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Pyrene-labeled deoxyguanosine as a fluorescence sensor to discriminate single and double stranded DNA structures: Design of ends free molecular beacons

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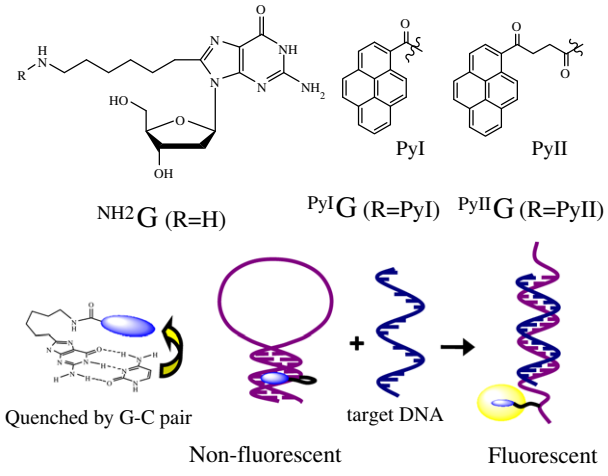
ABSTRACT

A novel fluorescent DNA probe containing pyrene-labeled C8 alkylamino-substituted 2′-deoxyguanosine was designed in order to discriminate single stranded and double stranded regions in DNA. This fluorescent sensor was used for the design of practically useful 3′- and 5′-ends free self-quenched molecular beacon (MB). Unique MB detectable by pyrene excimer fluorescence was also demonstrated.

There have been much current interests in the development of new fluorescent molecular sensors for genetic analysis.[1] In our continuous studies directed toward the design of practically useful fluorescent DNA probes,[2] we reported a new concept for the design of important fluorescent nucleosides such as base-discriminating fluorescent (BDF) nucleosides[3] and microenvironment-sensitive fluorescent nucleosides.[4] We now wish to report a unique fluorescent probe containing pyrene-labeled C8 alkylamino-substituted 2′-deoxyguanosine that can discriminate single stranded and double stranded regions in DNA. By using this strategy, we have developed extremely facile and practically useful 3′- and 5′-ends free self-quenched molecular beacons.[5]

We already reported a self-quenched molecular beacon (MB) with pyrene-labeled pyrrocytidine in the middle region of the stem.[6] Such 3′- and 5′-ends free MB are useful for homogeneous DNA assays as well as for heterogeneous assays using microarray technology, since ends free MBs, unlike conventional MBs containing fluorophore and quencher at both ends,[7] can be easily immobilized on the solid surface. However, for practical application of such ends free MB to high-throughput DNA analysis, a new development of more convenient, less expensive and versatile ends free self-quenched MB is highly desirable.

In our previous paper we demonstrated a facile synthesis of C8 alkylamino-substituted 2′-deoxyguanosine (^{NH2}G) which can be used as a guanine base possessing a flexible universal linker.[8] The easiness of the incorporation of the amino-modifier dG into oligonucleotides and subsequent post synthetic modification with various fluorophores allowed us to design a practically useful ends



Scheme 1. Concept for ends free MB using pyrene-labeled ^{NH2}G.

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free and quencher free MB, where the modifier dG in the stem serves as an efficient quencher for the intercalated fluorophore attached to the alkylamino side chain upon photoillumination (Scheme 1).

The incorporation of just one modified base ^{NH2}**G** into the stem region of MB makes is possible to design ends free MBs containing various fluorophores via post synthetic modification. With its simplicity and high 'on–off' sensitivity, this strategy will find widespread application in homogeneous and high-throughput microarray assays of nucleic acids.

We synthesized C8 aminohexyl-substituted dG (^{NH2}**G**) according to our reported procedure.[8] After converting to its phosphoroamidite derivative, ^{NH2}**G** was incorporated into oligonucleotides by automated DNA synthesizer to give ODN **1** (Table 1). Active ester of pyrene carboxylic acid (**PyI**) was reacted with ODN **1** at room temperature in aqueous $NaHCO_3$ (1 M) in the presence of triethylamine for 16 h and subsequent HPLC purification provided ODN **2** in high yield (Fig. S1).

We next examined the photophysical properties of ODN **2** upon duplex formation with complementary ODN **3**. UV spectra of duplex ODN **2/3** showed a blue shift (~3 nm) as compared with ODN **1/3** (Fig. 1a). Fluorescence spectra of single stranded ODN **2** and duplex ODN **2/3** were shown in Figure 1b. The fluorescence intensity was dramatically decreased by duplex formation, suggesting that the fluorescence of intercalated pyrene chromophore was effectively quenched by the parent dG of ^{PyI}**G** based paired with cytosine in the duplex.[5c] This is consistent with the remarkable thermal stability (ΔT_m = 4.2 °C) of duplex **2/3** as compared with duplex ODN **1/3** (Table 2). These results indicated that ^{PyI}**G** located in a single stranded region is highly emissive, whereas ^{PyI}**G**, once incorporated into duplex, exhibited extremely weak fluorescence due to the efficient quenching by the parent dG.[5c,6] These observations suggest that ^{PyI}**G** can be used as a sensor to discriminate single and double stranded DNA structures.

