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Base-discriminating fluorescent (BDF) nucleoside: distinction of thymine by fluorescence quenching†

Yoshio Saito,[a] Yohei Miyauchi,[b] Akimitsu Okamoto[b] and Isao Saito*[a]

[a] *NEWCAT Institute, Faculty of Engineering, Nihon University and SORST, Japan Science and Technology Corporation, Tamura, Koriyama 963-8642, Japan. E-mail: saito@mech.ce.nihon-u.ac.jp*

[b] *Department of Synthetic Chemistry and Biological Chemistry, Faculty of Engineering, Kyoto University, Kyoto 615-8510, Japan*

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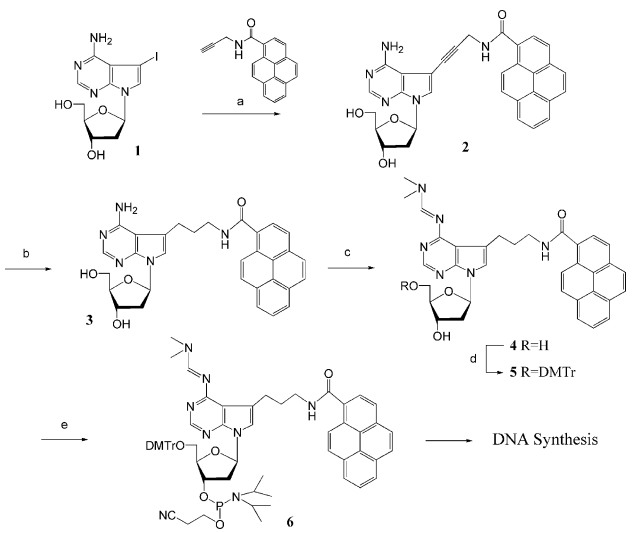
A novel fluorescence BDF probe containing pyrene-labeled 7-deaza-2′-deoxyadenosine has been developed for the detection of thymine base on a target DNA.

Single nucleotide polymorphisms (SNPs), the most common genetic variations found in the human genome, are important markers for identifying disease-associated loci and for pharmacogenetic studies.[1] SNPs appear in a human genome with an average density of once every 1000 bp[2] and numerous methods for SNPs typing have been described.[3] However, a new approach to develop cheap and efficient high-throughput genotyping is still necessary.

In our continuous efforts to develop base-discriminating fluorescent (BDF) nucleosides, we have recently demonstrated hybridization-based homogeneous assay that provides a clear distinction of the base on the complementary strand by the fluorescence change caused by the DNA microenvironment.[4] We report herein a conceptually new BDF probe containing 7-deaza-7-(1-pyrenecarboxamido)propyl-2′-deoxyadenosine (**^{Py}A**) for the discrimination of thymine base on a target DNA by a fluorescence quenching mechanism. While an oligonucleotide containing **^{Py}A** shows strong fluorescence emission when the bases opposite **^{Py}A** are mismatched bases (C, G, A), the fluorescence intensity of the perfectly matched duplex (T) is completely quenched. The drastic fluorescence change was successfully applied for the detection of thymine base located at a specific site on a target DNA.

The synthesis of a novel BDF nucleoside **3** is outlined in Scheme 1. 7-Deaza-7-iodo-2′-deoxyadenosine **1** was prepared according to the protocol of Seela and Zulauf.[5] Compound **1** was coupled with a pyrene substituted propalgyl amine under Sonogashira conditions by using $Pd(PPh_3)_4$ to afford **2** which was then hydrogenated on 10% Pd/C in MeOH to give **3**. Protection of the amino group with DMF acetal and deoxyribose 5′-hydroxyl group with 4,4′-dimethoxytrityl group afforded **5**. After conversion to phosphoramidite, **6** was incorporated into oligonucleotides by an automated DNA synthesizer. The newly synthesized ODNs are summarized in Table 1.



Scheme 1 Reagents and conditions: (a) $Pd(PPh_3)_4$, CuI, Et_3N, DMF, rt, 6 h, 88%; (b) 10% Pd/C, MeOH, rt, 2 h, 86%; (c) DMF acetal, DMF, 50 °C, 3h, 84%; (d) DMTrCl, pyridine, rt, 8 h, 75%; (e) $(i\text{-}Pr_2N)_2PO(CH_2)_2CN$, 1*H*-tetrazole, CH_3CN, rt, 1 h.

