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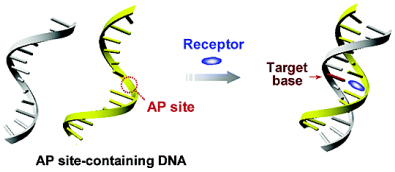
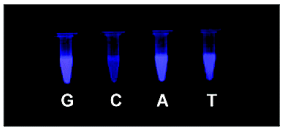
J. Am. Chem. Soc., **2003**, 125 (30), 8982-8983• DOI: 10.1021/ja029786m • Publication Date (Web): 04 July 2003





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Use of Abasic Site-Containing DNA Strands for Nucleobase Recognition in Water

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Recognition of molecules related to nucleic acids has attracted much attention the last few decades. Because hydrogen bonds are essential for complementary base-pairing in a biological system, many artificial ligands have been reported based on hydrogen-bonding motifs.[1,2] While excellent functions of these ligands have been demonstrated in organic media,[2] it is still a challenging task to use them in aqueous media because of significant interference from hydration of the binding site. On the other hand, Nakatani et al. recently reported that two synthetic hydrogen-bonding ligands can recognize a bulged base[3] and mismatch bases[4] in DNA duplexes, indicating that DNA and RNA double helixes provide unique microenvironments that allow abiotic hydrogen-bonding ligands to interact efficiently with an intrahelical nucleobase even in aqueous media.

Here, we propose a new strategy for nucleobase recognition in aqueous media, which is based on the construction of an abasic site (AP site) in DNA duplexes. As is schematically illustrated in Figure 1, an AP site-containing DNA strand is hybridized with a normal DNA strand so as to place the AP site opposite from, but facing toward, a target nucleobase, by which hydrophobic microenvironments are provided for ligands to recognize nucleobases through hydrogen bonding. Naturally occurring AP sites are one of the most common forms of DNA damage and are also produced in vivo as intermediates in the base excision repair pathway of damaged or modified bases.[5] In this study, a tetrahydrofuranyl residue (dSpacer) which lacks a nucleobase moiety is utilized for the design of an AP site and is incorporated with 11-mer oligodeoxynucleotides (5′-TCCAGXGCAAC-3′, X = dSpacer, A = adenine, C = cytosine, G = guanine, T = thymine). As a hydrogen-bonding ligand, we use naphthyridine and quinoline derivatives that have two or three hydrogen-bonding groups suitable for the nucleobase recognition. From the binding studies by melting temperature (T_m) measurements, 2-amino-7-methylnaphthyridine (AMND) is found to very strongly recognize target nucleobases at the AP site via the formation of hydrogen bonds.[6] Furthermore, a stacking interaction with nucleobases flanking the AP site significantly controls the binding properties of AMND. Potential use of the AP site is demonstrated as the field of nucleobase recognition to achieve unique functions of hydrogen-bonding ligands in aqueous media.

Table 1 shows the thermal stability of AP site-containing duplexes[7−9] (X = dSpacer and Y = A, C, G, T) in the presence and absence of AMND, which was assessed by determination of T_m. While hardly any changes in T_m are observed for normal duplexes,[6] significant increases in the melting temperature of AP site-containing duplexes are observed upon addition of AMND. The increase in T_m strongly depends on nucleobases opposite from the AP site and is in the order of C > T > G > A. In addition, no evidence for the interaction between AMND and normal duplexes was obtained in circular dichroism (CD) and fluorescence experiments. Apparently, AMND is incorporated into the AP site,

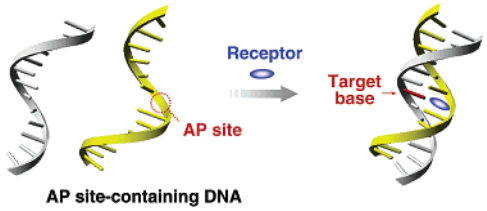
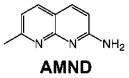


Figure 1. Schematic illustration of nucleobase recognition in water using an abasic site (AP site)-containing DNA strand.

Table 1. Melting Temperature (T_m) of AP Site-Containing Duplexes in the Presence ($T_{m(+)}$) and Absence ($T_{m(-)}$) of AMND[a]

5′-TCCAG**X**GCAAC-3′
3′-AGGTC**Y**CGTTG-5′
X= 



AMND

Y	$T_{m(-)}$ [°C]	$T_{m(+)}$ (ΔT_m) [°C]
G	34.9	39.8 (+ 4.9)
C	31.3	45.0 (+ 13.7)
A	34.8	37.8 (+ 3.0)
T	30.2	41.4 (+ 11.2)

[a] [DNA duplex], 30 μM; [AMND], 580 μM; [NaCl], 100 mM; [sodium cacodylate buffer], 10 mM; [EDTA], 1 mM, at pH 7.0; light-path length, 1 mm.

