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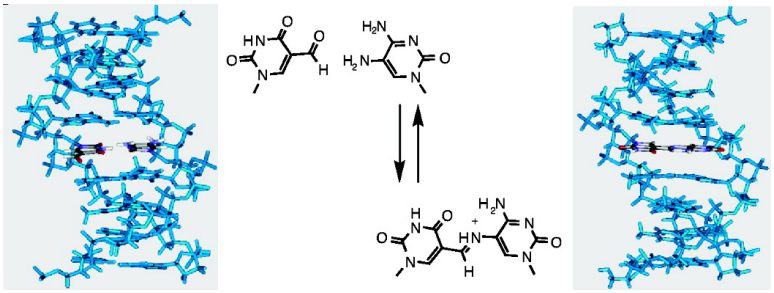

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Stable, Specific, and Reversible Base Pairing via Schiff Base

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Abstract: We here present a novel covalently linked base pair via Schiff base formation between 5-formyluracil (^{f}U) and 5-aminocytosine (^{Am}C). Formation of the Schiff base linkage proceeds reversibly and does not require any additives. The cross-linked DNA is very stable under denaturing conditions, whereas it completely dissociates upon heating at 90 °C. The pairing ability of ^{Am}C and ^{f}U is very specific and is applicable to the detection of ^{f}U, which is the major oxidative lesion of T in DNA. We propose the Schiff base linkage as a new artificial base pairing scheme to create functional DNAs.

Introduction

DNA consists of two polynucleotide chains held together by specific hydrogen bonds between the complementary bases, adenine and thymine (A:T), or guanine and cytosine (G:C). This specific base pairing underpins the essential function and structure of DNA. Construction of artificial DNA possessing non-hydrogen-bonding base pairs will add new functions to the DNA molecule. During the past decade, much effort has been devoted to developing artificial base pairs based not only on hydrogen bonding but also on hydrophobic interactions,[1] metal coordination,[2] or disulfide bonding.[3] Although there are many examples of covalently cross-linked base pairs,[4] the disulfide bonded base pairs are unique in terms of reversibility. The disulfide-linkage can be cleaved in the presence of reducing reagents such as dithiothreitol and mercaptoethanol.[3] Here we focus on the imine bond (Schiff base) as a reversible covalent bond for a novel base-pairing scheme. Schiff base formation is reversible under physiological conditions without any additives and can be fixed permanently as the corresponding amine by reduction. Efficient imine formation requires two spatially arranged functional groups, a nucleophilic amino group and an electrophilic carbonyl group. For the carbonyl part, we selected 5-formyluracil (^{f}U), which is known to form Schiff bases with various primary amines, including lysine side chains.[5] Amino groups of natural nucleobases are inadequate for Schiff base formation with ^{f}U, because of their low reactivity or improper location. Thus, 5-aminocytosine (^{Am}C),[6] where one additional amino group is attached at the C5 position, was used as the amine part of the Schiff base. Here, we report specific, stable, and reversible cross-linking via Schiff base between ^{f}U and ^{Am}C (Scheme 1). 5-Formyluracil is also known as the major oxidative lesion of T in DNA, and it is known to be mutagenic because of the increased base mispair formation. We describe the application of the specific pairing ability of ^{Am}C to the detection of ^{f}U in DNA.

Results and Discussion

Although synthesis of 2′-deoxy-5-aminocytidine has been reported previously,[6a] its incorporation into DNA has not been established. However, it has been reported that ^{Am}C-containing oligodeoxynucleotides (ODNs) were obtained as side products of 5-halocytidine-containing ODNs during deprotection with ammonia.[6b] We synthesized ^{Am}C-containing ODNs using this chemistry. 5-Bromocytidine (^{Br}C) -containing ODN was treated with concentrated ammonia and was incubated at 60 °C for extended time periods. The ^{Br}C-containing ODN was converted

