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# Detection of genetic polymorphisms with high sensitivity by DNA–perylene conjugate

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**Abstract**—Modified oligodeoxyribonucleotides (ODNs) involving two perylene moieties are synthesized. By using this ODN, one-base deletion can easily be distinguished with high sensitivity. In addition, emission color of the solution greatly changed so that the detection was possible even by naked eyes.
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In the human genome, there are more than 300,000 genetic polymorphisms including single nucleotide polymorphisms (SNPs) and insertion/deletion (indel) polymorphisms, and the relationship between these differences and genetic diseases has been elucidated.[1] Toward the tailor-made medicine, various kinds of fluorescent probes have been proposed to detect genetic polymorphisms for high through-put analysis.[2] Among the fluorescent dyes, pyrene is widely investigated because of its characteristic fluorescent behavior:[3] microenvironment around pyrene greatly affects the fluorescence intensity. Furthermore, pyrene exhibits monomer emission at around 390 nm whereas it shows excimer emission at around 480 nm when two pyrenes come close. Such fluorescent behavior based on the assembled structure made it possible to detect genetic polymorphisms by the change of emission maximum.[4] We have also reported that DNA probe that tethers pyrenes on D-threoninol could detect deletion polymorphism efficiently by the excimer or exciplex emission.[5] For the practical application, however, pyrene has two problems: its low quantum yield and emission maximum in near-UV region. Quantum yield of pyrene is usually below 0.2,[4] although it depends on the solution conditions. Furthermore, pyrene has its monomer emission at around 390 nm as described above. Such short wavelength emission is often disturbed by the background fluorescence from other natural molecules.[6]

Perylene is a promising candidate that can overcome these demerits, because its fluorescence exhibits in visible region (ca. 460 and 490 nm) with relatively high quantum yield.[7] By using perylene as a fluorophore instead of pyrene, improvement of sensitivity is expected.[8] Furthermore, perylene is also known to form excimer as pyrene does:[9] its emission maximum appears at ca. 530 nm. Although excimer emission of perylene has scarcely been applied to the detection of gene polymorphisms, utilization of monomer → excimer change will make it possible to detect the polymorphisms by naked eyes without serious background emission. In the present paper, we synthesize DNA–perylene conjugates and demonstrate that they can detect deletion polymorphisms as well as single nucleotide polymorphisms (SNPs) by naked eyes with high sensitivity.

Probe designs for detecting genetic polymorphisms are depicted in Scheme 1A, and sequences of ODNs are shown in Scheme 1B. Two perylene molecules are introduced into the middle of ODN and one base is inserted between them where deletion or substitution (SNPs) occurs (**EAE** in Scheme 1B). With wild type (**T1**), two perylene moieties intercalate between the base-pairs and thus they are separated by the intervening base-pair. As a result, only monomer emission should be observed. With one-base deletion (**N** in Scheme 1B), however, three-base bulge is formed and two perylene molecules come close enough to form excimer. Therefore, one-base deletion can be distinguished by monitoring emission maximum. Detection of substitution (SNPs) is also

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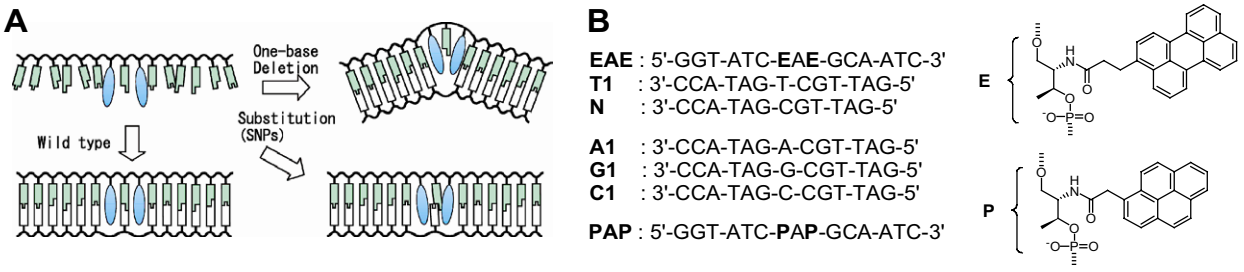



**Scheme 1.** (A) Schematic representation of the detection of genetic polymorphisms and (B) the sequences of ODNs synthesized in this study.

possible by this probe because mismatched intervening base-pair will associate the interaction of two perylenes.

