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A novel fluorescent guanine derivative distinguishable of three structures, single strand, duplex, and quadruplex

Akimitsu Okamoto,[a,b] Keiichiro Kanatani,[a] Yuji Ochi,[a] Yoshio Saito[b] and Isao Saito[b,c,*]

[a]Department of Synthetic Chemistry and Biological Chemistry, Faculty of Engineering, Kyoto University, Kyoto 615-8510, Japan
[b]SORST, Japan Science and Technology Corporation, Kyoto 615-8510, Japan
[c]NEWCAT Institute, Faculty of Engineering, Nihon University, Tamura, Koriyama 963-8642, Japan

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Abstract—We have constructed a pyrene-labeled guanine base, ^{8Py}G. ^{8Py}G is a novel fluorescent probe for monitoring the secondary structure of G-rich DNA. The fluorescence emitted from ^{8Py}G-labeled oligodeoxynucleotide clearly distinguished three structural states, single strand–duplex–quadruplex. Thus, the technique, which monitors the fluorescence of ^{8Py}G-labeled oligodeoxynucleotide, is a powerful tool for the investigation of DNA structural changes.
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Fluorescence labeling is a powerful tool to probe nucleic acid structure and protein–nucleic acid interactions.[1] One of the most attractive fluorophores for fluorescent DNA probes is a pyrene chromophore.[2] Due to the spatial requirement, pyrene excimer formation is very sensitive to its environment, and makes pyrene fluorescence a powerful tool for investigating nucleic acid interactions and structures.

G-rich DNA sequences are known to adopt various structures, such as B-DNA duplex, Z-DNA duplex, and parallel and antiparallel quadruplexes, which are biologically significant and structurally interesting.[3] Fluorescence could provide useful information about the folding of G-rich DNA. We have designed a guanine derivative in which a pyrene chromophore is tethered to C8 of guanine, ^{8Py}G. ^{8Py}G will be an effective fluorescent probe for the detection of a unique structure of G-rich DNA that contains a *syn*-conformation at the *N*-glycosyl bond,[3] because a bulky substituent at C8 of guanine will make a *syn*-conformation at the *N*-glycosyl bond predominant.[4]

Here we report the synthesis and fluorescence properties of an oligodeoxynucleotide (ODN) probe containing a novel fluorescent nucleobase, ^{8Py}G. ^{8Py}G-labeled ODN was efficiently synthesized from 8-bromo-2′-deoxyguanosine. The fluorescence emitted from ^{8Py}G-labeled ODN clearly distinguished three structural states, single strand–duplex–quadruplex of a G-rich DNA.

The synthetic route for ^{8Py}G-containing ODNs is outlined in Scheme 1. *O*,*O*′-Bis(*tert*-butyldimethylsilyl)-8-bromo-2′-deoxyguanosine (**1**)[5] was converted by Sonogashira coupling with *N*-propargyl-1-pyrenecarboxamide to give **2** (74%), which was then deprotected with tetrabutylammonium fluoride to produce ^{8Py}G (**3**) (94%, $\lambda_{max} = 342$ nm, $\Phi_{342} = 22,600$, $\Phi_F = 0.100$ in methanol). The NOESY spectrum of ^{8Py}G shows the correlation between the 1-imino proton and the 5′-hydroxy proton, strongly indicating that ^{8Py}G prefers a *syn*-conformation at the *N*-glycosyl bond (Fig. 1a). The 2-amino and 5′-hydroxy groups of ^{8Py}G were protected to give **5**[6] (52% in two steps), and then quantitatively converted to phosphoramidite **6** for DNA synthesis. ^{8Py}G-containing ODNs were efficiently synthesized via a conventional protocol. The crude ^{8Py}G-containing ODNs were purified by reverse phase HPLC. The composition of the ODN was determined by MALDI-TOF mass spectrometry.