followed by binding with nucleobases at the AP site. It is interesting to note that AMND shows the selectivity for C (ΔT_m = 13.7) over G (ΔT_m = 4.9) at the AP site, despite the hydrogen-bonding array of AMND being fully complementary to G. The selectivity for C over G is also observed in CD spectra of the AP site-containing duplexes, where the C-containing duplex shows the most noticeable changes upon addition of AMND.[6] It therefore seems that at the AP site another type of interaction rather than hydrogen bonding is also responsible for the binding selectivity of AMND. Indeed, determination of the complex stability between AMND and C indicates the significant role of stacking of AMND with nucleobases flanking the AP site. As is shown in Figure 2, AMND shows significant fluorescence quenching upon binding with the AP site-containing duplex (the base opposite from the AP site is C), in which a distinct titration endpoint is observed at a 1:1 ratio of [DNA duplex]/[AMND]. Surprisingly, the 1:1 binding constant, as calculated by the titration curve,[6] is greater than 10^6 M^{-1} in water even though the formation of only two hydrogen bonds is possible for the complexation of AMND with C. Therefore, AMND should bind to C in a cooperative fashion, that is, hydrogen bonding with C and stacking with nucleobases flanking the AP site.

Molecular modeling simulation supports the above consideration about the significant role the stacking effect plays. Figure 3 shows an energy-minimized model of the 1:1 complex between AMND and the duplex with C opposite from an AP site (5′-TCCAG**X**GCAAC-3′/5′-GTTGC**C**CTGGA-3′, X = dSpacer). As shown in

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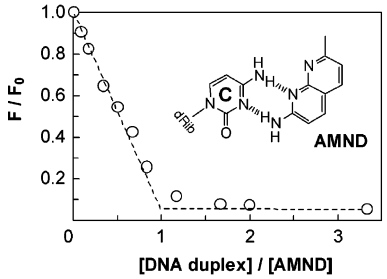

Figure 2. Fluorescence intensity of AMND at 405 nm in a titration with the AP site-containing duplex (5′-TCCAGXGCAAC-3′/5′-GTTGCCCT GGA-3′, X = dSpacer) in water. *F* and F_0 denote the fluorescence of AMND with and without DNA duplexes, respectively. [AMND], 30 μM; [NaCl], 100 mM; [sodium cacodylate buffer], 10 mM; [EDTA], 1 mM, at pH 7.0; excitation wavelength, 355 nm.

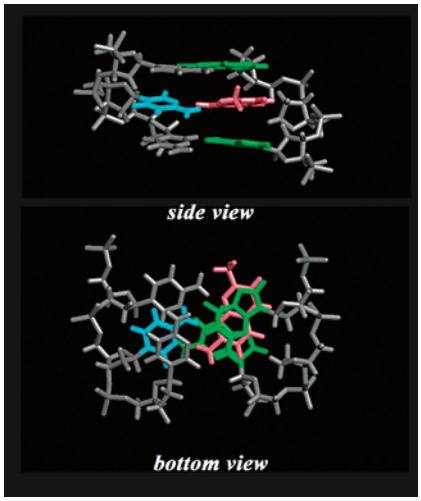

Figure 3. Energy-minimized structure, obtained using MacroModel (version 7.2), for the complex between AMND and the AP site-containing duplex with the cytosine base opposite from the AP site (5′-TCCAGXGCAAC-3′/5′-GTTGCCCTGGA-3′, X = dSpacer). AMND and the cytosine base opposite from an AP site are colored red and blue, respectively. The guanine bases flanking the AP site are colored green.

Figure 3, AMND is located at the AP site, where the formation of two hydrogen bonds is evident between AMND and C. Furthermore, AMND extensively stacks with two G's flanking the AP site. Similarly, in the energy-minimized structure of the complex between AMND and T, effective stacking with two G's flanking the AP site is also seen.[6] By contrast, probably due to a steric effect, such effective stacking is not obtained for the complex between AMND and purine bases (A and G), although hydrogen bonds are formed with these bases.[6] This molecular modeling study clearly indicates the importance of the stacking interaction in the binding event at the AP site, in accordance with the appearance of the selectivity for C as is observed by CD, fluorescence, and T_m measurements.

Preliminary experiments reveal that the quenching of AMND emission can be successfully utilized for selective, visible detection of single-base alternation related to C, as shown in Figure 4, and is applicable to the analysis of the C/G mutation sequences (P177R) of the cancer repression gene p53.[6]

In summary, we have achieved nucleobase recognition by the hydrogen-bonding ligand AMND in water, using the AP site as the field of molecular recognition. In addition to the hydrogen bonding, stacking with the nucleobases flanking the AP site plays an important role in the complexation of AMND at the AP site, which is indeed responsible for the binding stability and selectivity.

