

Synthesis and properties of a novel molecular beacon containing a benzene-phosphate backbone at a stem moiety

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ABSTRACT

This paper describes the synthesis and properties of a novel molecular beacon (MB) containing a benzene-phosphate backbone at the stem moieties. Fluorescent intensity of MBs was found to be stabilized by introducing a benzene-phosphate backbone at stem moieties.

INTRODUCTION

Sequence-specific probes for detecting target nucleic acids are becoming increasingly important as tools for monitoring various biological processes and for other biological applications. Molecular beacons (MBs) are self-reporting, nucleic acid probes with a structure including complementary terminal arm sequences and a loop that is complementary to a target sequence.¹ MBs carry a fluorophore and a quencher at the termini and, when not hybridized, form a closed structure that brings these labels into juxtaposition, thus quenching fluorescence. Upon binding to a complementary target, MBs adopt an open conformation in which the labels are spatially separated. As a result, MBs brightly fluoresce in the presence of nucleic acid target sequences. However, MB design is known to not be as simple as attaching arbitrary arm sequences onto previously designed linear probes. Stem arms can also interact with flanking target sequences, changing the hybridization specificity, and constantly adapting the arms to avoid such interactions, if undesired, increases design complexity.²

Conversely, we have recently reported the synthesis and properties of a nucleic acid analog consisting of a benzene-phosphate backbone.³ The building blocks of the nucleic acid analog are composed of bis(hydroxymethyl)benzene residues connected to nucleobases *via* the biaryl-like axis. Thermal denaturation study of duplexes revealed that a nucleic acid analog with a benzene-phosphate backbone forms a thermally and thermodynamically stable duplex in itself, whereas the analog does not form a thermally stable duplex with complementary natural DNA or RNA. We thus envisioned that, if we could introduce the analog into the stem moieties of an MB, fluorescent intensity of the MB would be stabilized, as unnecessary interactions between target RNA and stem moieties would be avoided, allowing

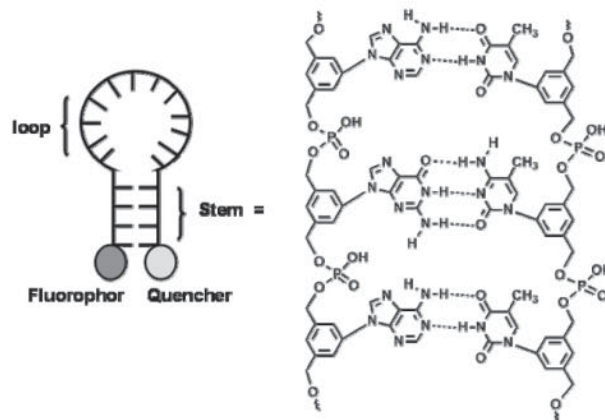


Fig. 1. A structure of a novel molecular beacon.

highly sensitive detection of target RNA. In this paper, we report the synthesis and properties of novel MBs containing the benzene-phosphate backbone at stem moieties (Fig. 1).

RESULTS AND DISCUSSION

Synthesis of MBs. We chose an mRNA of a human RNase H (position 140–156) as a target sequence. Sequences of MBs and target RNAs used in this study are shown in Fig. 2. MB1 comprised natural DNA and 2'-*O*-methyl-RNA, while MB2 comprised 2'-*O*-methyl-RNA and the nucleic acid analog with the benzene-phosphate backbone. RNA1 had a natural sequence, whereas RNA2 included a sequence complementary to the stem moiety of the MBs. RNA3 contained 4 mismatched bases.

UV Melting Studies of MBs. Stabilities of hairpin conformations of the MBs were studied by thermal denaturation. The T_m value (51.6 °C in 1.0 M NaCl) of MB2 containing the benzene-phosphate backbone was found to not differ substantially from that of MB1 composed of natural DNA and 2'-*O*-methyl-RNA (56.3 °C in 1.0 M NaCl). This indicates that the benzene-phosphate moieties of MB2 can form a thermally stable hybrid under test conditions.

