Fluorometric sensing of the salt-induced B–Z DNA transition by combination of two pyrene-labeled nucleobases†

Akimitsu Okamoto,*[a] Yuji Ochi[a] and Isao Saito*[b]

Received (in Cambridge, UK) 5th November 2004, Accepted 6th December 2004
First published as an Advance Article on the web 17th January 2005
DOI: 10.1039/b416965d

We have developed a new fluorescent DNA sensor containing two pyrene-labeled nucleobases, ^{Pet}G and ^{Py}C, and the fluorescence color was altered by the salt-induced B–Z DNA transtion.

Fluorescence labeling is a powerful tool for probing the structures and interactions of biomolecules.[1] One of the most attractive fluorophores for DNA probes is a pyrene chromophore.[2] Pyrene excimer formation is very sensitive to its environment because of the spatial requirement, and makes pyrene fluorescence a powerful tool for investigating nucleic acid interactions and structures. G-rich DNA sequences are known to often adopt unique conformations, such as Z-DNA duplex, which are biologically significant and structurally interesting.[3] Well-designed pyrene probes could provide useful information on such a conformational transition of G-rich DNA.[4]

Here, we report a new fluorescent DNA probe in which the fluorescence color is altered by the salt-induced B–Z DNA transition. We synthesized CG-alternative oligodeoxynucleotides (ODN) containing two pyrene-labeled nucleobases, and examined their fluorescence properties. Two conformational states of the doubly pyrene-labeled ODN, B-DNA and Z-DNA, showed different colors. This ODN is promising as a fluorescent DNA sensor sensitive to salt concentrations.

We have designed two fluorescent nucleobases for the detection of the B–Z transition, a guanine derivative in which a pyrene chromophore is tethered to the C8 position *via* an ethynyl linker, ^{Pet}G,[5] and a cytosine derivative in which a pyrene is tethered to the C5 position *via* a propargyl linker, ^{Py}C (Fig. 1a). ^{Pet}G will be a fluorescent nucleoside for inducing a unique structure of G-rich

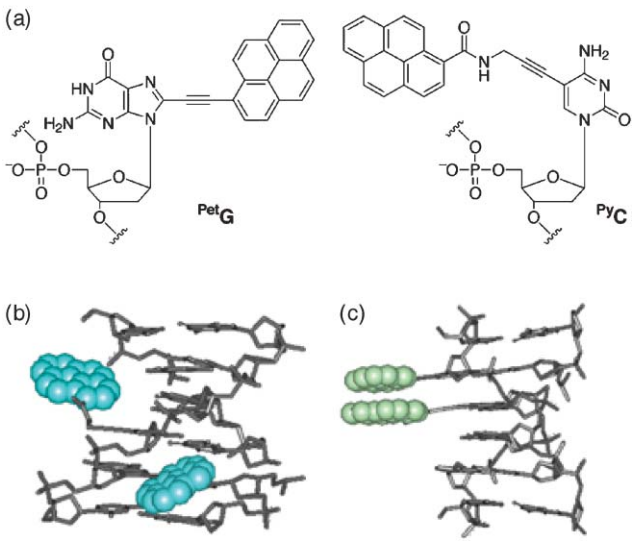
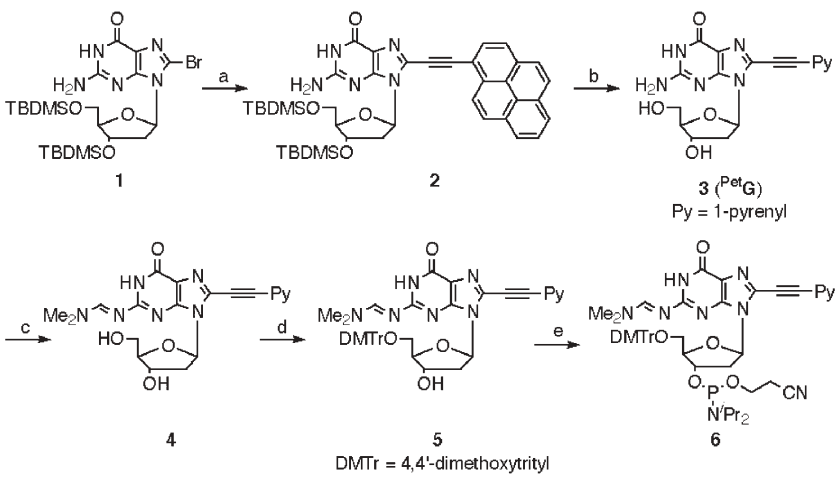