Initially, the fluorescence of **^{Py}A**-containing ODN was examined. The fluorescence spectra of the mismatched duplexes (**ODN(^{Py}A)/ODN(N)**, N = C, G, A) and single-stranded ODN (**ODN(^{Py}A)**) showed strong fluorescence emission at 390 nm (Φ_F is 0.097, 0.098, 0.054, and 0.064, respectively). In contrast, the fluorescence of the matched duplex (**ODN(^{Py}A)/ODN(T)**) was almost completely quenched (Φ_F = 0.006). The fluorescence quantum yield of **ODN(^{Py}A)/ODN(T)** was approximately 9 to 16 times less than that observed for mismatched duplexes (Fig. 1). The single strand **ODN(^{Py}A)** exhibited an absorption band at 349 nm arising from pyrene, and the absorption maxima shifted slightly to a longer wavelength by the duplex formation with complementary strands as shown in Table 2.

There are numerous examples in which fluorescence is quenched by the interaction of pyrene chromophore with duplex *via* intercalation. For example, Pederson and Christensen reported a

Table 1 Oligonucleotides (ODNs) used in this study

	Sequences
ODN(^{Py}A)	5′-d(CGCAAT^{Py}**AT**AACGC)-3′
ODN(T)	5′-d(GCGTTA**T**ATTGCG)-3′
ODN(C)	5′-d(GCGTTA**C**ATTGCG)-3′
ODN(A)	5′-d(GCGTTA**A**ATTGCG)-3′
ODN(G)	5′-d(GCGTTA**G**ATTGCG)-3′
ODN_{BRCA1}(^{Py}A)	5′-d(GGTACCA^{Py}**A**TGAAATA)-3′
ODN_{BRCA1}(T)	5′-d(TATTTCA**T**TGGTACC)-3′
ODN_{BRCA1}(C)	5′-d(TATTTCA**C**TGGTACC)-3′

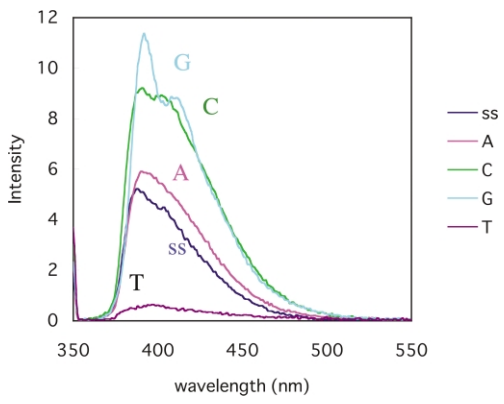


Fig. 1 Fluorescence spectra of **ODN(^{Py}A)**. Fluorescence spectra of **ODN(^{Py}A)** hybridized with 2.5 μM **ODN(A)**, **ODN(C)**, **ODN(G)**,or **ODN(T)** (50 mM sodium phosphate, 0.1 M sodium chloride, pH 7.0, room temperature). Excitation wavelength was 350 nm.

† Electronic supplementary information (ESI) available: Experimental procedures for new compounds, UV and excitation spectra. See http://www.rsc.org/suppdata/cc/b4/b405832a/

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decreased fluorescence emission of the pyrene-modified intercalating nucleic acid (INA) strands which allow the pyrene to intercalate into the double helixes upon hybridization with the complementary strand.[7] Furthermore, Netzel and co-workers reported the DNA duplexes containing covalently attached pyrenebutylate, where the fluorescence of the pyrene is almost completely quenched in the duplex because the pyrene chromophore is attached by a long and flexible linker to the base and thus could easily intercalate into DNA base pairs.[8] Taking account of these facts, the decreased fluorescence emission upon excitation at 350 nm of the duplex containing **^{Py}A** strongly suggests the intercalation of the pyrene chromophore into the duplex. Recently, we reported related pyrenecarboxamide-labeled BDF nucleosides that are highly sensitive to the solvent polarity.[9] We have indicated that the fluorescence quantum yield of pyrenecarboxamide decreased with decreasing solvent polarity.[9] Thus, the fluorescence quenching for the matched duplex **ODN(^{Py}A)/ODN(T)** is probably due to the intercalation of the pyrene unit. On the other hand, the mismatched base pair containing **^{Py}A** shows a strong fluorescence emission because the intercalation of the pyrene unit is less favorable as compared with the perfectly matched duplex. A slight red-shift and the hypochromicity of the pyrene chromophore also suggest the stacking interaction between the pyrene and DNA basepair (Fig. 1, Supplementary Information†).