Detection of target RNAs by MBs. Each MB was allowed to anneal with target RNAs. Fluorescence increased as a function of target RNA concentration (Fig. 3a, b). When RNA1 and RNA2 with complementary sequences were

MB1: 5'-Fluorescein-d(GCAAGC)-2'-O-Me(CCGGUCCACUUGUGCUC)-d(GCUUGC)-Dabcyl-3'

MB2: 5'-Fluorescein-b(GCAAGC)-2'-O-Me(CCGGUCCACUUGUGCUC)-b(GCUUGC)-Dabcyl-3'

RNA1: 3'-r(GUCGUCCUUUGGCCAGGUGAACACGAGACGUGAGUAA)-5'

RNA2: 3'-r(GUCG**CGUUC**GGGCCAGGUGAACACGAGACGUGAGUAA)-5'

RNA3: 3'-r(GUCGUCCUUUGG**ACAGCUGAU**CACCAGACGUGAGUAA)-5'

Fig. 2. Sequences of oligonucleotides used in this study. b indicates oligomers composed of a benzene-phosphate backbone. 2'-O-Me indicates oligomers composed of 2'-O-methylnucleosides. Underlined letters indicate complementary nucleosides to loop regions of MBs. Bold letters indicate complementary nucleosides to stem region of MBs. Italic letters indicate mismatched nucleosides.

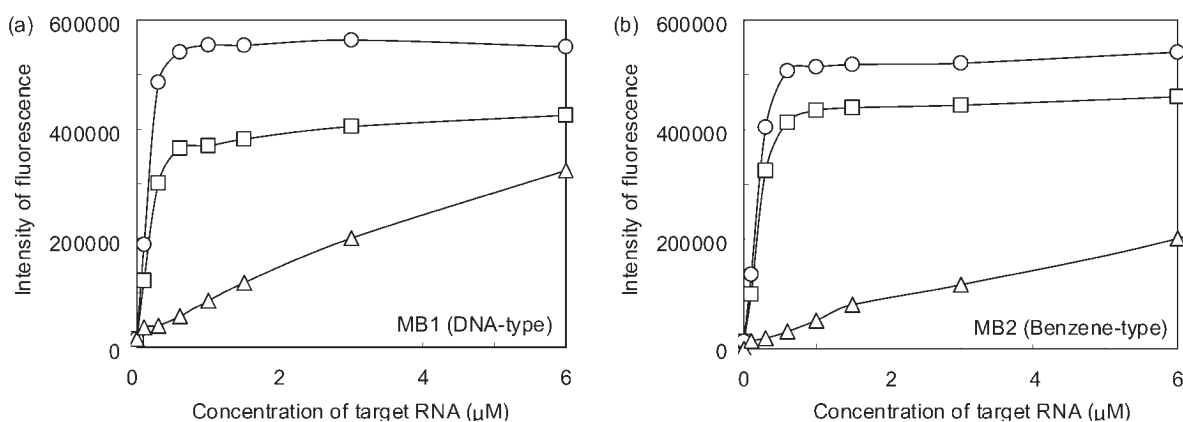


Fig. 3. Detection of target RNAs by MBs. Squares indicate intensities of fluorescence from MBs with RNA1 as a target. Circles indicate intensities of fluorescence from MBs with RNA2 as a target. Triangles indicate intensities of fluorescence from MBs with RNA3 as a target. Concentration of MB1 or 2 is 0.3 μM . Excitation wavelength: 485 nm. Emission wavelength: 535 nm. (a) MB1 was used as a probe. (b) MB2 was used as a probe.

used as targets, fluorescence plateaued near 0.5 μM of target RNA. On the other hand, when RNA3 containing 4 mismatched bases was used as the target, fluorescence increased gradually and did not reach a plateau even at 3.0 μM of target RNA. This indicates that these MBs can discriminate target RNAs from 4-nucleotide mismatched RNA. Furthermore, the difference in fluorescence intensities between MB2:RNA1 and MB2:RNA2 hybrids with excess amounts of RNA targets turned out to be smaller than that between MB1:RNA1 and MB1:RNA2 hybrids.

CONCLUSION

The present study demonstrated the synthesis and properties of novel MBs containing a benzene-phosphate backbone at the stem moieties. The problem of sequence dependency of fluorescence intensity from MBs was found to be improved by introducing the benzene-phosphate backbone at stem moieties.

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