Fig. 1 (a) Structure of ^{Pet}G and ^{Py}C. (b) Molecular modeling of B-DNA containing ^{Pet}G and ^{Py}C, and (c) Z-DNA containing ^{Pet}G and ^{Py}C.

† Electronic supplementary information (ESI) available: Experimental details. See http://www.rsc.org/suppdata/cc/b4/b416965d/

*okamoto@sbchem.kyoto-u.ac.jp (Akimitsu Okamoto)
saito@mech.ce.nihon-u.ac.jp (Isao Saito)



Scheme 1 Reagents and conditions: (a) 1-ethynylpyrene, $Pd(PPh_3)_4$, CuI, triethylamine, DMF, 55 °C, 5 h, 70%; (b) TBAF, THF, rt, 2.5 h, 90%; (c) *N*,*N*-dimethylformamide diethylacetal, DMF, rt, 1 h; (d) 4,4′-dimethoxytrityl chloride, pyridine, rt, 1 h, 60% in 2 steps; (e) $(^{i}Pr_2N)_2PO(CH_2)_2CN$, 1*H*-tetrazole, acetonitrile, rt, 1 h, quantitative.

DNA that contains a *syn* conformation at the *N*-glycosyl bond, because a bulky substituent at C8 of ^{Pet}G will make a *syn* conformation predominant.[6] In contrast, ^{Py}C, which we developed earlier,[7] has an *anti* conformation when ^{Py}C forms a base pair with G, and thus the pyrene chromophore of ^{Py}C was exposed to the outside of the major grove. Therefore, by using the combination of two pyrene-labeled nucleosides, we have designed a new system for fluorometrically monitoring the transition of B- and Z-DNA. In B-DNA, as shown in Fig. 1b, the two pyrenes of ^{Pet}G and ^{Py}C in a 5′-d(^{Pet}G^{Py}C)-3′/5′-d(GC)-3′ duplex are far apart, because the pyrene group of ^{Py}C is located at the major groove and the pyrene group of ^{Pet}G at the minor groove of the duplex as a result of the predominant *syn* conformation. On the other hand, in Z-DNA, where the bases alternate from G with a *syn* conformation to C with an *anti* conformation, both pyrenes of a 5′-d(^{Pet}G^{Py}C)-3′/5′-d(GC)-3′ duplex would be located at the same groove, and stacked together very closely (Fig. 1c). Thus, this pyrene-stacked structure is expected to give the emission of an excited complex.

We synthesized the ODNs containing ^{Pet}G and ^{Py}C bases. ^{Py}C was prepared according to the protocol reported earlier.[7] The synthetic route for ^{Pet}G-containing ODNs is outlined in Scheme 1. *O*,*O*′-Bis(*tert*-butyldimethylsilyl)-8-bromo-2′-deoxyguanosine (**1**)[8] was converted by Sonogashira coupling with 1-ethynylpyrene to give **2** (70%), which was then deprotected with tetrabutylammonium fluoride to produce ^{Pet}G (**3**) (90%, λ_{max} = 386 and 408 nm, ε_{386} = 39 800, ε_{408} = 38 900, λ_{fl} = 480 nm, Φ_F = 0.058 in methanol). The NOESY spectrum of ^{Pet}G shows a correlation between the 1-imino proton and the 5′-hydroxy proton, strongly indicating that ^{Pet}G prefers a *syn* conformation at the *N*-glycosyl bond. The 2-amino and 5′-hydroxy groups of ^{8Py}G were protected to give **5** (60% in two steps), and then quantitatively converted to phosphoramidite **6** for DNA synthesis. The ODNs containing ^{Pet}G and ^{Py}C were efficiently synthesized *via* a conventional protocol, and purified by reverse phase HPLC. The composition of the ODN was determined by MALDI-TOF mass spectrometry.