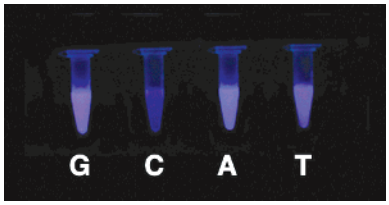

Figure 4. Changes in fluorescence of AMND in the presence of AP site-containing duplexes (5′-TCCAG**X**GCAAC-3′/5′-GTTGC**Y**CTGGA −3′, X = dSpacer, left to right: Y = G, C, A, and T) in water. The samples are excited with a UV lamp at 302 nm. [DNA duplex], 60 μM; [AMND], 30 μM; [NaCl], 100 mM; [sodium cacodylate buffer], 10 mM; [EDTA], 1 mM, at pH 7.0.

Although further studies are required for the effect of the neighboring bases on the binding behaviors of AMND, and also for the design of more sophisticated ligands for use at the AP site, our approach, utilizing an AP site-containing DNA strand, is expected to develop a ligand-based detection method for single-nucleotide polymorphisms (SNPs).

Acknowledgment. This work was partially supported by Grants-in-Aid for Scientific Research (B), No. 13554029, and Encouragement of Young Scientist, No. 13740417, from the Ministry of Education, Culture, Sports, Science and Technology, Japan. RFTF and the Japan-China Cooperative Research Program from the Japan Society for the Promotion of Science (JSPS) also provided support.

Supporting Information Available: Melting temperatures of normal duplexes, fluorescence spectra of AMND, CD spectra of AP site-containing duplexes, fitting procedures on binding constant calculation, and the energy-minimization structures of the complex between AMND and AP site-containing duplexes (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Král, V.; Sessler, L. J.; Furuta, H. *J. Am. Chem. Soc.* **1992**, *114*, 8704−8705. (b) Deslongchamps, G.; Galán, A.; Mendoza, d. J.; Rebek, J., Jr. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 61−63. (c) Rotrllo, M. V.; Vianik, A. E.; Deslongchamps, G.; Murray, A. B.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1993**, *115*, 797−798. (d) Kato, U.; Conn, M. M.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1994**, *116*, 3279−3284.

(2) (a) Inouye, M.; Kim, K.; Kitao, T. *J. Am. Chem. Soc.* **1992**, *114*, 778−780. (b) Abe, M.; Otsuki, J.; Araki, K. *Chem. Lett.* **1993**, 1541−1544. (c) Murray, J. T.; Zimmerman, C. S. *Tetrahedron Lett.* **1995**, *36*, 7627−7630. (d) Bell, W. T.; Hou, Z.; Zimmerman, C. S.; Thiessen, A. P. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 2163−2165. (e) Amemiya, S.; Buhlman, P.; Umezawa, Y. *Chem. Commun.* **1997**, 1027−1028. (f) Cho, L. Y.; Jeong, K.-S. *Tetrahedron Lett.* **1997**, *38*, 8337−8340. (g) Inouye, M.; Hyodo, Y.; Nakazumi, H. *J. Org. Chem.* **1999**, *64*, 2704−2710. (h) Inouye, M.; Itoh, M.; Nakazumi, H. *J. Org. Chem.* **1999**, *64*, 9393−9398. (i) Inouye, M.; Takase, M. *Angew. Chem., Int. Ed.* **2001**, *40*, 1746−1748.

(3) (a) Nakatani, K.; Sando, S.; Saito, I. *J. Am. Chem. Soc.* **2000**, *122*, 2172−2177. (b) Nakatani, K.; Sando, S.; Yoshida, K.; Saito, I. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 335−337.

(4) (a) Nakatani, K.; Sando, S.; Kumasawa, H.; Kikuchi, J.; Saito, I. *J. Am. Chem. Soc.* **2001**, *123*, 12650−12657. (b) Nakatani, K.; Sando, S.; Saito, I. *Bioorg. Med. Chem.* **2001**, *9*, 2381−2385. (c) Nakatani, K.; Sando, S.; Saito, I. *Nat. Biotechnol.* **2001**, *19*, 51−55.

(5) Demole, B.; Harrison, L. *Annu. Rev. Biochem.* **1994**, *63*, 915−948.

(6) Experimental results and fitting procedures are described in the Supporting Information.

(7) For reports on the effects of the AP site on the duplex stability and conformation: (a) Ide, H.; Shimizu, H.; Kimura, Y.; Sakamoto, S.; Makino, K.; Glackin, M.; Wallace, S. S.; Nakamura, H.; Sasaki, M.; Sugimoto, N. *Biochemistry* **1995**, *34*, 6967−6955. (b) Begar, D. R.; Bolton, H. P. *J. Biol. Chem.* **1998**, *273*, 15565−15573.

(8) For reports on the effects of base sequence on the AP site-containing duplex stability: (a) Sagi, J.; Hang, B.; Singer, B. *Chem. Res. Toxicol.* **1999**, *12*, 917−923. (b) Gelfand, A. C.; Plum, G. E.; Grollman, A. P.; Johnson, F.; Breslauer, K. J. *Biochemistry* **1998**, *37*, 7321−7327.

(9) For a report on the effects of neighbor bases at the AP site on the duplex stability and conformation: Sagi, J.; Guliaev, B. A.; Singer, B. *Biochemistry* **2001**, *40*, 3859−3868.

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