The sequence $d(T_3G_2)_4$ is known to form a stable quadruplex structure in the presence of potassium ion.[7] We incorporated ^{8Py}G into the sequence (Fig. 1b). The resulting ^{8Py}G-containing ODN, ODN1

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* Corresponding author. Tel.: +81-24-956-8911; fax: +81-24-956-8924; e-mail: saito@mech.ce.nihon-u.ac.jp

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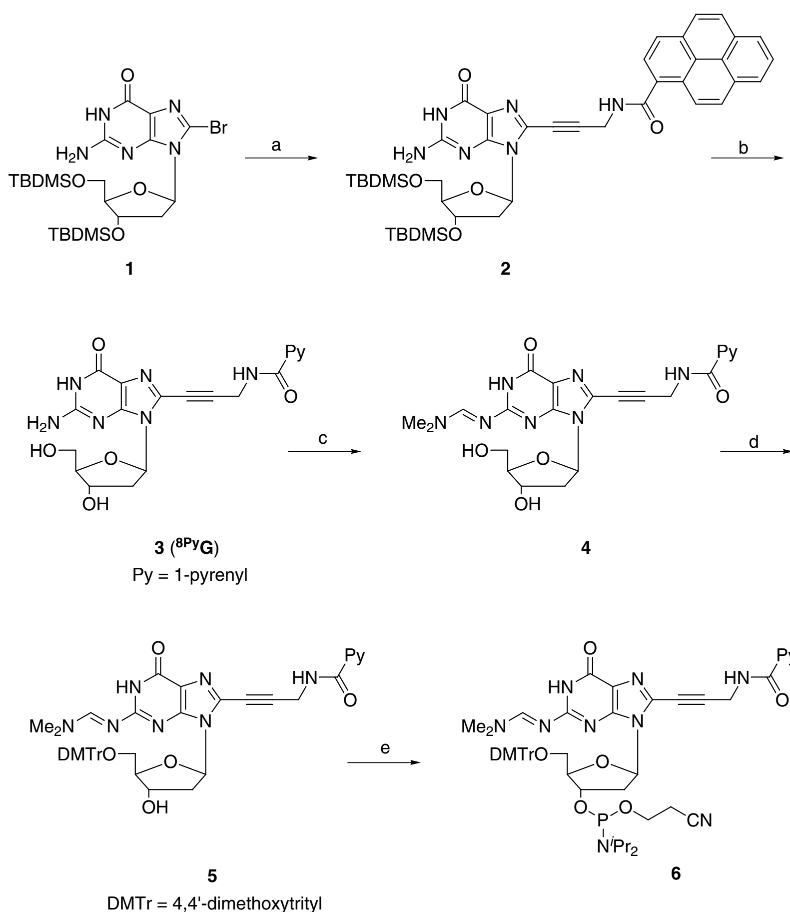

Scheme 1. Reagents and conditions: (a) *N*-propargyl-1-pyrenecarboxamide, $Pd(PPh_3)_4$, CuI, triethylamine, DMF, 55 °C, 1 h, 74%; (b) TBAF, THF, room temperature, 45 min, 94%; (c) *N*,*N*-dimethylformamide diethylacetal, DMF, room temperature, 1 h; (d) 4,4′-dimethoxytrityl chloride, pyridine, room temperature, 3 h, 52% in two steps; (e) $(^iPr_2N)_2PO(CH_2)_2CN$, 1*H*-tetrazole, acetonitrile, room temperature, 30 min, quantitative.