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(1) (a) Schweitzer, B. A.; Kool, E. T. *J. Org. Chem.* **1994**, *59*, 7288−7242. (b) Schweitzer, B. A.; Kool, E. T. *J. Am. Chem. Soc.* **1995**, *117*, 1863−1872. (c) Matray, T. J.; Kool, E. T. *J. Am. Chem. Soc.* **1998**, *120*, 6191−6192. (d) Lai, J. S.; Kool, E. T. *J. Am. Chem. Soc.* **2004**, *126*, 3040−3041. (e) McMinn, D. L.; Ogawa, A. K.; Wt, Y.; Liu, J.; Schultz, P. G.; Romesberg, F. E. *J. Am. Chem. Soc.* **1999**, *121*, 11585−11586. (f) Ogawa, A. K.; Wt, Y.; Berger, M.; Schultz, P. G.; Romesberg, F. E. *J. Am. Chem. Soc.* **2000**, *122*, 8803−8804. (g) Matsuda, S.; Romesberg, F. E. *J. Am. Chem. Soc.* **2004**, *126*, 14419−14427.
(2) (a) Tanaka, K.; Shionoya, M. *J. Org. Chem.* **1999**, *64*, 5002−5003. (b) Tanaka, K.; Yamada, Y.; Shionoya, M. *J. Am. Chem. Soc.* **2002**, *124*, 8802−8803. (c) Tanaka, K.; Tengeiji, A.; Kato, T.; Toyama, N.; Shionoya, M. *Science* **2003**, *299*, 1212−1213. (d) Meggers, E.; Holland, P. L.; Tolman, W. B.; Romesberg, F. E.; Schultz, P. G. *J. Am. Chem. Soc.* **2000**, *122*, 10714−10715. (e) Zimmerman, N.; Meggers, E.; Romesberg, F. E.; Schultz, P. G. *J. Am. Chem. Soc.* **2002**, *124*, 13684−13685. (g) Kuklenyik, Z.; Marzilli, L. G. *Inorg. Chem.* **1996**, *35*, 5654−5662.
(3) (a) Ferentz, A. E.; Verdine, G. L. *J. Am. Chem. Soc.* **1991**, *113*, 4000−4002. (b) Wang, H.; Osborne, S. E.; Zuiderweg, E. R. P.; Glick, G. D. *J. Am. Chem. Soc.* **1994**, *116*, 5021−5022. (c) Hatano, A.; Makita, S.; Kihara, M. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2459−2462.
(4) (a) Devadas, B.; Leonard, N. J. *J. Am. Chem. Soc.* **1986**, *108*, 5012−5014. (b) Bhat, B.; Leonard, N. J.; Robinson, H.; Wang, A. H.-J. *J. Am. Chem. Soc.* **1996**, *118*, 10744−10751. (c) Nagatsugi, F.; Kawasaki, T.; Usui, D.; Maeda, M.; Sasaki, S. *J. Am. Chem. Soc.* **1999**, *121*, 6753−6754. (d) Qiao, X.; Kishi, Y. *Angew.* Chem. Int. Ed. **1999**, *38*, 928−931. (e) Li, H.-Y.; Qiu, Y.-L.; Kishi, Y. *ChemBioChem* **2001**, *2*, 371−374.
(5) (a) Ono, A.; Okamoto, T.; Inada, M.; Nara, H.; Matsuda, A. *Chem. Pharm. Bull.* **1994**, *42*, 2231−2237. (b) Kittaka, A.; Horii, C.; Kuze, T.; Asakura, T.; Ito, K.; Nakamura, K. T.; Miyasaka, T.; Inoue, J. *Synlett*, **1999**, 869−872. (c) Sugiyama, T.; Kittaka, A.; Takayama, H.; Tomioka, M.; Ida, Y.; Kuroda, R. *Bioorg. Med. Chem.* Lett. **2003**. *13*. 2847−2851.
(6) (a) Goldman, D.; Kalman, T. I. *Nucleosides Nucleotides* **1983**, *2*, 175−187. (b) Ferrer, E.; Wiersma, M.; Kazimierczak, B.; Müller, C. W.; Eritja, R. *Bioconjugate Chem.* **1997**, *8*, 757−761.

