard plot to the noncooperative model of McGhee and von Hippel equation (Figure 1c).[10] While an intercalative binding of **1** to DNA was expected to be one of the major binding modes, topoisomerase unwinding assay of supercoiled DNA indicated that it was not the case for **1**, suggesting that the electrostatic interaction is a predominant force for the binding of **1** to duplex DNA.

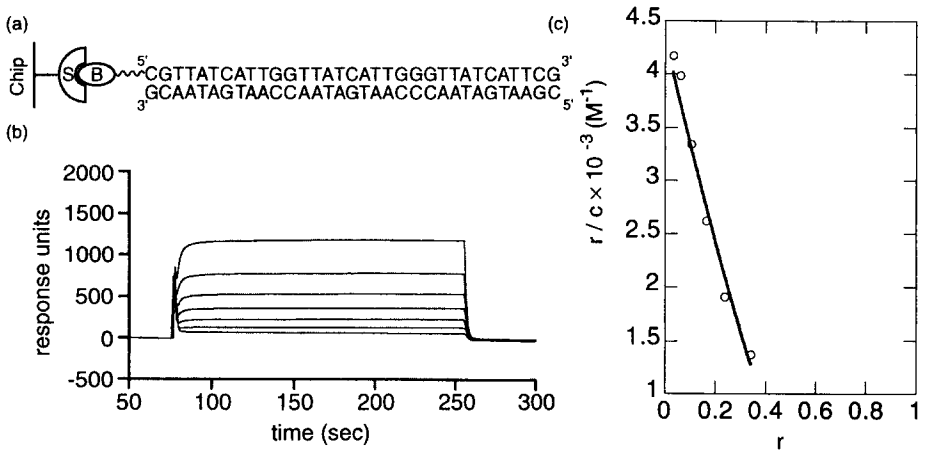


Figure 1. (a) 5'-Biothinylated DNA duplex (bio-ODN1) immobilized on a chip coated with streptavidine (S). (b) Sensorgrams with varying concentrations of **1**: 7.8, 15.6, 31.3, 62.5, 125, 250, and 500 μM, from bottom to top. Solution containing **1** was slowly injected (75 sec) and the value for the binding response was taken at equilibrium (250 sec). (c) Scatchard plot of the data shown in (b). The solid line represents the best fit isotherm generated using the noncooperative model of McGhee and von Hippel equation.[11]

Photoinduced DNA cleavage by **1** was next examined using ^{32}P 5'-end labeled ODN1 and its complementary strand (Figure 2). As clear from figure 2, G cleavage occurred selectively at 5' side G of 5'GG3' doublet after piperidine treatment of the photoirradiated mixture (lane 3). In the case of GGG triplet, the cleavage occurred at both 5' side and middle Gs together with a very weak cleavage at 3' side G (lane 3).

The G cleavage bands were not produced without piperidine treatment (lane 4), indicating that photoirradiation of **1** with DNA did not induce spontaneous strand cleavage but produced alkaline labile sites at the G residues. Such base sequence selectivity for DNA cleavage of GG doublet and GGG triplet is an indicator for the one-electron oxidation mechanism.[12]

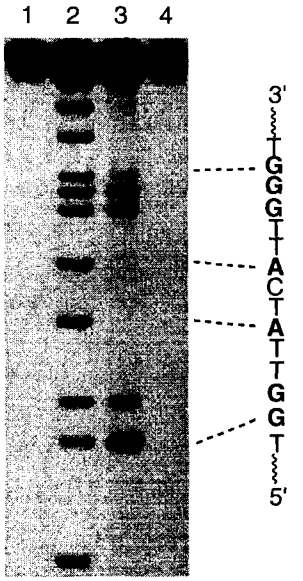


Figure 2. Photoinduced cleavage of ^{32}P 5'-end-labeled 33-mer ODN (ODN1) in the presence of **1** (100 μM). ^{32}P 5'-end-labeled ODN was annealed with a complementary strand in sodium cacodylate buffer (10 mM, pH 7.0) containing calf thymus DNA (10 μM base pair concentration) and the solution of the duplex was photoirradiated at 312 nm for 1 h in the presence of **1** at 0 °C. Recovered ODN by ethanol precipitation was analyzed by electrophoresis on denaturing sequencing gel containing 15% polyacrylamide and 7 M urea with or without piperidine treatment. Lane 1, photoirradiated ODN without **1**; lane 2, Maxam-Gilbert A+G sequencing reaction; lane 3, photoirradiated mixture with piperidine treatment (10% v/v, 90 °C, 30 min); lane 4, photoirradiated mixture without piperidine treatment.