5′-d(TTTGGTTT^{8Py}GGTTT^{8Py}GGTTTGG)-3′ (MALDI-TOF $[M-H]^-$ calcd 6784.62, found 6784.63), formed a quadruplex in the presence of potassium ion. The CD spectrum of ODN1 in the presence of potassium ion shows a positive peak at 293 nm and a negative peak at 261 nm, indicative of a typical antiparallel quadruplex (Fig. 1c).[8] The melting temperature (T_m) of the ODN1 quadruplex was 47.0 °C, which was determined by monitoring the change in the absorbance at 295 nm versus temperature. This quadruplex was more stable than $d(T_3G_2)_4$ ($T_m = 40.0$ °C), suggesting that the quadruplex was stabilized by the incorporation of ^{8Py}G, in which the *syn*-conformation is predominant. By the addition of the complementary strand, ODN1′5′-d(CCAAACCAAACCAAACCAAA)-3′, the CD spectrum changed to a positive peak at 264 nm and a negative peak at 243 nm, suggesting the formation of the B-type ODN1/ODN1′ duplex.[9] The T_m of ODN1/ODN1′ duplex was 51.7 °C, which was lower than that of $d(T_3G_2)_4/d(C_2A_3)_4$ ($T_m = 63.7$ °C).

The λ_{max} of the absorption of ODN1 in the presence of potassium ion was 372 nm (ε =55,000). Thus, the fluorescence spectra of ODN1 under various conditions were measured using excitation at 372 nm (Fig. 1d). The quadruplex state of ODN1 showed a strong fluorescence at 458 nm ($\Phi_F = 0.116$).[10] When the quadruplex was converted to a single-stranded state by heating at 60 °C, the fluorescence wavelength was shifted to 505 nm, which corresponds to a pyrene excimer fluorescence. On the other hand, the addition of the complementary strand ODN1′ greatly weakened the fluorescence at 458 nm. The fluorescence of the duplex showed a very weak monomer fluorescence at 428 and 405 nm ($\Phi_F = 0.007$). These results show that three structures of ODN1, single strand–duplex–quadruplex, are distinguishable by monitoring the change of the fluorescence wavelength and the intensity of ^{8Py}G incorporated into ODN1.

The weak fluorescence observed for the duplex state was easily converted to the strong fluorescence by removing the complementary strand. For example, the fluorescence of ODN1 quadruplex was rapidly quenched by mixing with 5′-overhanged complementary strand, ODN2 (Fig. 2a). However, by the addition of the fully complementary strand of ODN2 (ODN2′), ODN2 was removed from ODN1 to give ODN2/ODN2′ duplex, and the fluorescence of ODN1 at 458 nm recovered rapidly. Therefore, the blinking of the fluorescence of ODN1 became possible by using a DNA-based molecular motor system that was driven by thermodynamic