Introduction of perylene moiety to ODN was carried out through the corresponding phosphoramidite monomer synthesized from perylene as shown in Scheme 2. Perylene-3-carbaldehyde (compound **2** in Scheme 2) was synthesized from perylene according to the literature.[10] Then it was converted to 3-(3-perylenyl)propionic acid (compound **5**),[11] followed by the coupling with D-threoninol through amide bond. Obtained compound **6** was further converted to phosphoramidite monomer (compound **8**) for the incorporation into ODN.[12] Here, D-threoninol was used as a linker because most of non-natural molecules tethered on this linker are intercalated between base-pairs.[13] All the modified ODNs are characterized by MALDI-TOFMS.[14]

Single-stranded **EAE** involving two perylenes at both adjacent sides of adenosine showed distinct monomer emission at 461 and 491 nm as shown by the broken line in Figure 1.[15] Excimer emission, which usually appears at around 530 nm,[16] was scarcely observed. When **EAE** was hybridized with wild type DNA (**T1**), monomer emission of perylene was strongly enhanced (dotted line). Since two perylenes were intercalated between the hydrophobic base-pairs, monomer emission was fairly enhanced. It should be noted that this fluorescence behavior was entirely different from that of pyrene whose monomer emission was lowered by its intercalation.[17] In contrast, hybridization with one-base deletion mutant (**N**) greatly quenched its monomer emission (solid line in Fig. 1). Furthermore, weak but distinct excimer emission appeared at 529 nm due to the close

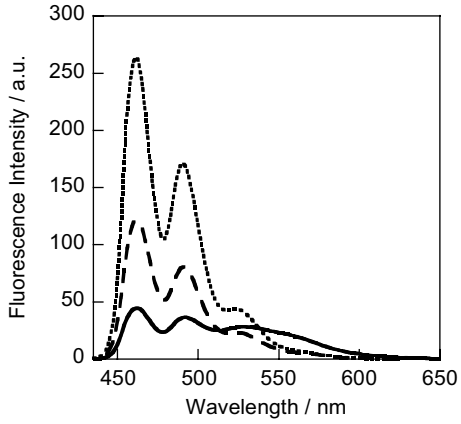
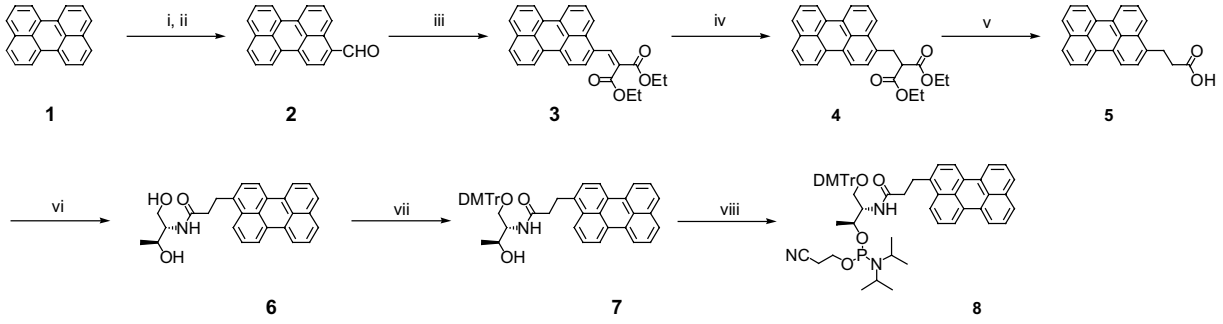



**Figure 1.** Fluorescent emission spectra of **EAE/N** (solid line), **EAE/T1** (dotted line) and single-stranded **EAE** (broken line) at 0 °C in the presence of 100 mM NaCl at pH 7.0 (10 mM phosphate buffer). Concentration of each DNA is 5 μM. Excitation wavelength was 425 nm. Melting temperatures ($T_m$s) of each duplex were **EAE/N**: 44.7 °C, **EAE/T1**: 48.9 °C.

proximity of two perylene chromophores. This change of fluorescence was quantified by the intensity ratio of excimer emission to monomer emission ($I_{550}/I_{461}$), which was 0.51 for **EAE/N** whereas 0.04 for **EAE/T1**.[18] Thus, one-base deletion could easily be detected from the change of its fluorescence.