Table 1
Sequences of oligonucleotides

ODNs	Sequence
1	5′-d(CGCAAT^{NH2}**G**TAACGC)-3′
2	5′-d(CGCAAT^{PyI}**G**TAACGC)-3′
3	5′-d(GCGTTACATTGCG)-3′
4 (MB **1**)	5′-d(TGACAGGTATCCAAGATTGAACT^{NH2}**G**TCA)-3′
5 (MB **2**)	5′-d(TGACAGGTATCCAAGATTGAACT^{PyI}**G**TCA)-3′
6 (MB **3**)	5′-d(TGACAGGTATCCAAGATTGAACT^{PyII}**G**TCA)-3′
7 (MB **4**)	5′-d(AGCAGCTGTATCCAAGATTGAAA^{NH2}**G**CT ^{NH2}**G**CT)-3′
8 (MB **5**)	5′-d(AGCAGCTGTATCCAAGATTGAAA^{PyI}**G**CT^{PyI}**G**CT)-3′
9	5′-d(TTCAATCTTGGATAC)-3′

Table 2
Thermal melting properties and quantum yields

ODNs	T_m (°C)	Φ_F
ODN **1**	–	–
ODN **2**	–	0.094
MB **1**	41.6	–
MB **2**	49.5	0.013
MB **3**	50.8	~0
MB **5**	56.6	0.015
ODN **1/3**	51.6	–
ODN **2/3**	55.8	0.020
MB **1**/ODN **9**	53.7	–
MB **2**/ODN **9**	56.9	0.264
MB **3**/ODN **9**	56.2	0.073
MB **5**/ODN **9**	57.5	0.064

This remarkable fluorescence property of ^{PyI}**G** was used for the design of 3′- and 5′-ends free self-quenched MBs.[5] Thus we incorporated ^{NH2}**G** into the stem region of MB. We also used pyrene derivative **PyII** as a fluorophore in order to get more efficient quenching efficiency by dG and for much longer wavelength of the fluorescence emission.[8] These MBs were prepared via post synthetic modification of ODN **4** (MB **1**) by treatment with active esters of **PyI** and **PyII** as described before (Table 1). As shown in Figure 2, MB **2** and MB **3** showed extremely weak fluorescence in their closed forms. When MB **2** and MB **3** were hybridized with target ODN **9**, strong fluorescence appeared at 400 nm and 460 nm, respectively, at their open forms, indicating that MBs containing ^{PyI}**G** and ^{PyII}**G** in the duplex of the stem serve as an efficient self-quenched MB with high signal to noise (S/N) ratio. Particularly, MB **3** possessing ^{PyII}**G** as a fluorophore is an excellent 'on–off' sensor for its long wavelength emission (~460 nm) and high S/N (73/1) selectivity.

In order to further improve the efficiency and the detection wavelength of ends free MB, the same strategy was applied to the design of an excimer emissive MB (MB **5**) containing two pyrene fluorophores separated by two bases in the middle region of the stem (Scheme 2). We were able to detect the target DNA via the characteristic pyrene excimer emission at 535 nm with a longer lifetime.

As shown in Figure 3a, MB **5** showed only monomer emission in its closed form, whereas in the presence of target ODN **9** excimer fluorescence appeared at 535 nm together with a monomer emission. The fluorescence intensity at 535 nm was specifically measured by means of fluorescence plate reader (Fig. 3b). With a large S/N ratio (53/1) and strong emission at 535 nm as visualized in fluorescence imaging system (Fig. S3), MB **5** can be used as an excellent ends free self-quenched MB. These observations can be explained by considering that in a closed form, two pyrene rings