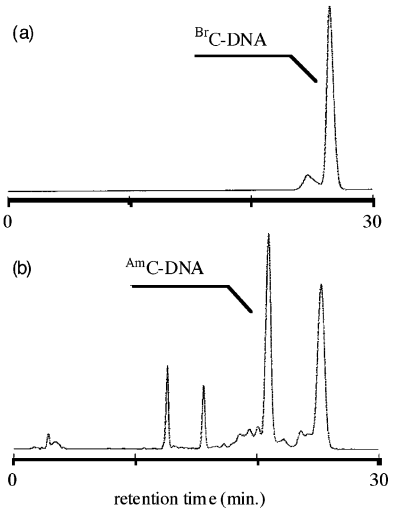


Figure 1. Synthesis of ^{Am}C-containing ODN. ^{Br}C-containing ODN was converted to the corresponding ^{Am}C-containing ODN by treatment with concentrated ammonia at 60 °C for 48 h. HPLC charts before (a) and after (b) ammonia treatment are shown. The new peak eluted that with a shorter retention time is the ^{Am}C-containing ODN.

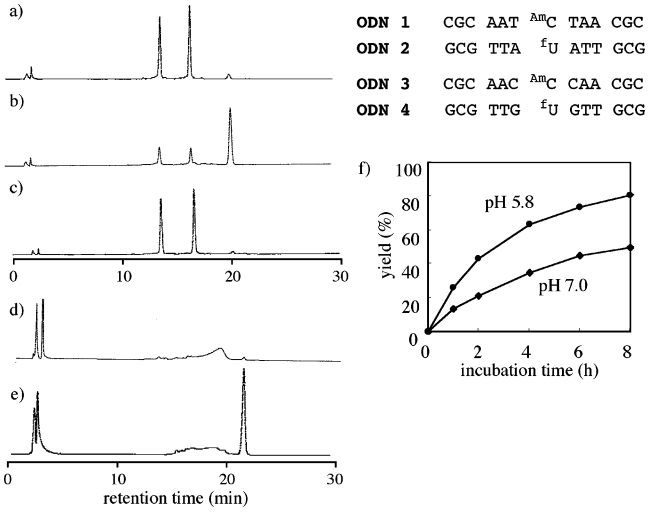


Figure 2. HPLC profiles of the duplexes **1**/**2** (a−c) and **3**/**4** (d, e) in 50 mM Na·phosphate (pH 5.8), 100 mM NaCl: (a) before incubation, (b) after incubation at 30 °C for 8 h, (c) after heating at 90 °C for 3 min, (d) before incubation, and (e) after incubation at 30 °C for 5 h. (f) Time course of cross-linked duplex **1**/**2**.

to the corresponding ^{Am}C-containing ODN by an addition−substitution−elimination reaction. The reaction was monitored by HPLC, showing that the ^{Am}C-containing ODN was eluted faster than the original ^{Br}C-containing ODN (Figure 1), and the desired product was obtained in moderate yield (∼50%). To examine cross-linking via Schiff base formation, ^{Am}C-containing ODN **1** was hybridized with its complementary ODN **2** in which ^{f}U was positioned opposite ^{Am}C. The synthesis of ^{f}U-containing ODNs was accomplished by a postsynthetic modification of ODN containing 5-(1,2-dihydroxyethyl)uridine with $NaIO_4$ according to a previously reported procedure.[7]

