

Design of a molecular beacon PNA

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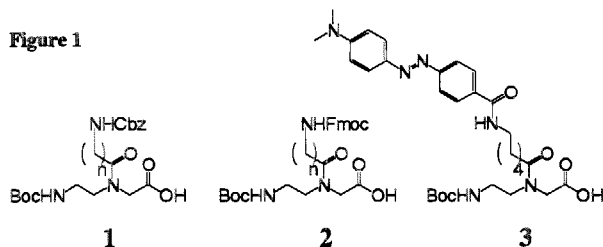
ABSTRACT

We have designed a novel dual-labeled PNA oligomer, having both a fluorescent dye and a quencher, by utilizing key compounds 1 and 2. We showed that the designed dual-labeled PNA oligomer works as a molecular beacon PNA. We also investigated the optimization of a stem-loop structure which can supersensitize the function as a molecular beacon PNA.

INTRODUCTION

A peptide nucleic acid (PNA) is a biopolymer in which the DNA sugar-phosphate backbone has been replaced by a pseudopeptide.(1) PNAs are superior to natural nucleic acids in numerous ways.(2) For example, (i) they can be easily synthesized by solid-phase *t*Boc/Fmoc chemistry, (ii) they are highly stable against cellular nucleases/proteases, and (iii) they can hybridize with complementary DNA with high affinity in comparison with conventional nucleic acids. Modifying conventional techniques that employ DNA as a medium so that they can be used with PNA can compensate for the defects of DNA that could not be overcome previously. Molecular beacons have been recently developed as probes that can detect a specifically recognized base sequence by fluorescence.(3) Since molecular beacons use DNA as a medium, which has poor enzymatic resistance, a PNA can be applied to the

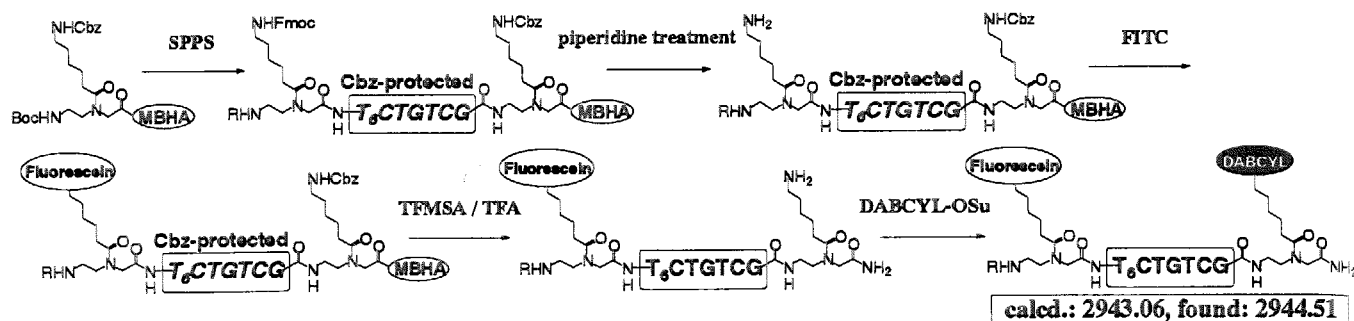
molecular beacon for higher enzymatic resistance. We have recently developed a strategic synthetic protocol which can prepare a number of photoactive PNA monomers.(4) In addition, we have found that many photoactive molecules having a carboxyl group can be introduced into the PNA backbone using the key compounds 1 and 2 (Fig. 1) and that the photoactive moiety can be incorporated into any position of the PNA oligomer.(5) We herein report that a novel molecular beacon PNA was designed using 1 and 2 and this could efficiently detect a specific target sequence by fluorescence.