To investigate this hypothesis, we examined the molecular modeling studies of the **^{Py}A**-containing duplex. The structure of the **^{Py}A**-containing matched duplex was obtained from optimization of 5′-d(CAAT **^{Py}A** TAAC)-3′/5′-d(GTTA**T**ATTG)-3′ using the AMBER* force field in a water employing MacroModel ver. 6.0 (Schrödinger Inc.). As shown in Fig. 2, the modeling studies supported the intercalation of the pyrene unit into the matched duplex to result in a remarkable stabilization of the duplex (Fig. 2). In melting temperature (T_m) measurements of the duplex, a high duplex stability was actually observed for **^{Py}A**-containing duplex (**ODN(^{Py}A)/ODN(T);**, T_m = 54.0 °C), which was more stable than a natural base pair (natural A/T base pairs: T_m = 51.2 °C, Table 2).

Table 2 Spectroscopic data and melting temperature (T_m)[a]

Sample	T_m/°C	λ_{max}/nm	$\varepsilon_{\lambda_{max}}$	λ_{em}/nm	Φ_F
ss	—	347	13,160	388	0.064
T	54.0	353	12,400	397	0.006
A	44.1	350	12,800	390	0.054
C	50.2	350	12,800	391	0.097
G	47.8	352	12,560	392	0.098

[a] Spectroscopic data and T_m. The fluorescence quantum yields (Φ) were calculated according to ref. 6.

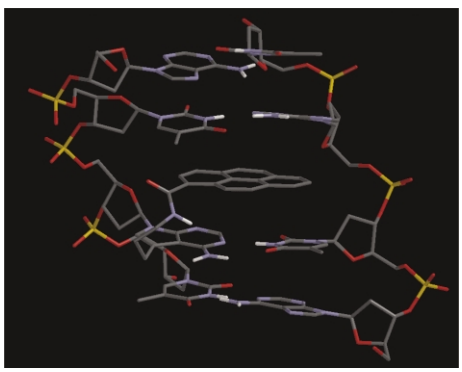

Fig. 2 Molecular model of perfectly matched duplex (5′-d(T**^{Py}A**TA)-3′/3′-(A**T**AT)-5′) viewed from the major groove. The model was optimized by AMBER* force field in a water using MacroModel ver. 6.0.

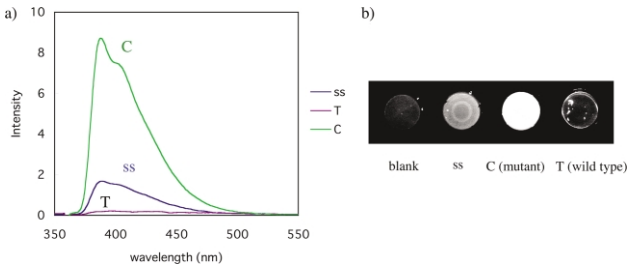

Fig. 3 Detection of the T/C allele type of BRCA1 using fluorescence change of BDF probe. (a) Fluorescence spectra of 2.5 μM **ODN$_{BRCA1}$(^{Py}A)** hybridized with 2.5 μM **ODN$_{BRCA1}$(T)** or **ODN$_{BRCA1}$(C)** (50 mM sodium phosphate, 0.1 M sodium chloride, pH 7.0, room temperature). Excitation wavelength was at 350 nm. (b) Detection of fluorescence image. A volume of 2.5 μM **ODN$_{BRCA1}$(T)** or **ODN$_{BRCA1}$(C)** was hybridized with 2.5 μM **ODN$_{BRCA1}$(^{Py}A)**. Fluorescence was observed with a fluorescence imager Versa Doc Imaging System (BioRad) equipped with a 290–365 nm transilluminator. The image was taken through a 380 nm long pass emission filter.