Schiff base formation between the paired ^{Am}C and ^{f}U was monitored by HPLC. Upon addition of the complementary ODN **2** to the ^{Am}C-containing ODN **1**, a new peak with slower mobility than that of the two starting ODNs was formed (Figure 2b). The new product was treated with $NaBH_3CN$ to fix the cross-link,[8] and the resulting product was then subjected to MALDI-TOF mass spectrometry. The observed mass (calculated mass 7897.2; found 7898.2) agreed with the cross-linked duplex in which the imine residue was reduced to the corresponding amine. We next examined the effect of sequence contexts around the cross-linking site on the Schiff base formation. ODN duplex **3**/**4** contains two G:C base pairs proximal to the unnatural base pair. As shown in Figure 2e, similar results were found for the duplex **3**/**4**,[9] and thus the cross-link formation was little affected by neighboring sequences. The time course of the cross-linking for the duplexes is shown in Figure 2f. The cross-linking proceeded faster at pH 5.8 (50% yield after 3 h) than at pH 7.0 (50% yield after 8 h). Because imine formation generally takes place faster at a slightly acidic pH, this pH dependence also supports the cross-linking via Schiff base formation.

The cross-linking can be analyzed by denaturing polyacrylamide gel electrophoresis (PAGE), since the Schiff base linkage is stable under the denaturing conditions. Among the various

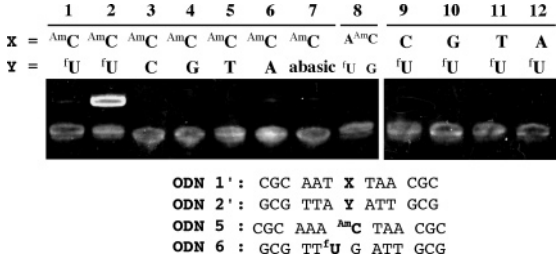


Figure 3. Electrophoretic mobility shift assay to monitor Schiff base formation. ODN duplexes **1′**/**2′** with various combinations of base pairs were incubated at 30 °C for 20 h in 50 mM Na phosphate (pH 7.0) and 100 mM NaCl. Samples were subjected to electrophoresis through 20% polyacrylamide containing 7 M urea gel and stained with SYBR Green I nucleic acid gel stain. Lane 1, without incubation; lane 8, ODN **5**/**6**.

combinations of base pairs we tested, only the ^{Am}C:^{f}U base pair produced a higher molecular weight band corresponding to the cross-linked duplex (Figure 3). Thus, all amino groups in A, G, and C are inactive toward Schiff base formation with the formyl group of the opposing ^{f}U (lanes 9−12), probably due to their low reactivity or improper location. Since 5-aminouracil-containing DNA also produced a similar cross-linked product (data not shown), the amino group at the C5 position of pyrimidine base would be suited for the Schiff base formation with the ^{f}U. The cross-linked band completely disappeared when ^{f}U was moved one base away from the position opposite ^{Am}C (lane 8). Schiff base formation is specific for duplexes with the paired ^{Am}C and ^{f}U.

Thermal denaturing experiments were performed to examine the stability of the cross-link (Figure 4a). As expected, the stability of the duplex **1**/**2** was dramatically increased after incubation of the duplex. Schiff base formation results in an increase by 31 °C in the melting temperature (T_m) of the duplex. The T_m value is 20 °C higher even than that of the natural duplex containing the C:G base pair. It is also noteworthy that, after denaturing of the cross-linked duplex by heating at 90 °C for 3 min, the thermal denaturing profile reverted to that before incubation. In this instance, the cross-linked duplex completely reverted to the original single-stranded ODNs **1** and **2** (Figure 2c). Similar results were obtained for the duplex **3**/**4**

(7) Sugiyama, H.; Matsuda, S.; Kino, K.; Zhang, Q.-M.; Yonei, S.; Saito, I. *Tetrahedron Lett.* **1996**, *37*, 9067−9070.
(8) Zhan, Z.-Y.; Lynn, D. G. *J. Am. Chem. Soc.* **1997**, *119*, 12420−12421.
(9) A broad peak in Figure 2d is attributed to the non-cross-linked duplex **3**/**4**. The duplex **3**/**4** (melting temperature = 49 °C) is not fully melted into the single-strand **3** and **4** under our HPLC conditions.

