http://dx.doi.org/10.5012/bkcs.2013.34.3.723

Modified Molecular Beacon for Detection of Protein and Its Application for Assay of Streptavidin

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Received January 8, 2013, Accepted January 17, 2013

Key Words : Molecular beacon, Protein sensor, Streptavidin

A molecular beacon is a widely used and well understood probe employed in oligonucleotide assays. The beacon is basically a modified oligonucleotide probe with a special loop-stem structure with a fluorophore and quencher attached on each end. The loop region comprises the oligonucleotide probe, which can detect a complementary target nucleic acid. When the beacon binds to a target molecule, the hairpin stem region unwinds and becomes fluorescent.[1-3] Recently, molecular beacons have been employed as biosensors for understanding biological processes, including real-time detection of PCR reactions, genetic analyses, and in molecular beacon probe array technology.[4-8]

Although the detection of a single-stranded DNA binding (SSB) protein has been reported, the major targets of molecular beacons have been limited to oligonucleotides.[9-12] In this study, a modified molecular beacon that has a ligand to bind the target protein was designed to expand the detection limits of the molecular beacon to proteins and develop a new facile protein assay system (Figure 1).

In the proposed molecular beacon, a modified T-base with an attached organic ligand is placed in a stem duplex sequence. In the absence of the target protein, the beacon is configured in a hairpin structure with no fluorescence. However, when the target protein is added, binding of the protein to the ligand molecule forms a complex between the molecular beacon and protein. Because the target protein is generally much larger in size than the molecular beacon, sufficient steric hindrance to destabilize the loop-stem structure should be induced. Finally, the binding of protein to the ligand molecule triggers the molecular beacon to open and become fluorescent.

To verify the concept experimentally, 6 molecular beacons, each having a biotin group as a ligand (to detect streptavidin as the target protein), were designed and synthesized.[13] All beacons have the same 5 base pair stem duplex sequence with different loop region length (Table 1). One biotin-modified thymidine (biotin-dT) is positioned in the stem region of each molecular beacon to maximize the steric effects of the bound protein. Following convention, each probe has a dual-labeled system: fluorescein, and quencher, dabcyl (4-(4'-dimethylaminophenyl-azo)benzoic acid).

Figure 1. General design of protein assay with a modified molecular beacon. The probe changes its conformation from a hybridized form (dark) to a dissociated form (bright) upon binding of the protein.

In order to perform a steptavidin assay, about 50 pmol of each molecular beacon was mixed with 0, 1/8, and 1/4 molar equivalent of streptavidin (0, 6.25, and 12.5 pmol, respectively) in a 96 well PCR plate (Bio-Rad, CA, USA). Tris buffer (10 mM, pH 8.5, containing 50 mM KCl and 2.5 mM $MgC1_2$) was added until the total volume was 50 uL. The PCR plate was incubated for about 30 min at 25 ºC. The temperature dependence of the fluorescence signal of each well was measured with an iCycler iQ real time PCR machine (Bio-Rad, USA) following the melting profile protocol.[14] Fluorescence measurement data of the six probes at different concentrations of streptavidin are shown in Figure 2. As expected, in the absence of the streptavidin, low fluorescence was observed for each molecular beacon at low temperature. This illustrates that each probe formed a loop-stem conformation (hybridized conformation). As temperature was increased, the fluorescence intensity increased due to melting of the double strand DNA in the stem region, and structural changes occurred to the dissociated conformation. Upon addition of the streptavidin, a substantially enhanced fluorescence signal was observed, even at low temperature.

This shows that binding of streptavidin to the stem region effectively destabilized the hairpin structure, enough to open the loop-stem conformation. Although steric hindrance induced

Table 1. Sequences of modified molecular beacon probes

Molecular Beacon	Sequence
MB5-4	5'-**F**[a]-GCAGGACTACC**B**[b]GC-**D**[c]-3'
MB5-6	5'-**F**-GCAGGACTTTACC**B**GC-**D**-3'
MB5-8	5'-**F**-GCAGGACTCATTACC**B**GC-**D**-3'
MB5-15	5'-**F**GCAGGATACTCATTACCATACC**B**GC-**D**-3'
MB5-25	5'-**F**-GCAGGATACTCATTAGCGACGAACACCATA CC**B**GC-**D**-3'
MB5-35	5'-**F**-GCAGGATACTTAGACCAACACATTAGCGAC GAACACCATACC**B**GC-**D**-3'

