Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 335–337

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Specific Binding of 2-Amino-1,8-naphthyridine into a Single Guanine Bulge as Evidenced by Photooxidation of GG Doublet

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

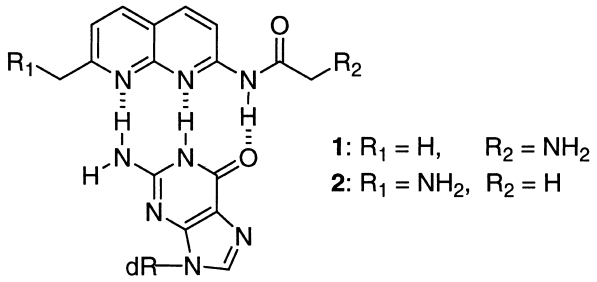
Department of Synthetic Chemistry and Biological Chemistry, Faculty of Engineering, Kyoto University, CREST, Kyoto 606-8501, Japan

Received 27 September 2000; accepted 15 November 2000

Abstract—Photoirradiation of 2-amino-1,8-naphthyridines in the presence of duplex DNA containing the GG doublet opposite a single bulge was examined. After hot piperidine treatment, DNA cleavage was observed preferentially at the GG opposite a single bulge. The cleavage efficiency was highly dependent on the nature of bulged base. The G cleavage at the GG opposite a single G bulge was exceptionally weak, suggesting an intercalative binding of 2-amino-1,8-naphthyridine chromophore into the GG step.

Accurate recognition of bulged bases that is indispensable for sensitive detection of genetic defects becomes increasingly important for post-sequence studies of the human genome.[1] DNA intercalators are known to bind preferentially to bulges as evidenced by specific cleavage at the site of binding.[2,3] We have succeeded in a highly selective alkylation of guanines (Gs) opposite a single bulge by an aglycon model for altromycin B.[4] We recently demonstrated that photoexcited riboflavin selectively oxidizes GG doublet opposite a bulge irrespective of the nature of bulged base.[5] However, the molecular design of a ligand that differentiates bulged bases is extremely difficult due to the lack of information for precise bulge structures. Very recently, we reported that the 2-amino-1,8-naphthyridine, which possesses hydrogen bonding groups fully complementary to G base and a planar bicyclic ring structure for binding, binds selectively to a single G bulge in 5′-d(C*G*C)-3′/5′-d(G_G)-3′ sequence.[5] We have proposed a structure of the complex where the naphthyridine intercalates into the GG step opposite a bulge and forms hydrogen bonds with the bulged G base (Fig. 1).

2-Amino-1,8-naphthyridine chromophore is known to oxidize G base under photoirradiation conditions via one electron transfer pathway.[6] It is therefore interesting from structural viewpoints to know whether the GG doublet opposite bulge is oxidized by the naphthyridine chromophore under photoirradiation conditions, since the oxidation efficiency of the GG doublet seems to be highly dependent on the stacking of two Gs.[7] Intercalation into the GG step would collapse the stacking of the GG doublet to result in an increase of the oxidation potential. We herein report that the GG doublet opposite a single G bulge is surprisingly inert to one electron photooxidation by 2-(2-aminoacetyl)amino-7-methyl-1,8-naphthyridine **1**, whereas strong cleavages at GGs opposite other bulges were clearly observed, supporting the proposed binding mechanism of **1** with a G bulge as shown in Figure 1.



For photocleavage studies, we used two isomeric naphthyridine derivatives **1** and **2**,[9] which differ only in the position of the primary amino group. A 29-mer **ODN1** 5′-d(CAC TAA CAA GCA TAT CCA **GG**C AAC TTA CC)-3′ was ^{32}P-5′-end-labeled and annealed with a 30-mer complementary strand **ODN2** 5′-d(GGT AAG TTG

*Corresponding author. Tel.: +81-75-753-4813; fax: +81-75-753-5676; e-mail: nakatani@sbchem.kyoto-u.ac.jp

