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DNA nanomotor using duplex-quadruplex conformational transition

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ABSTRACT

For DNA nanomotor, we synthesized 8-Py-dG and incorporated it into $d(T_3G_2)_4$ which can form quadruplex. We measured CD spectra for this ODN. The result suggested that this ODN was changed from quadruplex to duplex by the hybridization with the complementary strand. Further, this ODN had a very interesting property of fluorescence. The fluorescent intensity could be controlled whether the complementary strand was existed, or not.

INTRODUCTION

With the advancement of nanotechnology, nanomotors are required to power nanometer-scale devices.[1] Thus, there is a great need for various nanomotors. Recently, DNA is drawing attention as a new tool of nanotechnology[2], because, DNA have a high ability of molecular recognition, and various conformation of DNA can be controlled in nanoscale.

G-rich DNA sequences can form quadruplex. The glycosidic conformation of G residues in quadruplex alternates between *syn* and *anti* conformation.[3] Bulky substituents on G residues at 8-position shift the equilibrium conformation about the glycosidic bond to favor the *syn* conformation.[4] Therefore, G residues, possessing bulky substituents at 8-positon, would stabilize the quaduplex.

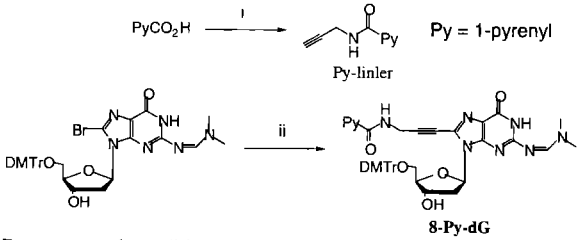
Here we report the synthesis of 8-Py-dG and the incorporation into ODN that can form a quadruplex. The ODN was changed from quadruplex to duplex by the hybridization with the complementary strand. In addition, the fluorescence of pyrene in the ODN dramatically changed with the conformational transition.

RESULTS and DISCUSSION

Synthesis of 8-Py-dG

Synthetic outline is shown in Scheme 1. Functional amide linker, prepared from propargylamine and 1-pyrene carboxylic acid, was coupled with 8-bromo-dG protected by 4,4'-dimethoxytrityl and DMF dimethylacetal. After conversion to the cyanoethyl phosphoramidite, the 8-Py-dG was efficiently incorporated into ODN with an automated DNA synthesizer.

Scheme 1. Synthesis of 8-Py-dG[a]



[a] Reagents and conditions: (i) propargylamine, EDCI, DMF, 34%. (ii) Py-linker, $(Ph_3P)_4Pd$, CuI, Et_3N, DMF, 47%.

Studies on $d(T_3G_2)_4$ containing 8-Py-dG

8-Py-dG was incorporated into the ODN (5'-d(TTTGGTTTGGTTTGGTTTGG)-3' = $d(T_3G_2)_4$), which forms a quadrupulex (**Figure 1a**) in the presence of KCl. When $d(T_3G_2)_4$ forms a quadruplex, the glycosidic conformation of G_3 and G_5 is *syn*, and 8-positions of these G are in the same groove. Therefore, we expected that **ODN-Py** (**Figure 1**), possessing 8-Py-dG at G_3 and G_5 position, could stabilize quadruplex and have characteristic fluorescence.

First, CD spectra were measured for **ODN-Py** (**Figure 1b**). In the absence of the complementary strand, a positive CD peak was observed in the region of 280-320 nm, whereas in the presence, the CD profile resembled to B-form DNA. The result suggested that **ODN-Py** could form quadruplex in the abcence of complementary strand, and the conformation of **ODN-Py** was changed from quadruplex to duplex by the addition of complementary strand.

Next, we measured fluorescence spectra for **ODN-Py** (**Figure 1c**). The remarkably strong fluorescence was observed for quadruplex, whereas the fluorescence of duplex was weak. The quantum yield for the quadruplex was 15 times larger than that of the duplex. The fluorescence of **ODN-Py** was controlled with the complementary strand.

CONCLUSION

In conclusion, we synthesized a bispyrene-modified quadruplex. The fluorescence of the quadruplex was controlled with the complementary ODN, and this fluorescence-switching system should act as a well-regulated nanomotor.

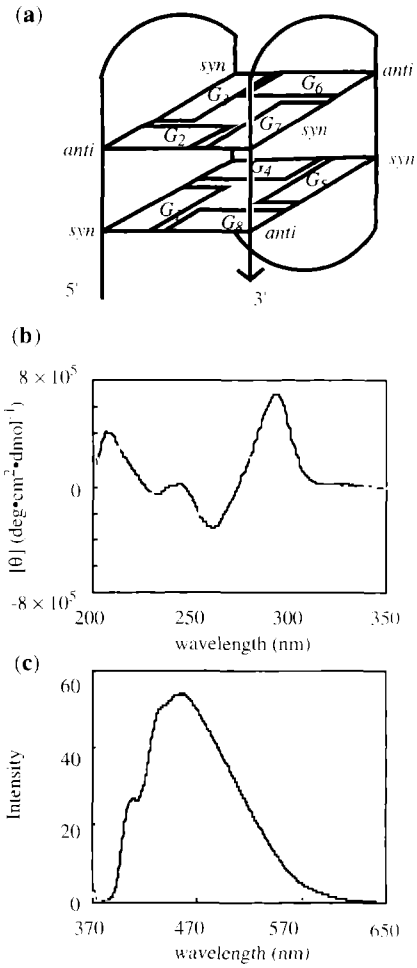


Figure 1. (a) Schematic illustration of the quadruplex. 5'-d(TTTG$_1$G$_2$TTTG$_3$G$_4$TTTG$_5$G$_6$TTTG$_7$G$_8$)-3'. (b) and (c) CD spectra and fluorescence spectra of **ODN-Py** 5'-d(TTTGGTTT8-PyGGTTT8-PyGGTTTGG)-3'. 5 μM ODN was measured in 20 mM potassium phosphate and 100 mM KCl (pH 7.0) and complementary strand is absent (black line) or present (gray line). Excited wavelength was 372 nm.

REFERENCES

1. Soong, R. K.; Bachand, G. D.; Neves, H. P.; Olkhovets, A. G.; Craighead, H. G.; Montemagno, C. D. *Science* **2000**, *290*, 1555.

2. Yurke, B.; Turberfild, A. J.; Mill, A. P.; Simmel, F. C.; Neumann, J. L. *Nature* **2000**, *406*, 605..

3. Dias, E.; Battiste, J. L.; Williamson, J. R. *J. Am. Chem. Soc.* **1994**, *116*, 4479.

4. Sarma, R. H.; Lee, C.H.; Evans, F. E.; Yathindra, N.; Sundaralingham, M. *J. Am. Chem. Soc.* **1974**, *96*, 7337.