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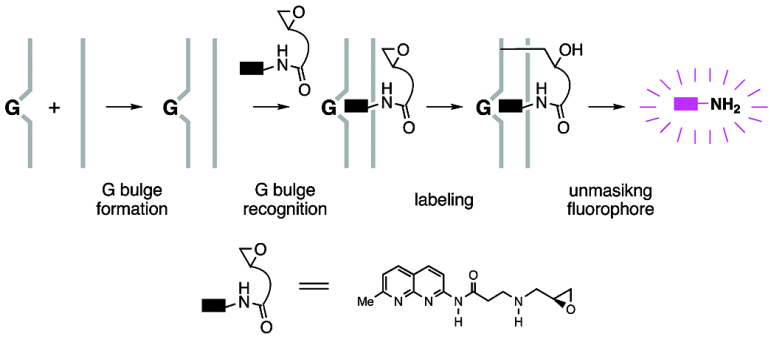

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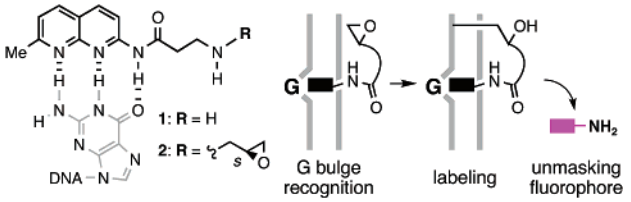
Affinity Labeling of a Single Guanine Bulge

Kazuhiko Nakatani,*[,†,‡] Souta Horie,[†] and Isao Saito*[,†]

Department of Synthetic Chemistry and Biological Chemistry, Faculty of Engineering, Kyoto University, Kyoto 606-8501, Japan, and PRESTO, Japan Science and Technology Corporation, Japan

Received March 10, 2003; E-mail: nakatani@sbchem.kyoto-u.ac.jp

In contrast to a fully matched duplex, DNA containing bulged bases and mismatched base pairs exhibits remarkable and unique reactivity toward drug binding. Due to the intrinsic thermodynamic instability, these structurally fluctuated sites in duplex are exceedingly susceptible for drug interactions.[1−10] The ligands selectively binding to the bulged and mismatched sites have been acknowledged as novel reagents for chemical-typing of genetic disorders such as single nucleotide polymorphisms.[11−13] We have reported that 2-amino-1,8-naphthyridine derivative **1** possessing hydrogen bonding groups fully complementary to a guanine selectively binds to and stabilizes a guanine bulge duplex.[8,9] Following the noncovalent binding to a guanine bulge, covalent bond formation between the ligand and the bulged DNA may provide a new method for chemical labeling of DNA containing a guanine bulge. Toward this end, we have designed electrophile-tethered naphthyridine **2** having DNA-alkylating epoxide. In addition to the role of a recognition element, *N*-acylated naphthyridine in **2** is envisioned to function as a masked fluorescent chromophore, because hydrolysis of the amide linkage producing 2-aminonaphthyridine induces a 15-fold increase in the fluorescence intensity at 396 nm. We here report that epoxide-tethered naphthyridine **2** selectively labels the guanine bulge duplex by formation of a **2**−DNA adduct. Subsequent hydrolysis of the **2**−DNA adduct unmasks the probe and releases fluorescent 2-aminonaphthyridine.



Epoxide-tethered naphthyridine **2** was synthesized by the reaction of (*S*)-epichlorohydrin with the primary amino group of **1**[8] and a subsequent conversion of the resulting chlorohydrin to the (*S*)-epoxide (SI, Scheme S1). Molecular modeling studies on the hydrogen-bonded pair of **2** and guanine bulge suggested that the electrophilic epoxide located in a major groove can reach nucleophilic N7 of purines and amino groups of adenine and cytosine within two base pairs from the bulged guanine. The labeling reaction of **2** was first examined on bulge-containing duplexes d(5′-GTT G**TXT**TG GA-3′)/d(3′-CAA C**A A** ACC T-5′) (**TXT**/**A_A**) where X denotes a bulged base. The reaction was monitored by HPLC, and major products were identified by MALDI-TOF MS. Incubation of the guanine bulge-containing duplex **TGT**/**A_A** with **2** produced an adduct of the **TGT** strand (**2**−**TGT**) (Figure 1a). MALDI-TOF MS of **2**−**TGT** (*m*/*z* 3702.13, calcd for [M − H] 3701.73) showed that one molecule of **2** was bound to **TGT**. The amount of intact