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PII: S0960-894X(00)00667-3

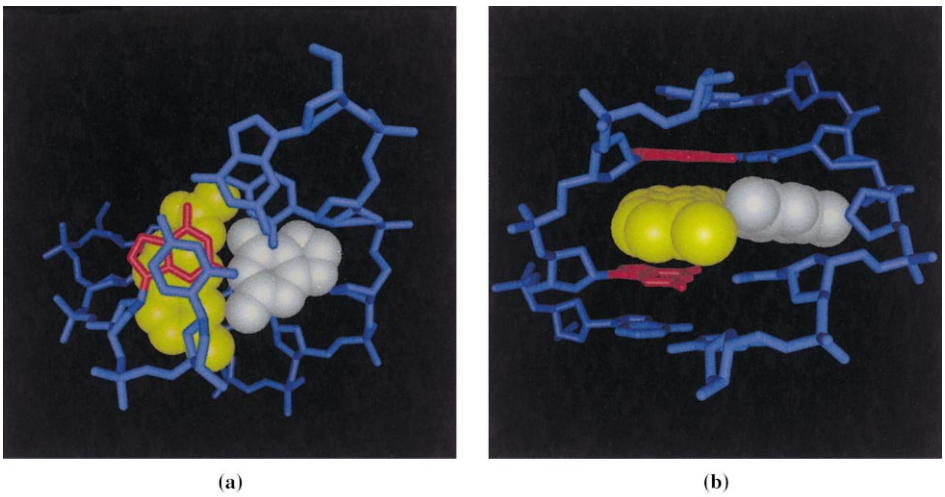

Figure 1. Proposed structure of the complex between the G bulge and 2-acetylamino-7-methyl-1,8-naphthyridine. Molecular modeling simulation was carried out with MacroModel (ver. 6.0) with Amber* force field. Initial structure of the complex was obtained by manually inserting 2-acetylamino-7-methyl-1,8-naphthyridine into the G bulge of duplex 5′-d(GC*G*CT)-3′/3′-d(CG_GA)-5′. Energy minimization was done for the initial structure of the complex with GB-SA treatment of water. Complexes viewed from the (a) top and the (b) minor groove are shown. Bulged G and flanking Gs are colored white and red, respectively. 2-Acetylamino-7-methyl-1,8-naphthyridine is colored yellow.

C*N*C T*GG* ATA TGC TTG TTA GTG)-3′, where *N* is a bulged base of A, C, G, or T, to produce a duplex containing a GG doublet (shown in a bold face) opposite a single nucleotide bulge. The duplex also contains a normal GG step as an internal standard on **ODN2** (shown in italic). Naphthyridine **1** or **2** was photoirradiated at 312 nm in the presence of the duplex, and then DNA cleavage was analyzed by PAGE after hot piperidine treatment (Fig. 2).

In the presence of naphthyridine **2**, strong cleavage occurred selectively at the GG opposite the bulge. We confirmed by a separate experiment using ^{32}P-5′-end-labeled **ODN2** that the oxidation of normal GG did not occur under the same conditions. Selective oxidation of the GG opposite the bulged site was fully consistent with the results of photooxidation with riboflavin,[5] showing that **2** binds preferentially to the bulged site. The GG cleavage efficiency was very similar for G, A, and T bulges, whereas somewhat weaker cleavage was observed for C bulge. In sharp contrast, the oxidation by **1** under the same conditions was highly dependent on a property of bulged base. While strong cleavage bands were detected for the GG doublets opposite A and T bulges, the oxidation was completely suppressed for the GG doublet opposite a G bulge. Oxidation of the GG doublet opposite a C bulge was moderately suppressed under the conditions.

