

Monitoring of Eco RI-catalyzed cleavage reaction of fluorescent-labeled heterochiral DNA

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ABSTRACT

We have found the unusual reactivity of a heterochiral oligodeoxynucleotide toward restriction endonuclease Eco RI. To conduct the kinetic analysis of the reaction, fluorescent-labeled single-stranded oligodeoxynucleotide molecular beacons were designed and synthesized. The beacons showed a remarkable fluorescence response by addition of Eco RI. The results promise that the beacon could be an effective tool for the kinetic analysis of Eco RI-catalyzed cleavage reaction of the heterochiral oligodeoxynucleotide.

INTRODUCTION

We have reported the solution structure of a heterochiral dodecadeoxynucleotide containing a nucleotide residue with unnatural L-configuration.¹ The self-complementary heterochiral 12-mer formed a relatively stable duplex and the L-nucleotide residue participated the Watson-Crick base-pairing with the complementary residue. Some of the heterochiral 12-mers, which contain an L-nucleotide residue in or around a canonical Eco RI recognition sequence, were found to be hydrolyzed by Eco RI. This result indicates that the heterochiral DNAs retain a B-form structure. One of the heterochiral 12-mers, which has an unnatural L-deoxyguanosine residue at the 3'-neighboring site of the Eco RI recognition sequence, showed much more enhanced susceptibility toward the Eco RI-catalyzed reaction than natural homochiral one. In order to demonstrate the origin of the enhanced susceptibility of the heterochiral 12mer toward Eco RI, we designed oligonucleotide molecular beacons² based on fluorescence resonance energy transfer (FRET)³ to establish a real-time monitoring system of the Eco RI-catalyzed reaction.⁴ The homo- (I) and heterochiral (II) oligonucleotide beacons form a stem-loop structure and contain the Eco RI recognition sequence in the stem region, which carry a 5'-fluorescent dye (FAM) and a 3'-

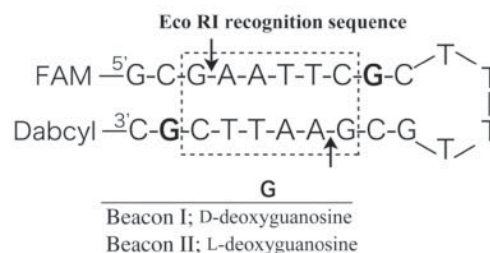


Fig. 1 Structures of the dye-labeled homo- and heterochiral beacons containing a canonical Eco RI recognition sequence. Arrows indicate the cleavage sites.

nonfluorescent quenching dye (Dabcyl) (Figure 1). We carried out the comparative study of Eco RI-catalyzed cleavage reaction of homo- and heterochiral DNAs by using Beacons I and II.

RESULTS AND DISCUSSION

A phosphoramidite unit of L-deoxyguanosine was synthesized by the reported procedure.⁵ FAM amidite and Dabcyl CPG were purchased from Glen Research Co. Oligonucleotides were synthesized by an Applied Biosystems DNA/RNA synthesizer Model 392.

The thermal stability of Beacons I and II was examined (Figure 2) and their T_m values were determined to be

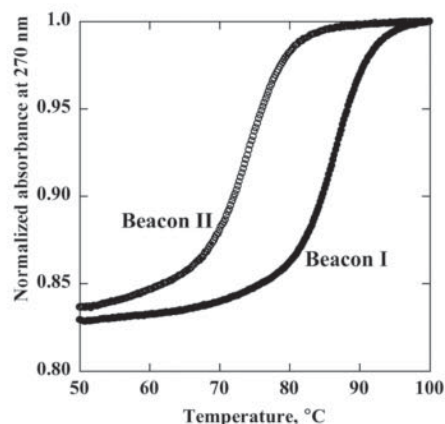


Fig. 2 UV melting curves of Beacons I and II. The beacon concentration was 4 μ M in 0.1 M NaCl, 10 mM MgCl₂, 1 mM DTT and 50 mM Tris-HCl (pH 7.5).

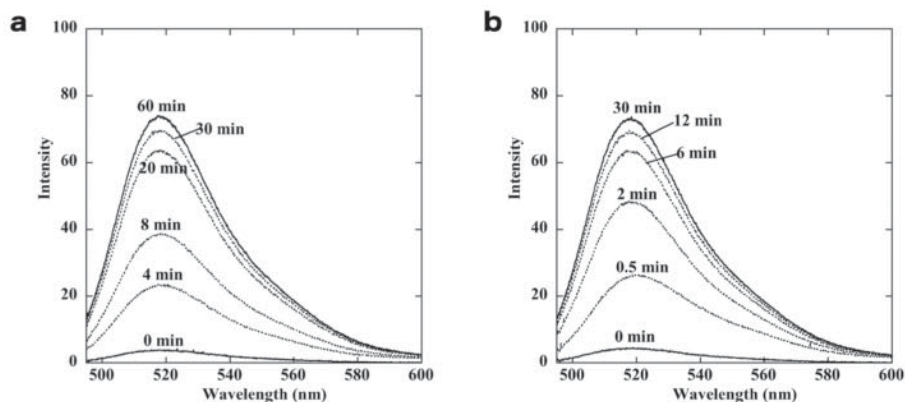


Fig. 3 Fluorescence spectral change (excitation at 485 nm) of Beacons I (a) and II (b) by addition of Eco RI. The beacon concentration was 3 nM in 0.1 M NaCl, 10 mM MgCl₂, 1 mM DTT and 50 mM Tris-HCl (pH 7.5) at 37°C.

86.7°C and 74.0°C, respectively. Thus, the stem-loop structure of the beacons has enough stability under the Eco RI-catalyzed cleavage reaction at 37°C. These beacons showed somewhat higher thermal stabilities compared to the parental non-labeled homo- and heterochiral oligonucleotides whose *T_m* values are 82.6°C and 70.3°C, respectively. Hydrophobic interactions between these dyes and/or between the dyes and hydrophobic portions of the oligonucleotides might raise the thermal stability of the beacons.

The Eco RI-catalyzed cleavage reaction was carried out at 3 nM beacon in the same buffer as UV melting experiments. The fluorescent emission of the FAM group of the beacons is quenched by the adjacent Dabcyl group when the beacons form a hairpin structure. However, scission at the cleavage sites in the stem by the enzyme leads to separation of the dyes from each other. Consequently, the increase of the fluorescent signal corresponding to the extent of DNA cleavage is observed. Indeed, the intensity of the fluorescence emission for Beacons I and II was increased in a time-dependent manner by addition of Eco RI (Figure 3). The intensity change of Beacon II was much faster than that of Beacon I. This result indicates that Beacon II is much more rapidly hydrolyzed by Eco RI than Beacon I, which is consistent with the earlier observation described above. To assess the origin of the unusual reactivity, the competition experiments are useful, in which mixtures of a fixed amount of the beacon and varying amounts of a non-labeled oligonucleotide that has the same sequence as the beacon are employed. We will describe the full details of the competition experiments and discuss the results.

CONCLUSION

In conclusion, we have synthesized the molecular beacons, which have a fluorescent FAM dye and a non-fluorescent Dabcyl quenching dye for monitoring Eco RI-catalyzed cleavage reaction of DNA. The beacons showed an effective fluorescent response for Eco RI-catalyzed reaction and could detect the difference between the susceptibilities of homochiral and heterochiral oligonucleotides toward Eco RI. The beacon could be a powerful tool for mechanistic study of Eco RI-catalyzed hydrolytic reaction.

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