A CG-alternative DNA is known to form a stable Z-DNA at high salt concentration.[9] We incorporated ^{Pet}G and ^{Py}C into the CG-repetitive sequence. The resulting ODN, ODN(^{Pet}G^{Py}C) 5′-d(CGCGCGCGC^{Pet}G^{Py}CGCG)-3′ (MALDI-TOF $[M-H]^-$ calcd 4771.31, found 4770.92), formed a self-complementary Z-DNA structure at high salt concentration. The CD spectrum of ODN(^{Pet}G^{Py}C) in an aqueous medium of 50 mM sodium phosphate (pH = 7.0) and 4.5 M sodium chloride shows a positive peak at 269 nm and a negative peak at 295 nm, indicative of a typical Z-DNA (Fig. 2a).[10] At a low salt concentration (50 mM sodium phosphate, pH = 7.0, and 0.1 M sodium chloride), the CD spectrum changed to a negative peak at 244 nm and a positive peak at 282 nm, suggesting the formation of the self-complementary B-DNA.[11]

Prior to the investigation of the fluorescence property of ODN(^{Pet}G^{Py}C), we measured the absorption and fluorescence spectra of single pyrene-labeled ODNs, ODN(^{Pet}G) 5′-d(CGCGCGCGC^{Pet}GCGCG)-3′ and ODN(^{Py}C) 5′-d(CGCGCGCG^{Py}CGCG)-3′. The λ_{max} of ODN(^{Py}C) was 346 nm (ε = 17 200) in 50 mM sodium phosphate (pH = 7.0) and 0.1 M sodium chloride, and the λ_{fl} was observed at 400 nm on 350 nm excitation (Fig. 2b). The λ_{fl} of ODN(^{Pet}G) were 38 nm longer than those of ODN(^{Py}C) (λ_{max} = 392, 402, and 423 nm, λ_{fl} = 438 nm on 420 nm excitation). On excitation at 350 nm, which is the λ_{max} of ODN(^{Py}C), the fluorescence intensity of ODN(^{Pet}G) at 438 nm was *ca.* 3.4 times lower than that obtained on 420 nm excitation.