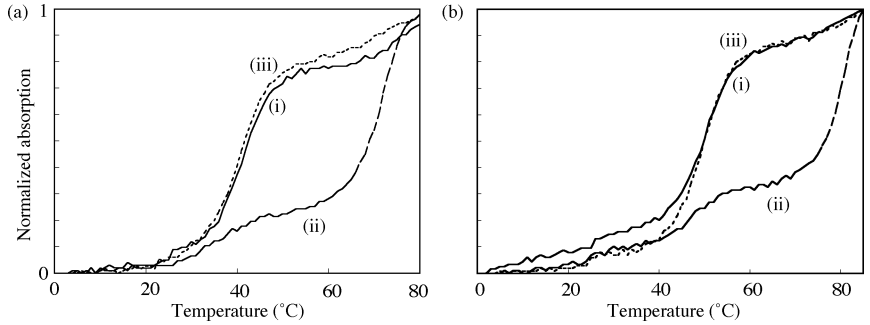
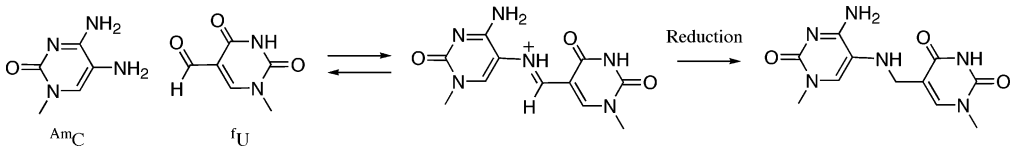


Figure 4. UV melting curves of the duplexes **1**/**2** (a) and **3**/**4** (b) (i) before and (ii) after incubation at 30 °C for 24 h. Experiments were performed in 50 mM Na·phosphate (pH 7.0), 100 mM NaCl. (iii) Melting profile of curve ii reverted to that of curve i after heating at 90 °C for 3 min.

Scheme 1



(Figure 4b). The thermal reversibility of the cross-link is easily understandable, because the Schiff base can undergo hydrolysis in aqueous solution to produce the corresponding amine and aldehyde.

To examine the structure of the base pairing, we performed a molecular modeling study of the duplex containing the Schiff base linkage (Figure 5). Molecular modeling simulation was carried out by using the MacroModel v8.1 software package and the AMBER* force field. In the optimized structure, introduction of a Schiff base linkage causes little distortion in the global structure of DNA. The Schiff base is buried in the helical stack and is poorly accessible to solvent, leading to a shift of the equilibrium toward Schiff base formation. Schiff base formation requires that both ^{Am}C and ^{f}U must adopt unusual syn conformations. Since the syn glycosidic conformation is energetically unfavorable for pyrimidine bases, this will explain the fact that Schiff base formation takes several hours in this system. Improvement in the rate of the Schiff base formation will be required for further application of Schiff base linkage as a functional interbase interaction in DNA.

5-Formyluracil is one of the major oxidative T lesions in DNA formed by ionizing radiation, the Fenton reaction, and quinone-mediated photosensitized reactions.[10] The ^{f}U residues in DNA promote various types of base mispair formation, due to the electron-withdrawing formyl group substituent that increases the acidity of the N3 proton.[11] In addition to the miscoding properties, the ^{f}U could form covalent adducts with proteins and small molecules via Schiff base formation.[5,10] Accumulating evidence suggests that the ^{f}U is an abundant, mutagenic, and cytotoxic lesion in DNA.[10,11] Since ^{Am}C-containing DNA dramatically stabilizes the duplex only when ^{f}U is located at the site opposite ^{Am}C, we examined the detection of ^{f}U formation in DNA. A single T residue containing ODN **7** (5′-GCG UUA T AUU GCG) was photoirradiated in the presence of 2-methyl-1,4-naphthoquinone (menadione), which had been reported to produce ^{f}U as the oxidation product of T in DNA.[10b] HPLC analysis of the enzymatically digested ODN indicated that approximately 3% of thymine was converted to ^{f}U under these conditions. The oxidized ODN was incubated with ^{Am}C-containing DNA at 30 °C for 24 h and then analyzed by denaturing PAGE. As is clearly shown in Figure 6, a single lower mobility band, corresponding to cross-linked product, was formed only in the case of incubation after photoirradiation (lanes 4 and 5). Because the loss of bases produces a deoxyribose residue that can exist in an aldehyde form, we tested the Schiff base formation between ^{Am}C and the abasic site.[12] Interestingly, no cross-linking was observed between the abasic site and its opposing ^{Am}C (Figure 3, lane 7); therefore, ^{Am}C reacts specifically with ^{f}U among the various thymine oxidation products. In comparison with the ^{f}U detection by HPLC analysis, this method enables apparently easier discrimination of ^{f}U at a