As described above, merits of perylene against pyrene are its high quantum yield and emission in visible region. These merits were apparently demonstrated when these changes were observed with naked eyes. Photographs of **EAE/N** and **EAE/T1** are depicted in Figure 2A and




**Scheme 2.** Synthesis of phosphoramidite monomer involving perylene. Reagents and conditions: (i) $SnCl_4$, dichloromethyl methyl ether, 1,2-dichloroethane, 80 °C, 18 h; (ii) $H_2O$, rt, 3 h, 72%; (iii) diethyl malonate, piperidine, dry benzene, 80 °C, 48 h, 75%; (iv) $H_2$, Pd–C, THF, rt, overnight, 73%; (v) KOHaq, benzene, ethanol, *o*-dichlorobenzene, 80 °C, 1 h, 91%; (vi) D-threoninol, dicyclohexylcarbodiimide, 1-hydroxybenzotriazole, DMF, rt, 5 h, quant.; (vii) dimethoxytrityl chloride, pyridine, $CH_2Cl_2$, rt, 2 h, 67%; (viii) $(i\mathrm{Pr}_2\mathrm{N})_2\mathrm{PO}(\mathrm{CH}_2)_2\mathrm{CN}$, 1*H*-tetrazole, acetonitrile, rt, 1 h, 68%.

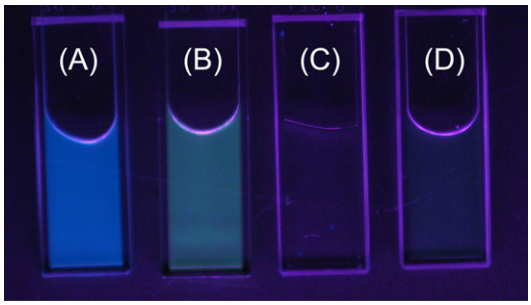



**Figure 2.** A photograph of (A) **EAE/T1**, (B) **EAE/N**, (C) **PAP/T1** and (D) **PAP/N**. Concentration of each DNA is 5 μM. This photo was taken at room temperature in the presence of 100 mM NaCl at pH 7.0 (10 mM phosphate buffer). Fluorescence is observed over a UV transilluminator (365 nm).

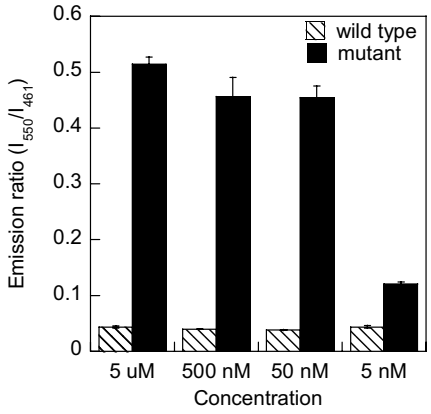



**Figure 3.** Intensity ratio of the emission at 550 nm and 461 nm ($I_{550}/I_{461}$) of wild type (**EAE/T1**) and one-base deletion mutant (**EAE/N**) at various concentrations. Fluorescence intensity was measured at 0 °C in the presence of 100 mM NaCl at pH 7.0 (10 mM phosphate buffer).

B, which correspond to the dotted and solid lines in Figure 1, respectively. Blue emission was observed from **EAE/T1** over UV illuminator because monomeric emission appeared at 461 and 491 nm (see Fig. 2A). In contrast, **EAE/N** emitted green light due to the quenching of monomer emission (blue light) and appearance of excimer emission at 529 nm (see Fig. 2B). Such shift was distinctly observed as color change with naked eyes (compare Fig. 2A with B). In order to compare perylene with pyrene, same DNA probe involving two pyrenes, **PAP**, (see Scheme 1B) was synthesized. With this pyrene probe, one-base deletion could also be detected in a same manner as **EAE** as reported previously:[5a] **PAP/T1** exhibited monomer emission at 380 and 400 nm whereas **PAP/N** did excimer emission at 480 nm (see Supplementary Fig. S-1). But as shown in Figure 2C and D, such shift was not observed clearly because of its low quantum yield. In addition, since emission from **PAP/T1** occurred in near-UV region, we could hardly see it with naked eyes. Although excimer emission appeared at visible 470 nm for **PAP/N**, its emission was very dark (see Fig. 2D) due to the low quantum yield. It should be noted that **EAE/T1** showed 7-times stronger emission than **PAP/N**, each of which emitted in similar wavelength range.[19] With perylene probe of high quantum yield, one-base deletion could be detected even at lower concentration. The intensity ratio of excimer emission (550 nm) to monomer emission (461 nm) was depicted in Figure 3 as a function of concentration. Even at 5 nM, wild type and deletion mutant are distinguishable. Thus, sensitivity was substantially improved by using perylene instead of pyrene.[20]