The clear change in the fluorescence that depends on the type of base on the complementary strand would be very useful for SNP typing.[4] We tested the SNP detection of the T/C (wild type/mutant) SNP sequence of human breast cancer 1 gene (BRCA1)[10] by a **^{Py}A**-containing BDF probe method. We added BDF probe **ODN$_{BRCA1}$(^{Py}A)** to the solution of the target sequence, **ODN$_{BRCA1}$(T)** (wild type) and **ODN$_{BRCA1}$(C)** (mutant), and incubated these solutions at room temperature for one minute. The sample solutions were then illuminated at 350 nm, and the fluorescence images were taken through a 380 nm cutoff filter. An efficient quenching was observed with the **ODN$_{BRCA1}$(^{Py}A)/ODN$_{BRCA1}$(T)** duplex, whereas the mismatched duplex (**ODN$_{BRCA1}$(^{Py}A)/ODN$_{BRCA1}$(C)**) showed a strong emission (Fig. 3). The fluorescence quantum yield of the matched duplex (**^{Py}A**/T, Φ_F = 0.002) was approximately 47 times less than that observed for mismatched one (**^{Py}A**/C, Φ_F = 0.094). This **^{Py}A**-containing BDF probe facilitates the distinction of thymine on a target DNA by the drastic fluorescence change.

In conclusion, we have developed a novel BDF probe based on fluorescence quenching, which can clearly distinguish thymine base on the complementary strand. The present method using **^{Py}A**-containing BDF probe constitutes a different type of homogeneous assay using BDF probe.

Notes and references

1 H. Zhang, H. Zhao and K. Merikangas, *Ann. Med.*, 1997, **6**, 493.
2 E. Lai, J. Riley, I. Purvis and A. Roses, *Genomics*, 1998, **54**, 31.
3 (*a*) M. Shi, *Clin. Chem.*, 2001, **47**, 164; (*b*) U. Landegren, M. Nilsson and P.-Y. Kwok, *Genome Res.*, 1998, **8**, 769; (*c*) S. Tyagi, D. P. Bratu and F. R. Kramer, *Nat. Biotechnol.*, 1998, **16**, 49; (*d*) S. Sauer, D. Lechner, K. Berlin, H. Lehrach, J. L. Escary, N. Fox and I. G. Gut, *Nucleic Acids Res.*, 2000, **28**, e13.
4 (*a*) A. Okamoto, K. Tainaka and I. Saito, *J. Am. Chem. Soc.*, 2003, **125**, 4972; (*b*) A. Okamoto, K. Tanaka, T. Fukuta and I. Saito, *J. Am. Chem. Soc.*, 2003, **125**, 9296.
5 F. Seela and M. Zulauf, *Synthesis*, 1996, 726.
6 J. V. Morris, M. A. Mahaney and J. R. Huber, *J. Phys. Chem.*, 1976, **80**, 969.
7 (*a*) U. B. Christensen and E. B. Pedersen, *Nucleic Acids Res.*, 2002, **30**, 4918; (*b*) U. B. Christensen and E. B. Pedersen, *Helv. Chim. Acta*, 2003, **30**, 2090.
8 (*a*) T. L. Netzel, *J. Phys. Chem.*, 1995, **99**, 17461; (*b*) J. Telser, K. A. Cruickshank, L. E. Morrison and T. L. Netzel, *J. Am. Chem. Soc.*, 1989, **111**, 6966; (*c*) J. Telser, K. A. Cruickshank, L. E. Morrison, T. L. Netzel and C.-K. Chan, *J. Am. Chem. Soc.*, 1989, **111**, 7226.
9 A. Okamoto, K. Kanatani and I. Saito, *J. Am. Chem. Soc.*, 2004, **126**, 4820.
10 H. Haga, Y. Yamada, Y. Ohnishi, Y. Nakamura and T. Tanaka, *J. Hum. Genet.*, 2002, **47**, 605.