We have already reported that the 2-amino-1,8-naphthyridine selectively increases the thermal stability of G bulge-containing duplex.[5] In order to compare the capability of the GG photooxidation with bulge stabilization by **1** and **2**, we examined the thermal stability of bulge-containing duplex in the presence of **1** and **2**. The thermal stability was examined by measuring the melting temperature (T_m) of oligomer duplex 5′-d(TCCA-G_GCAAC)-3′/3′-d(AGGTC*N*CGTTG)-5′ containing a single nucleotide bulge (N = A, C, G, or T) in the presence of drug (**1** or **2**) according to the previously reported procedure (Table 1).[5]

Increase of T_m (ΔT_m) in the presence of **2** (100 μM) was only 0.4 °C for the G bulge-containing duplex, showing a drastic contrast to ΔT_m of 4.9 °C in the presence of **1**. In the cases of A and T bulges, both **1** and **2** showed a little increase of T_m by 0.2–0.6 °C, whereas no T_m increase was observed for normal duplex. In addition, a weak but distinct increase of T_m of C bulge-containing duplex was observed in the presence of both **1** (ΔT_m = 1.8 °C) and **2** (0.6 °C).

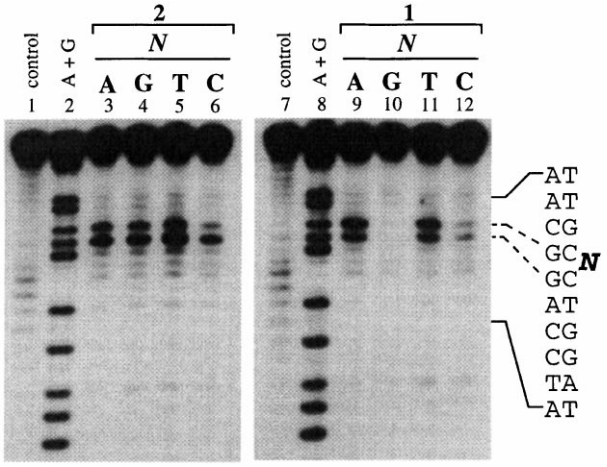

Figure 2. Cleavage of ^{32}P-5′-end-labeled 29-mer duplexes containing A, G, T, and C bulges by photoirradiation of 2-amino-7-methyl-1,8-naphthyridines.[8] Lanes 1 and 7, photoirradiated duplex containing the G bulge in the absence of drug; lanes 2 and 8, Maxam-Gilbert A + G sequencing reaction; lanes 3–6, duplex containing A, G, T, and C bulges under photoirradiation of **2**, respectively; lanes 9–12, duplex containing A, T, G, and C bulges under photoirradiation of **1**, respectively. Target bulged sites are shown on the right of the gel.

Table 1. Melting temperature (T_m) of bulge-containing duplexes in the presence and absence of drug[a]

Duplex[b]	$T_{m(-)}$	**1**[c]		**2**[c]	
		$T_{m(+)}$	ΔT_m (°C)	$T_{m(+)}$	ΔT_m (°C)
5′-TCCAG_GCAAC-3′ 3′-AGGTC**G**CGTTG-5′	32.3	37.2	4.9	32.7	0.4
5′-TCCAG_GCAAC-3′ 3′-AGGTC**C**CGTTG-5′	33.7	35.5	1.8	34.3	0.6
5′-TCCAG_GCAAC-3′ 3′-AGGTC**A**CGTTG-5′	32.6	32.8	0.2	33.0	0.4
5′-TCCAG_GCAAC-3′ 3′-AGGTC**T**CGTTG-5′	31.2	31.8	0.6	31.6	0.4
5′-TCCAGGCAAC-3′ 3′-AGGTCCGTTG-5′	46.2	46.2	0.0	46.2	0.0

[a]The UV-melting curve was measured at a total base concentration of 100 μM in 10 mM sodium cacodylate buffer (pH 7.0) containing 0.1 M NaCl. Melting temperature in the absence ($T_{m(-)}$) or presence ($T_{m(+)}$) of the drug was calculated as the maximum in a plot of $\Delta Abs_{260}/\Delta T$ versus temperature. Temperature was increased at a rate of 1 °C/min.
[b]Bulged bases are shown in bold face.
[c]Drug concentration was 100 μM.