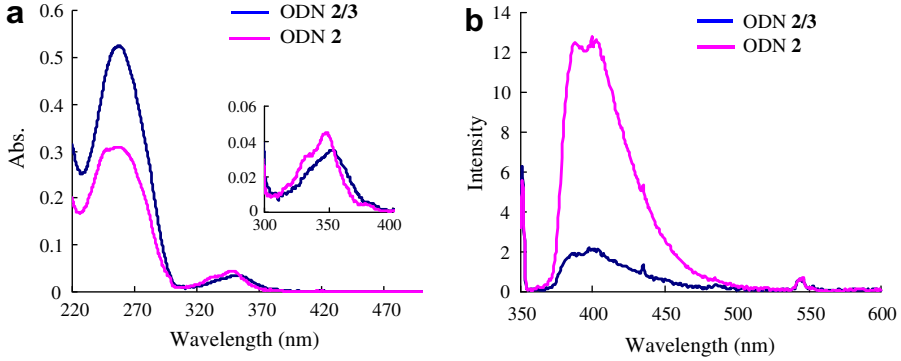


Figure 1. (a) UV–visible spectra of ODN **2** and the duplex formed by hybridization with ODN **3**. (b) Fluorescence spectra of ODN **2** and the duplex formed by hybridization with ODN **3** (2.5 μM, 50 mM sodium phosphate, 0.1 M sodium chloride, pH 7.0, rt). Excitation wavelength was 350 nm.

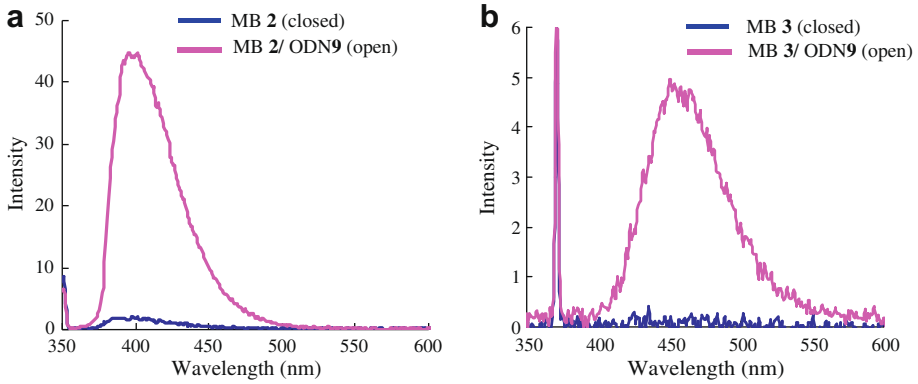
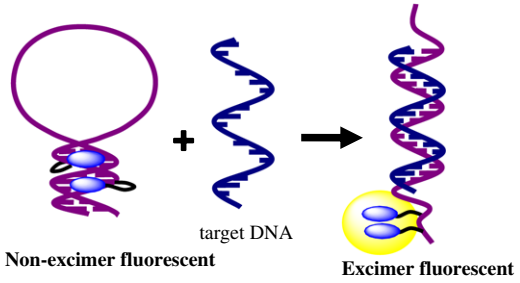


Figure 2. (a) Fluorescence spectra of MB **2** and the duplex with ODN **9** (2.5 μM, 50 mM sodium phosphate, 0.1 M sodium chloride, pH 7.0, rt). (b) Fluorescence spectra of MB **3** and the duplex with ODN **9**. Excitation wavelength was 350 nm for MB **2** and 370 nm for MB **3**.



Scheme 2. Schematic illustration of MB detectable by pyrene excimer emission.

in an opposite site of the stem cannot sterically interact with each other and the excimer formation is prohibited by the intercalation of the pyrene rings into the stem duplex. In contrast, in an open form two pyrene rings located in a single stranded region can easily interact with each other to result in an excimer formation.

In conclusion, we have devised a new fluorescence sensing system using C8 alkylamino-modifier dG to discriminate single and double stranded DNA structures. By using this strategy we have developed extremely facile ends free MBs. We also demonstrated unique MB that is capable of sensing specific DNA sequence by emitting characteristic pyrene excimer fluorescence with a long lifetime.[9] These ends free MBs showed an excellent 'on–off' signals that can be used in homogeneous assays as well as for microarray assays.

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Supplementary data

Supplementary data (experimental procedures, spectroscopic data, and visualized fluorescence image) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.09.060.

