Exciplex Formation between Pyrene and *N*,*N*-Dimethylaniline in DNA for the Detection of One-base Deletion

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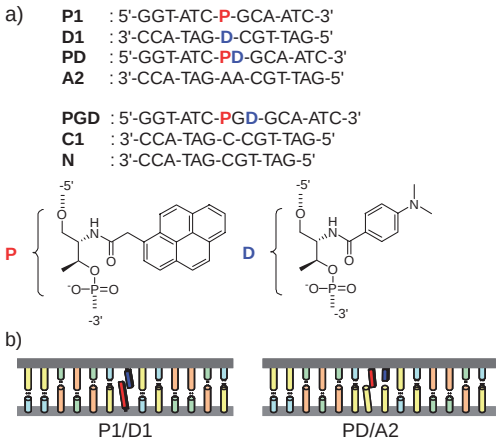
Exciplex emission from pyrene and *N*,*N*-dimethylaniline moieties was observed in aqueous solution by incorporating them into DNA. By using this exciplex formation, one-base deletion was successfully detected.

Exciplex is a transient complex composed of two kinds of molecules, and some of them exhibit characteristic fluorescence at longer wavelength in hydrophobic environment.[1] Its fluorescence spectrum is controllable by changing the distance between the chromophores, or selecting a pair of the complex. Although the exciplex emission has been applied to the design of efficient probes in some fields,[2] little has been reported on the DNA detection with the exciplex. In the field of DNA analyses, FRET (fluorescence resonance energy transfer), molecular beacon (fluorophore–quencher combination), or excimer emission has been mainly adopted.[3] One of the reasons is that exciplex easily dissociates to ionic pair in hydrophilic environment and thus emission becomes rather small in water, whereas FRET or excimer emission efficiently works in aqueous media. Although Bichenkova et al. has recently applied exciplex to the detection of SNPs,[4] addition of trifluoroethanol was inevitably required to facilitate the exciplex emission because chromophores were introduced at the terminal of oligodeoxyribonucleotides (ODNs).

In this paper, we located the chromophores of exciplex at the inside of the duplex to facilitate the exciplex emission even in water. Interestingly, distinct exciplex emission was observed without the assistance of trifluoroethanol or other organic solvent. By designing the position of chromophores in the ODN, one-base deletion was efficiently detected from the change of exciplex emission in aqueous media.

Pyrene (**P** in Scheme 1a) and *N*,*N*-dimethylaniline (DMA; **D**) were chosen as chromophores, and were incorporated into the middle of ODN because they are known as a typical pair for exciplex emission.[5] D-Threoninol was used as a linker to incorporate them into ODN because our earlier studies have demonstrated that it is a convenient linker for the chromophores to intercalate between the base-pairs and stabilize the duplex.[6] Corresponding two phosphoramidite monomers were synthesized from D-threoninol, and each chromophore was incorporated at the middle of the sequence as shown in Scheme 1.[7,8] In **P1**/**D1**, **P** and **D** residues were located to face each other in the duplex (see Scheme 1b). As shown by the solid line in Figure 1, strong exciplex emission appeared at around 480 nm when **P** and **D** residues were contacted each other in the duplex.[9] As expected, exciplex emission disappeared on elevating the temperature above T_m.[8,10] Strong exciplex emission was also observed when **P** and **D** residues were consecutively introduced into the duplex (**PD**/**A2**, see broken line in Figure 1).[11,12] Exciplex emission was observed only when **P** and **D** residues were contacted each other, and accordingly lack of **D** residue (such as **P1**/**N** duplex, see Supporting Information Figure S2) showed no exciplex emission.[8,13] Interestingly, even single-stranded **PD** also exhibited exciplex emission, although its intensity was small (see Supporting Information Figure S3).[8] These results demonstrate that exciplex emission can be observed even in aqueous solution by introducing chromophores into the middle of ODN. It should be noted that such exciplex emission did not occur without the addition of organic solvent when chromophores



Scheme 1. a) The sequences of modified ODNs synthesized in this study and b) schematic illustration of the structures of **P1**/**D1** and **PD**/**A2**.

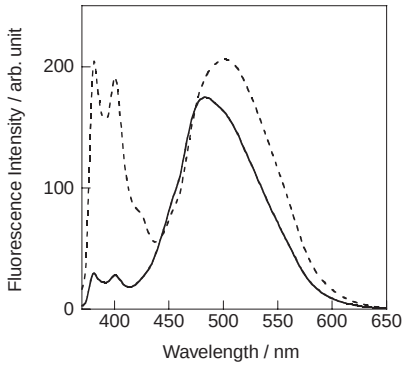
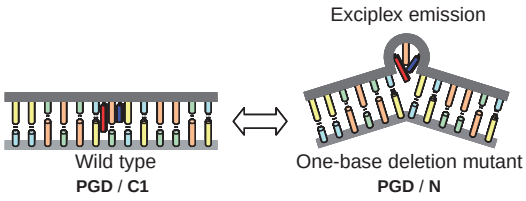


Figure 1. Fluorescent emission spectra of **P1**/**D1** (solid line), **PD**/**A2** (broken line) at 0 °C. Excitation wavelength was 345 nm. Melting temperatures (T_ms) were **P1**/**D1**: 46.5 °C and **PD**/**A2**: 37.8 °C.