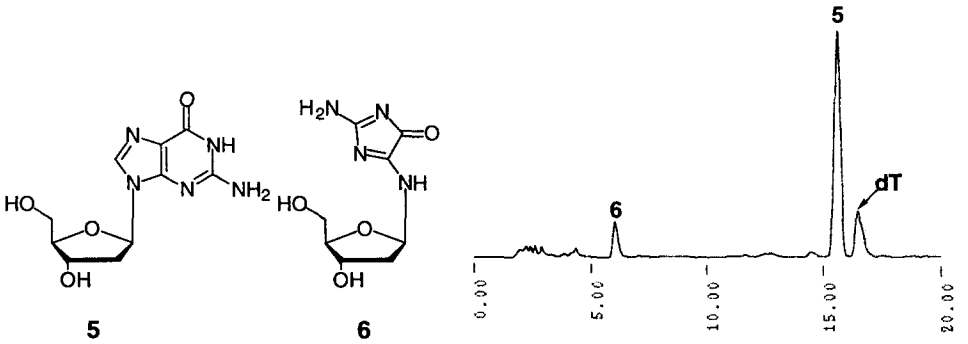


Figure 3. HPLC profile of the crude reaction mixture obtained from the photoirradiation of **1** (2.4 mM) in the presence of **5** (0.8 mM) in sodium cacodylate buffer (10 mM, pH 7.0) at 312 nm for 2 h at 0 °C. dT was added as an internal standard. HPLC analysis was carried out on a CHEMCOBOND 5-ODS-H column (6 × 150 mm) eluted with 0.1 M triethylammonium acetate buffer containing 0–8% acetonitrile linear gradient over 20 min at a flow rate of 1.0 mL/min, detected at 254 nm.

It has also been demonstrated that one-electron oxidation of monomer deoxyguanosine **5** resulted in a formation of imidazolone **6**.[5,13] Photoirradiation of **1** with **5** at 312 nm produced one major product eluted at 6.0 min (Figure 3). The product comigrated with authentic imidazolone **6** on HPLC.[14] Selective cleavage at 5'

side G of GG doublet and the specific formation of imidazolone **6** strongly support that photoinduced DNA cleavage by **1** proceeds via one-electron transfer process.

In conclusion, the present studies have demonstrated that water-soluble 2-amino-1,8-naphthyridine derivative **1** is a novel class of DNA binding photosensitizer which can be used for one-electron oxidation of DNA. Moreover, 2-amino-1,8-naphthyridine is a very attractive chromophore for designing artificial electron-accepting nucleobases. Further work on this line is now in progress.

References and Notes

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(8) **1**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 10.27 (br, 1H), 8.51 (d, 1H, J = 8.8 Hz), 8.13 (d, 1H, J = 8.8 Hz), 7.99 (d, 1H, J = 8.4 Hz), 7.26 (d, 1H, J = 8.4 Hz), 3.54 (s, 2H), 2.75 (s, 3H); ^{13}C NMR ($CDCl_3$, 400 MHz) δ 173.2, 164.1, 155.7, 153.9, 139.9, 137.2, 122.5, 119.5, 115.0, 46.6, 26.8; UV(H_2O) 366.0 (ε 280), 312.0 (ε 6710); FABMS (NBA) (relative intensity), *m/e* 217 [(M+H)$^+$] (100, base peak), 200 (20); HRMS calcd for $C_{11}H_{13}ON_4$ [(M+H)$^+$] 217.1089, found 217.1087.

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(10) Scatchard plot for the binding of **1** to DNA ($r < 0.4$) was fit by nonlinear least square analysis to the McGhee and von Hippel equation[11] governing random noncooperative binding to a lattice:

$$r/c = K(1-lr)[(1-lr)/\{1-(l-1)r\}]^{l-1}$$

where r is the ratio of bound concentration of **1** to the concentration of DNA phosphate; c is the concentration of **1** free in solution; K is the association constant; l is the neighbor exclusion value.

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