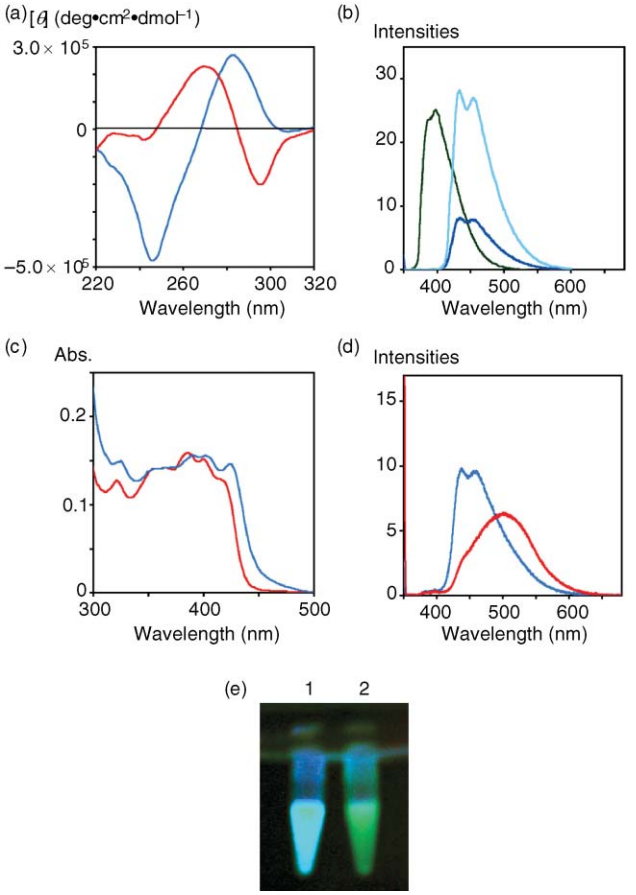


Fig. 2 (a) CD spectra of 5 μM ODN(^{Pet}G^{Py}C) in 50 mM sodium phosphate (pH = 7.0). Blue, with 0.1 M sodium chloride; red, with 4.5 M sodium chloride. (b) Fluorescence spectra of 5 μM each of ODN(^{Pet}G) and ODN(^{Py}C) in 50 mM sodium phosphate (pH = 7.0) and 0.1 M sodium chloride. Excitation wavelength was 350 nm for ODN(^{Py}C) (green) and 350 (blue) and 420 nm (light blue) for ODN(^{Pet}G). (c) and (d) Absorption and fluorescence spectra of 5 μM ODN(^{Pet}G^{Py}C) in 50 mM sodium phosphate (pH = 7.0), respectively. Blue, with 0.1 M sodium chloride; red, with 4.5 M sodium chloride. (e) Fluorescence from a solution of 5 μM ODN(^{Pet}G^{Py}C) in 50 mM sodium phosphate (pH = 7.0). The fluorescence was observed using a transilluminator at 366 nm. Sample 1, with 0.1 M sodium chloride; sample 2, with 4.5 M sodium chloride.

Next, the absorption and fluorescence spectra of a doubly pyrene-labeled ODN, ODN(^{Pet}G^{Py}C) were measured. The absorption spectrum of ODN(^{Pet}G^{Py}C) contained absorption peaks arising from both ^{Pet}G and ^{Py}C (Fig. 2c). For the ODN(^{Pet}G^{Py}C) at high salt concentration, a fluorescence was observed at 507 nm on excitation at 350 nm (Φ_F = 0.028) (Fig. 2d). The fluorescence wavelength from ODN(^{Pet}G^{Py}C) was much longer than those from ODN(^{Pet}G) or ODN(^{Py}C). This fluorescence wavelength was very close to that of a typical pyrene excimer,[12] suggesting that the excited complex of ^{Pet}G and ^{Py}C was formed in the duplex. On the other hand, the B-form ODN(^{Pet}G^{Py}C) at low salt concentration showed a fluorescence at 440 nm (Φ_F = 0.033). Although the duplex was excited at 350 nm where ^{Py}C was effectively excited as shown in Fig. 2b, the fluorescence at 400 nm originating from ^{Py}C

was not observed, and only the fluorescence emission at 438 nm, arising from ^{Pet}G, was observed. This fluorescence wavelength indicated that the fluorescent emission process *via* fluorescence resonance energy transfer from ^{Py}C to ^{Pet}G was predominant in B-DNA. Therefore, B- and Z-form structures of ODN($^{Pet}G^{Py}C$) induced by salt concentration are easily distinguishable, by monitoring the change in the fluorescence wavelength.

Two conformations of duplex ODN($^{Pet}G^{Py}C$) induced by changing the salt concentration showed different visible colors (Fig. 2e). For B-DNA, a strong pale blue fluorescence was observed, whereas a bluish-green fluorescence was emitted by Z-DNA. The change in the fluorescence color facilitates the monitoring of salt-induced conformational transition of ODN($^{Pet}G^{Py}C$).

In conclusion, we were able to monitor B–Z DNA transition using the fluorescence from pyrene-labeled bases. Two pyrene-labeled bases, ^{Pet}G and ^{Py}C, were designed for monitoring the structural change. The fluorescence emitted from the labeled ODN was distinguishable for two conformational states, B- and Z-DNA. This pyrene-labeled CG-alternative ODN is promising as a new optical DNA sensor in which the fluorescence color is altered by changing the salt concentration, and would also be applicable to DNA–protein interaction assays in which CD spectroscopy is not suitable.