This probe is also applicable to the detection of substitution mutants (SNPs) from the change of $I_{550}/I_{461}$.[21] As listed in Table 1, $I_{550}/I_{461}$ of mismatched sequence (**C1**, **G1**, or **A1**) was higher than that of full-matched one (**T1**). Increase of $I_{550}/I_{461}$ by the mismatch would be attributable to the increased interaction between the two perylenes due to the disorder (see Scheme 1A). Although the difference is still small, optimization of the linker and length of methylene chain between the perylene and main chain would improve the difference.

In conclusion, one-base deletion as well as substitution mutants was detected with high sensitivity by using perylene-modified ODN. Wild type and one-base deletion mutant could easily be discriminated from the change of emission even by naked eyes.

**Table 1.** The ratios of excimer emission to the monomer emission of **EAE** with various counter strands at 0 °C[a]

| Counter strand | N | T1 | C1 | G1 | A1 |
|---|---|---|---|---|---|
| $I_{550}/I_{461}$[b] | 0.51 | 0.04 | 0.14 | 0.06 | 0.09 |

[a] Concentration of each ODN was 5 μM.
[b] The intensity ratio of the emission at 550 nm to that at 461 nm.

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## Supplementary data

The fluorescence emission spectra of **EAE/N**, **EAE/T1**, **PAP/N** and **PAP/T1** (excited at 365 nm) are presented in Supplementary data. UV–vis spectra of these duplexes are also presented. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2007.07.084.

## References and notes

1. Tsongalis, G. J.; Coleman, W. B. *Clin. Chim. Acta* **2006**, *363*, 127.
2. (a) Kool, E. T. *Acc. Chem. Res.* **2002**, *35*, 936; (b) Okamoto, A.; Saito, Y.; Saito, I. *J. Photochem. Photobiol. C* **2005**, *6*, 108, and references cited therein.
3. Winnik, F. M. *Chem. Rev.* **1993**, *93*, 587.