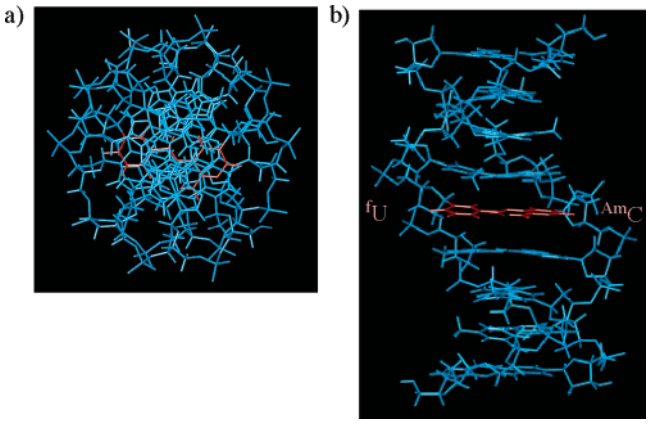


Figure 5. Molecular modeling of the conformations of the duplex containing a Schiff base linkage, 5′-d(CAAT^{f}UTAAC)/3′-d(GTTA^{Am}-CATTG). The model structure was optimized by use of the AMBER* force field in water with MacroModel version 8.1. Top views (a) and side view from the major groove (b) are shown. The paired ^{Am}C and ^{f}U is represented in red color.

(10) (a) Kasai, H.; Iida, A.; Yamaizumi, Z.; Nishimura. S.; Tanooka, H. *Mutat. Res.* **1990**, *243*, 249−253. (b) Bjelland, S.; Eide, L.; Time, R. W.; Stote, R.; Eftedal, I.; Volden, G.; Seeberg, E. *Biochemistry* **1995**, *34*, 14758−147674. (c) Douki, T.; Delatour, T.; Paganon, F.; Cadet, J. *Chem. Res. Toxicol.* **1996**, *9*, 1145−1151.

(11) (a) Privat, E. J.; Sowers, L. C. *Mutat. Res.* **1996**, *354*, 151−156. (b) Zhang, Q.-M.; Sugiyama, H.; Miyabe, I.: Matsuda, S.; Saito, I.; Yonei, S. *Nucleic Acids. Res.* **1997**, *25*, 3969−3973. (c) Masaoka, A.; Terato, H.; Kobayashi, M.; Ohyama, Y.; Ide, H. *J. Biol. Chem.* **2001**, *276*, 16501−16510. (d) Bjelland, S.; Ånensen, H.; Knævelsrud, I.; Seeberg, E. *Mutat. Res.* **2001**, *486*, 147−154. (e) Kamiya, H.; Murata-Kamiya, N.; Karino, N.; Ueno, Y.; Matsuda, A.; Kasai, H. *Mutat. Res.* **2002**, *513*, 213−222.

(12) The abasic site was generated in an uracil-containing ODN by removing the uracil using uracil-DNA glycosylase according to a previously reported procedure. Hoehn, S. T.; Turner, C. J.; Stubbe, J. *Nucleic Acids* Res. **2001**, *29*, 3413−3423.