Akimitsu Okamoto,*[a] Yuji Ochi[a] and Isao Saito*[b]
[a]*Department of Synthetic Chemistry and Biological Chemistry, Faculty of Engineering, Kyoto University, Kyoto, 615-8510, Japan. E-mail: okamoto@sbchem.kyoto-u.ac.jp; Fax: +81 75 383 2759; Tel: +81 75 383 2757*
[b]*NEWCAT Institute, School 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; Fax: +81 24 956 8924; Tel: +81 24 956 8911*

Notes and references

1 C. Wojczewski, K. Stolze and J. W. Engels, *Synlett*, 1999, 1667; M. E. Hawkins, *Cell Biochem. Biophys.*, 2001, **34**, 257.
2 Y. Saito, Y. Miyauchi, A. Okamoto and I. Saito, *Chem. Commun.*, 2004, 1704; G. T. Hwang, Y. J. Seo, S. J. Kim and B. H. Kim, *Tetrahedron Lett.*, 2004, **45**, 3543; Y. Saito, Y. Miyauchi, A. Okamoto and I. Saito, *Tetrahedron Lett.*, 2004, **45**, 7827; A. Mahara, R. Iwase, Y. Sakamoto, K. Yamana, T. Tamaoka and A. Murakami, *Angew. Chem., Int. Ed.*, 2002, **41**, 3648; F. D. Lewis, Y. Zhang and R. L. Letsinger, *J. Am. Chem. Soc.*, 1997, **119**, 5451.
3 A. H.-J. Wang, G. J. Quigley, F. J. Kolpak, J. L. Crawford, J. H. van Boom, G. A. van der Marel and A. Rich, *Nature*, 1979, **282**, 680; W. Saenger, *Principles of Nucleic Acid Structure*; Springer-Verlag, New York, 1984; B. Hartmann and R. Lavery, *Q. Rev. Biophys.*, 1996, **29**, 309; A. Herbert and A. Rich, *Genetica*, 1999, **106**, 37.
4 T. Kimura, K. Kawai and T. Majima, *Chem. Commun.*, 2004, 268; R. Tashiro and H. Sugiyama, *Angew. Chem., Int. Ed.*, 2003, **42**, 6018; C. Mao, W. Sun, Z. Shen and N. C. Seeman, *Nature*, 1999, **397**, 144.
5 E. Mayer, L. Valis, C. Wagner, M. Rist, N. Amann and H.-A. Wagenknecht, *ChemBioChem*, 2004, **5**, 865.
6 T. D. Son, W. Guschlbauer and M. Gueron, *J. Am. Chem. Soc.*, 1972, **94**, 7903; R. H. Sarma, C.-H. Lee, F. E. Evans, N. Yathindra and M. Sundaralingam, *J. Am. Chem. Soc.*, 1974, **96**, 7337.
7 A. Okamoto, K. Kanatani and I. Saito, *J. Am. Chem. Soc.*, 2004, **126**, 4820.
8 A. Okamoto, K. Kanatani, Y. Ochi, Y. Saito and I. Saito, *Tetrahedron Lett.*, 2004, **45**, 6059; L. C. J. Gillet and O. D. Schärer, *Org. Lett.*, 2002, **4**, 4205.
9 K. Kawai, I. Saito and H. Sugiyama, *J. Am. Chem. Soc.*, 1999, **121**, 1391; S. K. Wolk, C. C. Hardin, M. W. Germann, J. H. van de Sande and I. Tinoco, Jr., *Biochemistry*, 1988, **27**, 6960; A. Rich, A. Nordheim and A. H.-J. Wang, *Ann. Rev. Biochem.*, 1984, **53**, 791.
10 J. H. Riazance, W. A. Baase, W. C. Johnson, Jr., K. Hall, P. Cruz and I. Tinoco, Jr., *Nucleic Acids Res.*, 1985, **13**, 4983.
11 V. A. Bloomfield, D. M. Crothers and I. Tinoco, Jr., *Nucleic Acids: Structures, Properties, and Functions*; University Science Books: Sausalito, CA, 2000.
12 A. Okamoto, T. Ichiba and I. Saito, *J. Am. Chem. Soc.*, 2004, **126**, 8364; K. Yamana, T. Iwai, Y. Ohtani, S. Sato, M. Nakamura and H. Nakano, *Bioconjugate Chem.*, 2002, **13**, 1266.