4. (a) Lewis, F. D.; Zhang, Y.; Letsinger, R. L. *J. Am. Chem. Soc.* **1997**, *119*, 5451; (b) Paris, P. L.; Langenhan, J. M.; Kool, E. T. *Nucleic Acids Res.* **1998**, *26*, 3789; (c) Kostenko, E.; Dobrikov, M.; Pyshnyi, D.; Petyuk, V.; Komarova, N.; Vlassov, V.; Zenkova, M. *Nucleic Acids Res.* **2001**, *29*, 3611; (d) Yamana, K.; Iwai, T.; Ohtani, Y.; Sato, S.; Nakamura, M.; Nakano, H. *Bioconjugate Chem.* **2002**, *13*, 1266; (e) Okamoto, A.; Ichiba, T.; Saito, I. *J. Am. Chem. Soc.* **2004**, *126*, 8364.
5. (a) Kashida, H.; Asanuma, H.; Komiyama, M. *Chem. Commun.* **2006**, 2768; (b) Kashida, H.; Komiyama, M.; Asanuma, H. *Chem. Lett.* **2006**, *35*, 934.
6. Martí, A. A.; Li, X.; Jockusch, S.; Li, Z.; Raveendra, B.; Kalachikov, S.; Russo, J. J.; Morozova, I.; Puthanveettil, S. V.; Ju, J.; Turro, N. J. *Nucleic Acids Res.* **2006**, *34*, 3161.
7. Quantum yield of perylene is 0.98 in acetonitrile solution: Komfort, M.; Löhmannsröben, H. G.; Salthammer, T. *J. Photochem. Photobiol. A* **1990**, *51*, 215.
8. (a) Balakin, K. V.; Korshun, V. A.; Mikhalev, I. I.; Maleev, G. V.; Malakhov, A. D.; Prokhorenko, I. A.; Berlin, Y. A. *Biosens. Bioelectron.* **1998**, *13*, 771; (b) Gao, J.; Watanabe, S.; Kool, E. T. *J. Am. Chem. Soc.* **2004**, *126*, 12748; (c) Skorobogatyi, M. V.; Malakhov, A. D.; Pchelintseva, A. A.; Turban, A. A.; Bondarev, S. L.; Korshun, V. A. *ChemBioChem* **2006**, *7*, 810; (d) Bag, S. S.; Saito, Y.; Hanawa, K.; Kodate, S.; Suzuka, I.; Saito, I. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 6338; (e) Wagner, C.; Wagenknecht, H. A. *Org. Lett.* **2006**, *8*, 4191.
9. (a) Tanaka, J. *Bull. Chem. Soc. Jpn.* **1963**, *36*, 1237; (b) Birks, J. B. *Rep. Prog. Phys.* **1975**, *38*, 903.
10. Asseline, U.; Cheng, E. *Tetrahedron Lett.* **2001**, *42*, 9005.
11. Yamamura, K.; Miyake, H.; Murata, I. *J. Org. Chem.* **1986**, *51*, 251.
12. Compound **6**: $^1$H NMR [$CDCl_3$, 500 MHz] $\delta$ 8.24–8.12 (m, 4H), 7.91 (m, 1H), 7.68–7.40 (m, 6H), 6.02 (d, 1H), 4.10 (m, 1H), 3.81–3.69 (m, 3H), 3.43 (t, 2H), 2.72 (m, 2H), 1.09 (d, 3H). Compound **7**: $^1$H NMR [$CDCl_3$, 500 MHz] $\delta$ 8.20–6.75 (m, 24H), 5.82 (d, 1H), 4.06 (m, 1H), 3.98 (m, 1H), 3.71 and 3.70 (s, 6H), 3.39 (m, 2H), 3.22 and 3.15 (m, 2H), 2.67 (m, 2H), 1.06 (d, 3H).
13. (a) Liang, X. G.; Asanuma, H.; Kashida, H.; Takasu, A.; Sakamoto, T.; Kawai, G.; Komiyama, M. *J. Am. Chem. Soc.* **2003**, *125*, 16408; (b) Kashida, H.; Tanaka, M.; Baba, S.; Sakamoto, T.; Kawai, G.; Asanuma, H.; Komiyama, M. *Chem. Eur. J.* **2006**, *12*, 777.
14. Perylene moiety was quantitatively incorporated into DNA as judged from the coloration of released tritylcation. MALDI-TOFMS for **EAE**: obsd. 4904 (calcd for protonated form: 4904), **PAP**: obsd 4776 (calcd 4776).
15. Conditions of the sample solutions were as follows: [NaCl] = 0.1 M, pH 7.0 (10 mM phosphate buffer), [DNA] = 5 μM. The $T_m$ value was determined from the maximum in the first derivative of the melting curve, which was obtained by measuring the absorbance at 260 nm as a function of temperature. The temperature ramp was 1 °C min$^{-1}$.
16. Akimoto, S.; Ohmori, A.; Yamazaki, I. *J. Phys. Chem. B* **1997**, *101*, 3753.
17. Christensen, U. B.; Pedersen, E. B. *Nucleic Acids Res.* **2002**, *30*, 4918.
18. We also measured the ratios ($I_{550}/I_{461}$) at 20 °C, which were almost the same as those measured at 0 °C.
19. The difference of absorbance at 365 nm between pyrene and perylene was not so large. See Supplementary data (Fig. S-2) for the actual UV–vis spectra.
20. Fluorescence quantum yields of **EAE/T1** and **PAP/N** were 0.25 and 0.04, respectively, as determined from the quantum yield of 9,10-diphenylanthracene in ethanol (0.95) as a reference: Morris, J. V.; Mahaney, M. A.; Huber, J. R. *J. Phys. Chem.* **1976**, *80*, 969.
21. Similar DNA probe detecting SNPs has been reported by Pedersen et al. who utilized pyrene as a fluorophore: Christensen, U. B.; Pedersen, E. B. *Helv. Chim. Acta* **2003**, *86*, 2090.