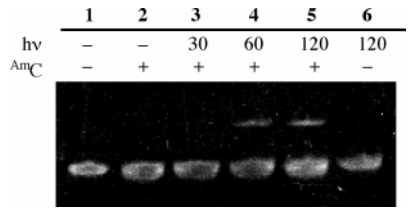


Figure 6. Detection of ^{f}U formation. Single-stranded ODN **7** (5′-GCG UUA T AUU GCG, 10 μM) was irradiated at 366 nm in the presence of menadione (500 μM), 50 mM Na phosphate (pH 7.0), and 100 mM NaCl, for the indicated time (min). Then photooxidized ODNs were incubated with ODN **1** (10 μM) at 30 °C for 24 h. Samples in lanes 1 and 6 were incubated in the absence of the ODN **1**. Samples were subjected to electrophoresis through 20% polyacrylamide containing 7 M urea gel and stained with SYBR Green I nucleic acid gel stain.

specific position in DNA. The ^{Am}C-containing ODN is an effective probe for the detection of ^{f}U in DNA.

Conclusions

The above results demonstrate that paired ^{Am}C and ^{f}U form stable and reversible covalent linkage via Schiff base formation. The cross-link is very stable, even under the denaturing PAGE condition, whereas it completely dissociates upon simple heating. Although natural bases, A, G, and C, have amino groups, they are inactive toward condensation with the formyl group of the ^{f}U. In addition, an aldehyde group of an abasic site does not undergo Schiff base formation with the opposing ^{Am}C. This specific pairing ability of ^{Am}C and ^{f}U can be used for the detection of ^{f}U formed at a specific position in DNA. Although there is room for improvement, especially in the rate of the Schiff base formation, this new type of base pairing will expand the possibilities of functional artificial DNA.

Experimental Section

General. The reagents for DNA synthesis including 5-BrdC phosphoramidite were purchased from Glen Research. ODNs were synthesized by a conventional phosphoramidite method by using an Applied Biosystems 392 DNA/RNA synthesizer. Uracil DNA glycosylase was purchased from Invitrogen. Masses of oligodeoxynucleotides were determined with a MALDI-TOF mass (Perseptive Voyager Elite, acceleration voltage 21 kV, negative mode) with 2′,3′,4′-trihydroxyacetophenone as a matrix, using T_8 ([M − H]$^-$ 2370.61) and T_{17} ([M − H]$^-$ 5108.37) as internal standards. All aqueous solutions utilized purified water (Millipore, Milli-Q sp UF). Reversed-phase HPLC was performed on CHEMCOBOND 5-ODS-H columns (10 × 150 mm, 4.6 × 150 mm) with a Gilson chromatograph, Model 305, using a UV detector, Model 118, at 254 nm.

Synthesis of ^{Am}C-Containing ODN.[6b] ODNs containing 5-bromocytidine were synthesized on a DNA synthesizer by a conventional phosphoramidite method. The ^{Br}C-containing ODNs were treated with concentrated ammonia at 60 °C for 35−48 h. The resulting mixture was evaporated and purified by reverse-phase HPLC on a CHEMCOBOND 5-ODS-H column (10 × 150 mm) eluting with 3−14% (40 min) acetonitrile in 0.05 M ammonium formate, at a flow rate 3.0 mL/min. ODN1 5′-d(CGC AAT ^{Am}C TAA CGC)-3′: [(M − H)$^-$] calcd 3917.6, found 3917.4.