Scheme 2. Schematic illustration of the discrimination strategy for one-base deletion.

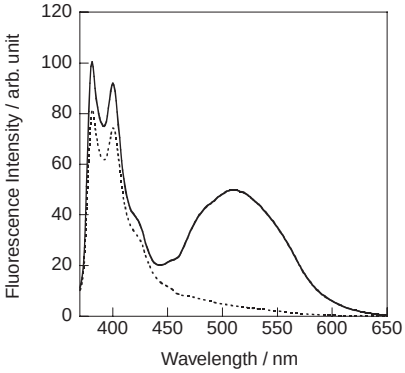


Figure 2. Fluorescent emission spectra of **PGD**/**N** (solid line) and **PGD**/**C1** (dotted line) at 0 °C. Excitation wavelength was 345 nm. T_ms of duplexes were **PGD**/**N**: 37.0 °C and **PGD**/**C1**: 47.4 °C.

were located at the terminal as reported by Bichenkova et al.[4]

Next, we applied exciplex emission to the detection of one-base deletion. Some insertion/deletion polymorphisms (*indel*s) are known to cause genetic diseases. Especially, one-base deletion is frequently found in the human genome.[14] Here, we applied exciplex emission to the detection of one-base deletion. Our design is depicted in Scheme 2. **P** and **D** were introduced to both sides of the nucleotide (in this case, G) that we want to detect the deletion (**PGD**). When **PGD** is hybridized with wild type ODN (**C1** in Scheme 1a), **P** and **D** intercalate between base pairs. Therefore, interaction between **P** and **D** is suppressed by the intervening GC base pair and no exciplex emission will be observed. However, with one-base deletion mutant (**N**), a three-base bulge is formed. In this bulge, **P** and **D** are close enough to form exciplex and its emission is expected.

Figure 2 shows fluorescent emission spectra of **PGD**/**N** and **PGD**/**C1**. When **PGD** was hybridized with wild type ODN (**C1**), no exciplex emission was observed (dotted line in Figure 2), indicating that interaction between **P** and **D** was completely suppressed by the intervening GC base pair. On the other hand, when one-base deletion mutant (**N**), which lacks cytosine from **C1**, was hybridized, strong exciplex emission was observed at around 500 nm (solid line in Figure 2). This result demonstrated that **P** and **D** came close together and formed exciplex as we designed.[8,15] The intensity of **PGD**/**N** at 550 nm was 17-times larger than **PGD**/**C1**. Deletion of cytosine was successfully detected by the intensity of exciplex emission.[16]

This design is applicable to the deletion mutants of other bases. By designing ODN probes, deletions of thymine, guanine, and adenine were successfully detected (See Supporting Information Figure S5).[8,17] Exciplex probe presented in this paper showed little sequence dependences, indicating that quenching by bases was not so large in our system.

In conclusion, exciplex emission from pyrene and *N*,*N*-dimethylaniline pair was observed in aqueous solution by incorporating chromophores into the middle of ODN. One-base deletion could be detected by using exciplex emission. Our study showed that exciplex emission was observed even in aqueous solution by locating chromophores inside the duplex. Other biological probe utilizing exciplex emission would be also possible by using ODN to provide sufficient hydrophobic environments for exciplex formation.

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References and Notes

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7 See Supporting Information Scheme S1 for synthesis of phosphoramidite monomer containing DMA. All the modified ODNs listed in Scheme 1a were purified by reversed-phase HPLC and characterized by MALDI-TOFMS. MALDI-TOFMS for **P1**: obsd. 4051 (calcd for $[\mathbf{P1}+H]^+$: 4054), **D1**: obsd. 3959 (calcd for $[\mathbf{D1}+H]^+$: 3959), **PD**: obsd. 4369 (calcd for $[\mathbf{PD}+H]^+$: 4368), **PGD**: obsd. 4697 (calcd for $[\mathbf{PGD}+H]^+$: 4697).
8 Supporting Information is available electronically on the CSJ-Journal web site, http://www.csj.jp/journals/chem-lett/.
9 Conditions of the sample solutions were as follows: [NaCl] = 0.1 M, [DNA] = 5 μM, pH 7.0 (10 mM phosphate buffer). 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}.
10 See Supporting Information Figure S1 for the temperature dependence of fluorescence emission spectra of **P1**/**D1**.
11 Reasons for the difference of exciplex emission between **P1**/**D1** and **PD**/**A2** are not so clear at present, because exciplex emission is strongly affected by the microenvironment such as solvent polarity, geometry and quenching by bases as evidenced in Ref. 12.
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15 Single-stranded **PGD** did not show exciplex emission, demonstrating that bulge formation is necessary for this emission. See Supporting Information Figure S4.
16 Excimer emission from two pyrenes is also available for the detection of one-base deletion based on this probe design. One of the advantages for the exciplex emission is that we can control the emission spectra by changing the chromophores.
17 **PGD**/**N** showed larger exciplex emission than other sequences, such as **PAD**/**N**. This may be attributed to the difference in the structure of bulges because melting temperature of **PGD**/**N** was slightly lower than those of other sequences.

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