These thermal denaturation studies indicate that a very stable complex is formed specifically between **1** and G bulge-containing duplex. Suppression of photooxidation of the GG doublet opposite a bulged G strongly suggests that the two Gs are no more stacked each other in the complex formed with **1**. While the exact structure of G bulge-**1** complex is not yet determined, our proposed structure of the complex where **1** intercalates into the GG step making three hydrogen bonds with the bulged G (Fig. 1) well rationalizes the suppression of the GG photooxidation. In addition, both photooxidation and thermal denaturation studies indicate that the binding of **1** to the duplex containing a G bulge is much more efficient than that of **2**, although the structural difference between **1** and **2** is only the position of an amino substituent. The marked difference of the G bulge recognition may implicate the presence of a extra hydrogen bond between the amino group of **1** and carbonyl group of bulged G in addition to normal Watson–Crick type hydrogen bonds.

An important insight into C bulge recognition was also obtained in this study. Both **1** and **2** weakly stabilized the C bulge-containing duplex and moderately suppressed the oxidation of the GG doublet opposite a C bulge. In this case, it is rather difficult to estimate the structure of the naphthyridine–C bulge complex, since in the 5′-d(G_G)-3′/3′-d(C$\underline{C}$C)-5′ sequence three cytosines compete for base pairing with two complementary guanines to result in a formation of three alternative C bulge structures. Nevertheless, the results strongly suggest that both **1** and **2** bind to the C bulge, although the binding is not so strong as that observed for **1** to the G bulge. The present study provides an important insight into the molecular design of a new C bulge specific ligand.

References and Notes

1. For a recent report on bulge detection using array-based hybridization assay, see: Favis, R.; Day, J. P.; Gerry, N. P.; Phelan, C.; Narod, S.; Barany, F. *Nature Biotechnol.* **2000**, *18*, 561.
2. (a) Williams, L. D.; Goldberg, I. H. *Biochemistry* **1988**, *27*, 3004. (b) Kappen, L. S.; Goldberg, I. H. *Biochemistry* **1997**, *36*, 14861. (c) Stassinopoulos, A.; Gao, J. J.; Goldberg, I. H. *Science* **1996**, *272*, 1943. (d) Kappen, L. S.; Goldberg, I. H. *Biochemistry* **1995**, *34*, 5997. (e) Kappen, L. S.; Goldberg, I. H. *Science* **1993**, *261*, 1319.
3. (a) For recent reports on synthetic ligands that selectively cleave DNA near a bulge, see: Cheng, C.-C.; Kuo, Y.-N.; Chuang, K.-S.; Luo, C.-F.; Wang, W. J. *Angew. Chem., Int. Ed.* **1999**, *38*, 1255. (b) Shin, H.-C.; Tang, N.; Burrows, C. J.; Rokita, S. E. *J. Am. Chem. Soc.* **1998**, *120*, 3284.
4. Nakatani, K.; Okamoto, A.; Saito, I. *Angew. Chem., Int. Ed.* **1999**, *38*, 3378.
5. Nakatani, K.; Sando, S.; Saito, I. *J. Am. Chem. Soc.* **2000**, *122*, 2172.
6. Nakatani, K.; Sando, S.; Yoshida, K.; Saito, I. *Tetrahedron Lett.* **1999**, *40*, 6029.
7. (a) Part, F.; Houk, K.; Foote, C. S. *J. Am. Chem. Soc.* **1998**, *120*, 845. (b) Sugiyama, H.; Saito, I. *J. Am. Chem. Soc.* **1996**, *118*, 7063.
8. ^{32}P-5′-end-labeled ODN 5′-d(CAC TAA CAA GCA TAT CCA GGC AAC TTA CC)-3′ was annealed with a complementary strand 5′-d(GGT AAG TTG C*N*C TGG ATA TGC TTG TTA GTG)-3′ (bulged base N = A, C, G, or T) in sodium cacodylate buffer (10 mM, pH 7.0) containing calf thymus DNA (10 μM base pair concentration) and NaCl (100 mM). The solution of the duplex was photoirradiated at 312 nm for 1 h in the presence of either **1** or **2** (100 μM each) at 0 °C. Recovered ODN by ethanol precipitation was analyzed by electrophoresis on denaturing sequencing gel containing 15% polyacrylamide and 7 M urea after piperidine treatment.
9. Kelly, T. R.; Bridger, G. J.; Zhao, C. *J. Am. Chem. Soc.* **1990**, *112*, 8024.