RESULTS AND DISCUSSION

We examined the synthetic protocol for a dual-labeled PNA oligomer in which a fluorescent dye such as TAMRA and a quencher such as a DABCYL group were introduced at opposite ends. We first tried to synthesize a dual-labeled PNA oligomer using a DABCYL-tethered PNA monomer unit 3 (Fig. 1). However, the DABCYL moiety decomposed at the release step from the MBHA resin using a TFMSA/TFA solvent system, although each coupling

Scheme 1. Synthetic protocol of a dual-labeled PNA oligomer using the key compounds 1 and 2



reaction of PNA monomer in solid phase synthesis was fine. This result suggested that the DABCYL moiety would need to be post-synthetically introduced into the PNA oligomer after the release step. We have already designed 1 which can be deprotected by the TFMSA/TFA solvent system. We therefore synthesized a precursor PNA oligomer containing 1. Simultaneously, we also selected 2 as a precursor for the post-synthetic introduction of a fluorescent moiety into the PNA oligomer (Scheme 1). MALDI-TOF MS of the designed PNA oligomer, which was purified by HPLC, suggested that a dual-labeled PNA oligomer, having both a fluorescent dye and a quencher, could be effectively prepared by utilizing 1 and 2.

We next investigated whether or not the designed dual-labeled PNA oligomer could work as a molecular beacon PNA. A photoactive PNA 16 mer 4, which consisted of 4 base pairs for the stem region and 6 bases for the loop region in addition to a fluorescent dye and a quencher at the opposite ends, was synthesized as a molecular beacon PNA. When 4 hybridized with the complementary DNA oligomer in 10 mM Tris-HCl buffer (pH 8.0), the fluorescence intensity increased (Fig. 2). In addition, the fluorescence intensity rose gradually until the ratio of PNA (10 μ M) to DNA was ca. 1 : 1 and it then remained at that level even if extra DNA was added (Fig. 2). These results suggest that the designed dual-labeled PNA oligomer 4 became a molecular beacon PNA having sequence specificity.

Optimization of the molecular beacon PNA is currently being carried out using several dual-labeled PNA oligomers having different stem-loop structures.

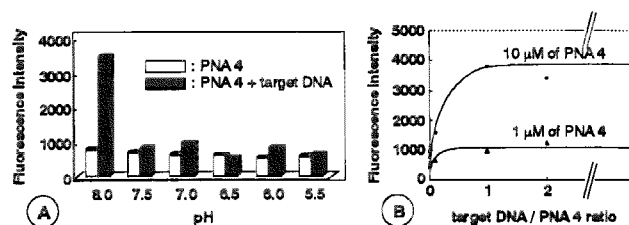


Figure 2. (A) Effects of pH on the formation of the molecular beacon PNA. (B) Effects of ratio on the hybridization of PNA 4 with target DNA. Conditions: PNA 4 (H-DCATGTCTAAGCATGT-NH₂; D = DABCYL, T = TAMRA) in 10 mM TE buffer.

ACKNOWLEDGEMENT

This study was supported by a Grant-in-Aid from the New Energy and Industrial Technology Development Organization (NEDO), Japan.

REFERENCES

- Nielsen, P. E.; Egholm, M.; Berg, R. H.; Buchardt, O. *Science* **1991**, *254*, 1497-1500.
- For most recent review, see: Nielsen, P. E. *Curr. Opin. Biotechnol.* **2001**, *12*, 16-20.
- Tyagi, S.; Kramer, F. R. *Nature Biotechnology* **1996**, *14*, 303-8.
- (a) Ikeda, H.; Fujimori, F.; Murakami, Y.; Nakamura, Y. *Nucleic Acids Symp. Ser.* **2001**, *45*, 177-8. (b) Ikeda, H.; Nakamura, Y.; Saito, I. *Tetrahedron Lett.* **2002**, *43*, 5525-8.
- (a) Ikeda, H.; Saito, I. *Japanese patent application* No. 2000-268638 and No. 2000-394669. (b) Ikeda, H.; Saito, I.; Nakamura, Y. *International patent application* PCT/JP01/07696. (c) Ikeda, H.; Saito, I.; Kitagawa, F. *International patent application* PCT/JP01/08120. (d) Ikeda, H.; Tonosaki, M. *Japanese patent application* No. 2001-285191 and No. 2002-121667.