Synthesis of ^{f}U-Containing ODN.[7] The synthesis of ^{f}U-containing ODNs were accomplished by a postsynthetic modification of ODN containing 5-(1,2-dihydroxyethyl)uridine with $NaIO_4$. ODNs containing 5-(1,2-dihydroxyethyl)uridine were synthesized on a DNA synthesizer. Phosphoramidite of 5-(1,2-dihydroxyethyl)uridine was prepared according to previously reported procedure.[7] After the automated synthesis, the ODNs were cleaved from the resign and deprotected by treating with concentrated ammonia at 55 °C for 8 h. The ODNs dissolved in water were treated with aqueous $NaIO_4$ (500 mM) at room temperature for 2 min. The resulting mixture was purified by reverse-phase HPLC on a CHEMCOBOND 5-ODS-H column (10 × 150 mm) eluting with 7−14% (35 min) acetonitrile in 0.1 M triethylamine acetate (TEAA), pH 7.0, at a flow rate 3.0 mL/min). ODN2 5′-d(GCG TTA ^{f}U ATT GCG)-3′: [(M − H)$^-$] calcd 3993.6, found 3994.5.

Synthesis of Abasic-Site-Containing ODN.[12] The abasic site was generated in an uracil-containing ODN by removing the uracil using uracil-DNA glycosylase according to previously reported procedure.[12] Uracil-containing ODN (5′-d(GCG TTA U ATT GCG)-3′) was synthesized on a DNA synthesizer and purified by reverse-phase HPLC. The ODN (100 μM) was treated with the uracil-DNA glycosylase (0.01 unit/μL, Invitrogen) in 10 mM Na·phosphate (pH 7.0) at 37 °C for 2 h, and then purified by Micropure-EZ and Microcon YM-3 (Millipore). 5′-d(GCG TTA ϕ ATT GCG)-3′: [(M + Na − 2H)$^-$] calcd 3893.5, found 3894.0.

Schiff Base Formation between the ^{Am}C- and ^{f}U-Containing ODNs. ODNs **1** and **2** (7.5 μM each) were mixed in buffered solution (20 μL) of 50 mM Na·phosphate (pH 5.8 or 7.0) and 100 mM NaCl. The solution was incubated at 30 °C for 8−24 h. The Schiff base formation was monitored by reverse-phase HPLC on a CHEMCOBOND 5-ODS-H column (4.6 × 150 mm) elution with 7−19% (30 min) acetonitrile in 0.1 M triethylamine acetate, pH 7.0, at a flow rate 1.0 mL/min). For PAGE experiment, the samples (5 μL) were mixed with formamide loading buffer (1.25 μL) and were subjected to electrophoresis through 20% polyacrylamide/7 M urea gel at 200 V. The gels were stained for 10 min with SYBR Green I nucleic acid gel stain (Takara). Images of the gels were collected by using a VersaDoc 3000 imaging system (Bio-Rad).

Thermal Denaturing Profiles. All melting temperatures (T_m) of the ODNs (2.5 μM, final duplex concentration) were taken in sodium phosphate buffers (50 mM, pH 7.0) containing sodium chloride (100 mM). Absorbance vs temperature profiles were measured at 260 nm using a Shimadzu UV-2550 spectrophotometer equipped with a Peltier temperature controller using a 1 cm path length cell. The absorbance of the samples was monitored at 260 nm from 0 to 85 °C with a heating rate of 1 °C/min.

Detection of ^{f}U Formation. Single-stranded ODN **7** (10 μM) was irradiated at 366 nm in the presence of menadione (500 μM; TCI), 50 mM Na phosphate (pH 7.0), and 100 mM NaCl under air bubbling. Photoirradiation was carried out with a Cosmo BIO CSF-20AF transilluminator. Then ODN **1** (10 μM) was added, and the samples were incubated at 30 °C for 24 h. The samples were subjected to electrophoresis through 20% polyacrylamide containing 7 M urea gel and stained with SYBR Green I nucleic acid gel stain. Yield of the ^{f}U formation was determined from the amount of d^{f}U formed by enzymatic digestion of the photooxidized ODN. A cocktail of three enzymes, calf intestinal alkaline phosphatase (Promega), *Crotalus adamanteus* venom phosphodiesterase I (USB), and *Penicillium citrinum* nuclease P1 (Roche), was used for the enzymatic digestion.

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