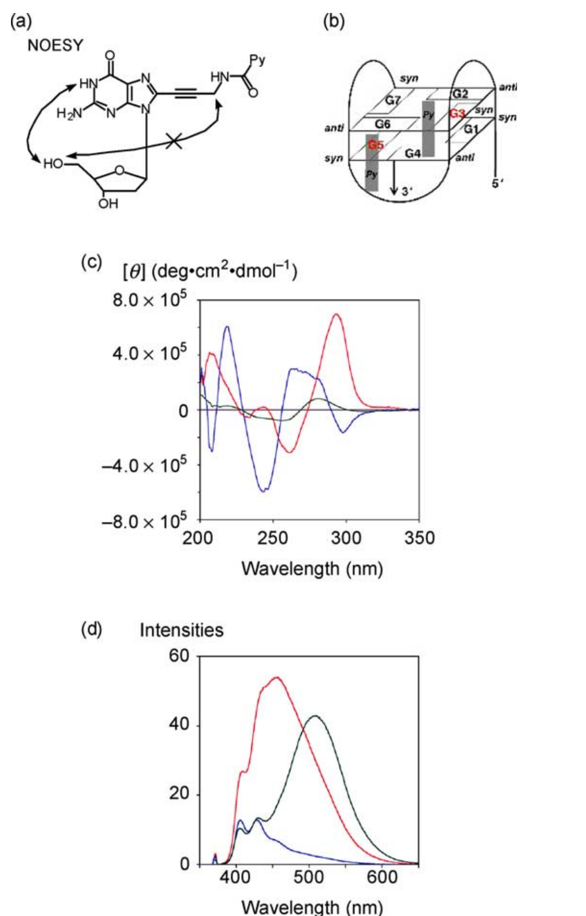

Figure 1. Structure and properties of ^{8Py}G and ^{8Py}G-labeled ODN. (a) Conclusions from the NOESY experiment for ^{8Py}G in DMSO-d_6. The *syn*-conformation at the *N*-glycosyl bond was confirmed. (b) Schematic illustration of ODN1 quadruplex. (c) CD spectra of 5 μM ODN1 in 20 mM potassium phosphate and 100 mM potassium chloride (pH = 7.0). Red, at room temperature; green, at 60 °C; blue, in the presence of ODN1′ at room temperature. (d) Fluorescence spectra of 5 μM ODN1 in 20 mM potassium phosphate and 100 mM potassium chloride (pH = 7.0). Excitation wavelength was 372 nm. Red, at room temperature; green, at 60 °C; blue, in the presence of ODN1′ at room temperature.

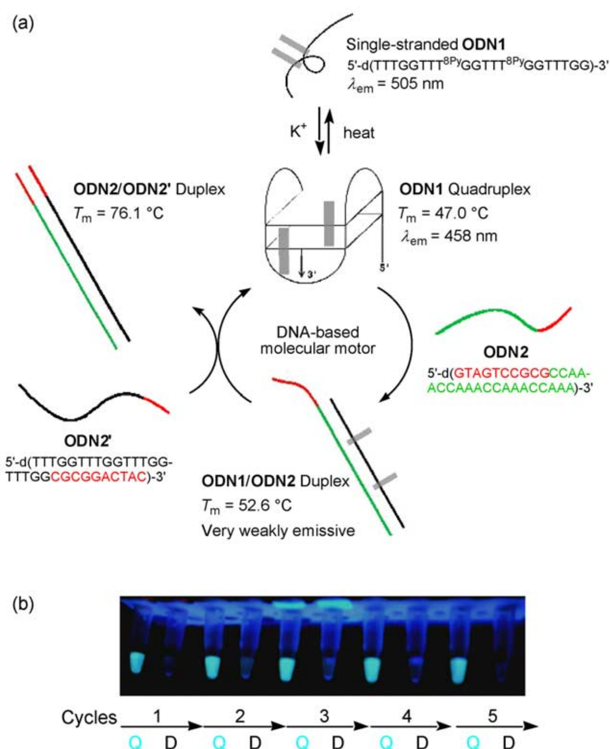

Figure 2. Blinking of the fluorescence of ODN1 using the DNA-based molecular motor system. (a) Schematic illustration of the fluorescence-blinking system. (b) Fluorescence blinking of ODN1 during five cycles. To a solution of 5 μM ODN1 in 20 mM potassium phosphate and 100 mM potassium chloride (pH = 7.0, 1 mL), a solution of 1 mM ODN2 (5 μL) and a solution of 1 mM ODN2′ (5 μL) were added alternately. All operations were performed at room temperature without heating for annealing. Sample solutions were illuminated with a 366 nm transilluminator. Q = ODN1 quadruplex, D = ODN1/ODN2 duplex.

demand,[11] as shown in Figure 2a. As shown in Figure 2b, the repetition of the emission and the quenching of the fluorescence of ODN1 for each cycle of the molecular motor was observed.

In conclusion, we have constructed a pyrene-labeled guanine base, ^{8Py}G. ^{8Py}G is a novel fluorescent probe for monitoring the secondary structure of G-rich DNA. The pyrene chromophore at C8 of ^{8Py}G made a *syn*-conformation predominant. Thus, the conformation of ^{8Py}G facilitated the detection of a unique structure at G-rich DNA that contains a *syn*-conformation. The fluorescence emitted from ^{8Py}G-labeled ODN clearly distinguished three structural states, single strand–duplex–quadruplex. Thus, the technique, which monitors the fluorescence of ^{8Py}G-labeled ODN, can be a powerful method for investigating DNA structural changes.

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