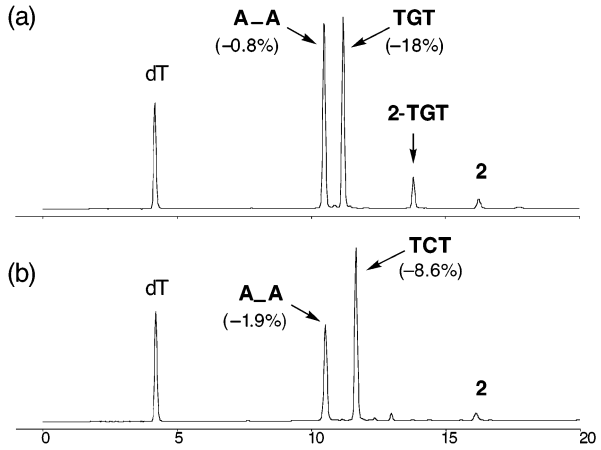


Figure 1. HPLC profiles for the reaction of (a) **TGT**/**A_A** and (b) **TCT**/**A_A** (62.5 μM) with **2** (125 μM) in 50 mM sodium cacodylate buffer (pH 7.0) at 4 °C for 18 h. The numbers in parentheses are the relative amounts of the oligomers consumed by the reaction. dT was added as an internal standard.

TGT relative to the internal standard dT was decreased by 18%, whereas the other strand **A_A** was nearly unchanged. Beside the guanine bulge duplex, the reaction with **2** was observed with a reduced efficiency for the cytosine bulge duplex **TCT**/**A_A** (Figure 1b) but not at all for those containing adenine and thymine bulges (Figure S1). The observed preference of the guanine bulge duplex for the labeling with **2** is consistent with the binding preference of **1** to a single nucleotide bulge (e.g., G > C ≫ A, T).[8] We have evaluated the labeling reaction of the (*R*)-enantiomer of **2**, which was slower than that of the (*S*)-enantiomer (Figure S2).

The site of covalent bond formation in **2**−**TGT** was unambiguously determined by identifying the oligomer fragments upon strand cleavage. Thus, isolated **2**−**TGT** was heated in an aqueous solution producing apurinic DNA d(GTT ΦTG TTG GA) (**apTGT**) where Φ denotes an apurinic site, accompanied by an isomerization to **2**−**TGTx** eluting more slowly than **2**−**TGT** (Figure 2a). Molecular weight of **apTGT** (*m*/*z* 3282.67, calcd for [M − H] 3282.55) is consistent with a release of **2**-guanine adduct from **2**−**TGT**, whereas **2**−**TGTx** shows the same molecular weight (*m*/*z* 3702.23) with **2**−**TGT**. We could not detect **2**-guanine adduct by HPLC. Heating **2**−**TGTx** regenerated **apTGT** and **2**−**TGT**, indicating that **2**−**TGT** and **2**−**TGTx** equilibrated under the conditions and slowly produced **apTGT**.[14] Strand cleavage of isolated **apTGT** at the apurinic site was carried out by heating in an aqueous piperidine. Subsequent dephosphorylation of the products with alkaline phosphatase produced two oligomer fragments (Figure 2b). One fragment was identified as d(GTT) by MS (*m*/*z* 874.31, calcd for [M − H] 874.18) and coelution with the authentic oligomer, whereas the other fragment was d(TGTTGGA) (*m*/*z* 2149.38, calcd [M − H] 2149.39). These analyses confirmed that the covalent bond

[†] Kyoto University.
[‡] PRESTO, JST.

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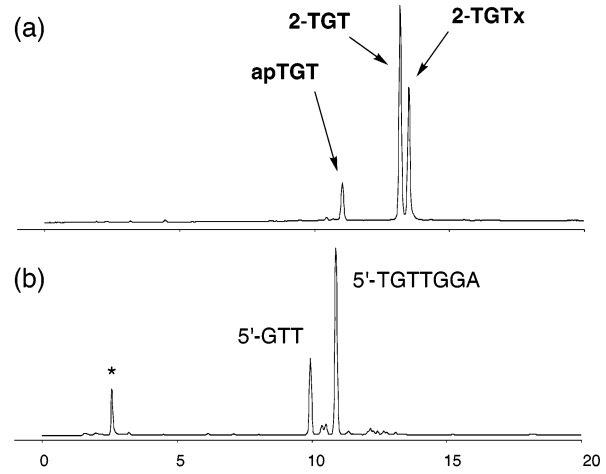

Figure 2. HPLC profiles for (a) the degradation of **2**−**TGT** at 90 °C for 30 min and (b) oligomer fragments produced from isolated **apTGT** by treating with 10% piperidine at 90 °C for 20 min and subsequent dephosphorylation with alkaline phosphatase (1 unit/μL, 10% v/v) at 37 °C for 2 h. The peak marked with an asterisk was impurity.