References and notes

1. (a) Ranasinghe, R. T.; Brown, T. *Chem. Commun.* **2005**, 5487; (b) Tor, Y., Ed., *Tetrahedron, symposium in print*, **2007**, *63*, *17*.; (c) Wang, K.; Tang, Z.; Yang, C. J.; Kim, Y.; Fang, X.; Li, W.; Wu, Y.; Medley, C. D.; Cao, Z.; Li, J.; Colon, P.; Lin, H.; Tan, W. *Angew. Chem., Int. Ed.* **2009**, *48*, 856.
2. Okamoto, A.; Saito, Y.; Saito, I. *Photochem. Photobiol. C: Photochem. Rev.* **2005**, *6*, 108.
3. Okamoto, A.; Kanatani, K.; Saito, I. *J. Am. Chem. Soc.* **2004**, *126*, 4820.
4. Tainaka, K.; Tanaka, K.; Ikeda, S.; Nishiza, K.-i.; Unzai, T.; Fujiwara, Y.; Saito, I.; Okamoto, A. *J. Am. Chem. Soc.* **2007**, *129*, 4776.
5. (a) Hwang, G. T.; Seo, Q. Y. J.; Kim, B. H. *J. Am. Chem. Soc.* **2004**, *126*, 6528; (b) Seo, Y. J.; Ryu, J. H.; Kim, B. H. *Org. Lett.* **2005**, *7*, 4931; (c) Saito, Y.; Mizuno, E.; Bag, S. S.; Suzuka, I.; Saito, I. *Chem. Commun.* **2007**, 4492; (d) Friedrich, A.; Hoheisel, J. D.; Marme, N.; Knemeyer, J.-P. *FEBS Lett.* **2007**, *581*, 1644; (e) Venkatesan, N. Y.; Seo, J.; Kim, B. H. *Chem. Soc. Rev.* **2008**, *37*, 648.
6. Saito, Y.; Shinohara, Y.; Bag, S. S.; Takeuchi, Y.; Matsumoto, K.; Saito, I. *Tetrahedron* **2009**, *65*, 934.

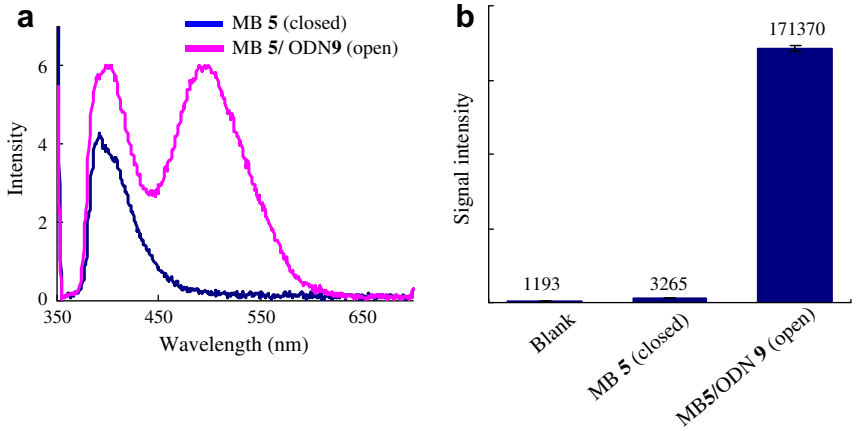

Figure 3. (a) Fluorescence spectra of MB **5** and the duplex formed by hybridization with ODN **9** (2.5 μM, 50 mM sodium phosphate, 0.1 M sodium chloride, pH 7.0, rt). Excitation wavelength was 350 nm. (b) Fluorescence intensity of MB **5** and the duplex formed by hybridization with ODN **9** as determined by fluorescence plate reader. Excitation and detection wavelength were 355 nm and 535 nm, respectively.

7. (a) Tyagi, S.; Kramer, F. R. *Nat. Biotechnol.* **1996**, *14*, 303; (b) Tyagi, S.; Bratu, D. P.; Kramer, F. R. *Nat. Biotechnol.* **1998**, *16*, 49; (c) Sandhya, S.; Chen, W.; Mulchandani, A. *Anal. Chim. Acta* **2008**, *614*, 208.
8. Saito, Y.; Matsumoto, K.; Bag, S. S.; Ogasawara, S.; Fujimoto, K.; Hanawa, K.; Saito, I. *Tetrahedron* **2008**, *64*, 3578.
9. (a) Tong, G.; Lawlor, J. M.; Tregear, G. W.; Haralambidis, J. *J. Am. Chem. Soc.* **1995**, *117*, 12151; (b) Yamana, K.; Iwai, T.; Ohtani, Y.; Sato, S.; Nakamura, M.; Nakano, H. *Bioconjugate Chem.* **2002**, *13*, 1266; (c) Okamoto, A.; Ichiba, T.; Saito, I. *J. Am. Chem. Soc.* **2004**, *126*, 8364; (d) Yamana, K.; Ohshita, Y.; Fukunaga, Y.; Nakamura, M.; Maruyama, A. *Bioorg. Med. Chem.* **2008**, *16*, 78; (e) Conlon, P.; Yang, C. J.; Wu, Y.; Chen, Y.; Martinez, K.; Kim, Y.; Stevens, N.; Marti, A. A.; Jockusch, S.; Turro, N. J.; Tan, W. *J. Am. Chem. Soc.* **2008**, *130*, 336. and references cited therein.