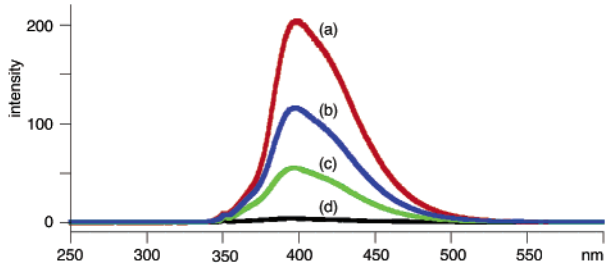

Figure 3. Fluorescence spectra recorded in 10 mM sodium cacodylate buffer (pH 7.0) excited at 352 nm. Key: crude hydrolysate of (a) **2**−**TGT** (red line) and (b) **2·2-CGC** (blue) in 700 μL of buffer solution, (c) 2-amino-1,8-naphthyridine (10 μM) (green), and (d) **1** (10 μM) (black).

formation between duplex **TGT**/**A_A** and **2** occurred at the N7 of the guanine two bases away the guanine bulge.[15]

Affinity labeling using **2** was also examined on duplex d(5′-GTT G**CGC**TG A-3′)/d(3′-CAA C**G G** ACT-5′) containing the guanine bulge flanking two G−C base pairs (**CGC/G_G**). The reaction of **CGC/G_G** with **2** produced an adduct containing two molecules of **2** on the **CGC** strand (**2·2-CGC**) (*m*/*z* 3630.26, calcd for [M − H] 3628.82) (Figure S3). Adenine, cytosine, and thymine bulges with two G−C flanking base pairs did not produce distinct **2** adduct. Since **2·2-CGC** underwent neither depurination nor strand cleavage reaction upon heating, the labeling reaction is most likely to occur at the amino group of two cytosines flanking the guanine bulge (Scheme S3).

Having confirmed the selective labeling of guanine bulge DNA, unmasking of the fluorescent chromophore was next investigated. **2**−**TGT** prepared from 156 nmol of **TGT**/**A_A** was treated with 35% hydrochloric acid for 15 min at 40 °C.[16] After concentration of the reaction mixture to dryness, the crude hydrolysate was dissolved in 10 mM sodium cacodylate buffer (pH 7.0). The fluorescence spectra of the hydrolysate were recorded with an excitation at 352 nm (Figure 3). The spectral curve and the emission maximum at 396 nm were identical to the authentic spectra of 2-amino-1,8-naphthyridine (10 μM) in the same buffer solution, confirming the affinity labeling of the guanine bulge DNA by **2**. Comparing the fluorescence intensity of the hydrolysate with that of authentic solution, the concentration of 2-amino-1,8-naphthyridine in the hydrolysate was about 37 μM (in 750 μL, 27 nmol), indicating the overall yield for the covalent bond formation and hydrolysis to be 17%. This yield is in a good agreement with the oligomer consumption (18%) obtained by HPLC with internal standard of dT (cf. Figure 1a). By the same procedure of unmasking, hydrolysate of **2·2-CGC** prepared from 44 nmol of **CGC/G_G** and **2** showed a fluorescence of 2-amino-1,8-naphthyridine with a concentration of 21 μM (in 700 μL, 15 nmol). The overall yield for the labeling of **CGC/G_G** and unmasking was about 17%.

The affinity labeling targeting the guanine bulge is a conceptually novel method for the postsynthetic labeling of DNA. Two examples described here showed the labeling of the guanine bulge-containing strand. Hybridization of a probe DNA possessing one extra guanine especially between two cytosines to the target sequence provides a unique site for the labeling by masked fluorophore **2**.[17] Because synthesis of fluorescent-labeled oligomers is a bottleneck in reducing the cost of most genetic typing, the technique we described here may have broad application in the genetic typing without using a conventional synthesis of fluorescent-labeled DNA oligomers.

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Supporting Information Available: Details for the synthesis of **2**, HPLC profiles for the labeling reaction of **TXT/A_A** and **CXC/G_G** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(14) A proposed reaction scheme involving a nucleophilic addition of the secondary amino nitrogen to C8 of the alkylated guanine for the isomerization of **2**−**TGT** to **2**−**TGTx** is shown in Scheme S2.
(15) N7 of the guanine directly hydrogen bonded to **2** was found not to be nucleophilic to the epoxide. This is most likely due to an electron-withdrawing effect of naphthyridine chromophore. HOMO energies of guanine hydrogen-bonded to cytosine and **2** calculated at B3LYP/6-31G(d) are −4.82 and −5.11 eV, respectively, showing a considerable lowering of the HOMO energy level upon **2**-binding.
(16) It was confirmed separately that 2-amino-1,8-naphthyridine was stable under the conditions, and hydrolysate of **TGT** under the conditions did not produce any fluorescence at 396 nm.
(17) The use of an excess amount of **2** or hybridization probe results in an increase of the background level of the labeling reaction.

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