[a]F: fluorescein, [b]B: biotin-modified thymidine (biotin-dT), [c]D: dabcyl

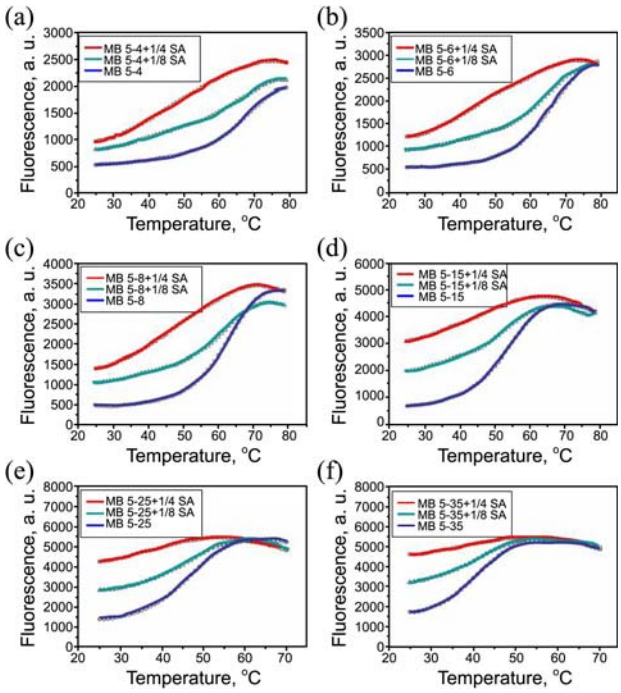

Figure 2. Temperature dependence of the fluorescence measurement of molecular beacons at different streptavidin concentrations.

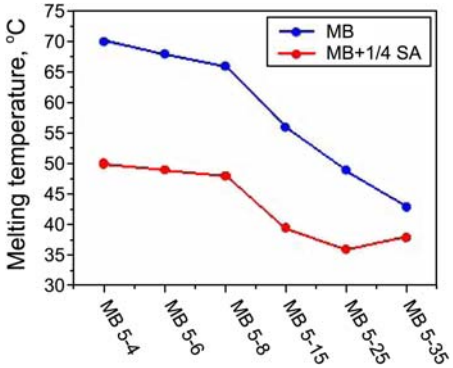

Figure 3. Melting temperature of the molecular beacon probes.

by introduction of a large protein adjacent to the loop-stem structure can be considered as a major destabilizing force, the exact mechanism has yet to be determined. It is worth noting that T_m (melting temperature) values estimated in Figure 2 in the presence of streptavidin are slightly lower than those obtained in the absence of protein (Figure 3).

This illustrates that protein binding should destabilize DNA hybridization in the stem region. More precise and systematic T_m measurements to explain the effects of protein binding are being carried out, and the results will be reported in the near future. Additionally, at a certain temperature, the increase in the fluorescence signal was proportional to the amount of added streptavidin. This provides additional evidence that opening of the hairpin structure is dependent on the protein binding. Under optimized conditions, this methodology can be applied for quantitative analysis of proteins.

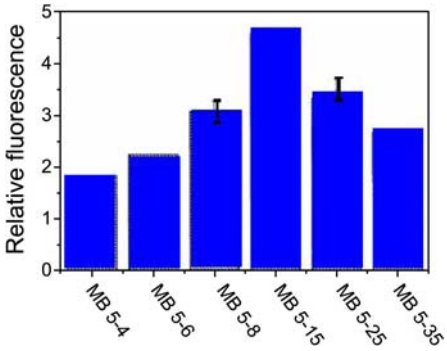

Figure 4. Relative fluorescence of molecular beacon probes.

Relative fluorescence of each molecular beacon, *i.e.*, the fluorescence intensity with 1/4 molar equivalent of streptavidin at 25 °C divided by that without protein, is shown in Figure 4.

The data presented in this figure illustrates that enhancement of the fluorescence signal is sensitive to the loop length, and the maximum value is observed when the number of nucleotides in the loop region is 15. It is also suggested that adjusting the length of the loop region can additionally optimize the efficiency of the molecular beacon.

In conclusion, structurally modified molecular beacons for protein detection were designed and successfully applied for a streptavidin assay. We hope the present study will be a first step toward expanding the detection capabilities of molecular beacons to various proteins and other biomolecules. Further optimization studies on the structural design of molecular beacons and choice of dye and quencher should improve the efficiency of modified molecular beacons as protein assay probes.

References

1. Goel, G.; Kumar, A.; Puniya, A. K.; Chen, W.; Singh, K. *J. Appl. Microbio.* **2005**, *99*, 435.
2. Tan, W.; Fang, X.; Li, J.; Liu, X. *Chem. Eur. J.* **2000**, *6*, 1107.
3. Bonnet, G.; Tyagi, S.; Libchaber, A.; Kramer, F. R. *Proc. Natl. Acad. Sci.* **1999**, *96*, 6171.
4. Komurian-Pradel, F.; Perret, M.; Deiman, B.; Sodoyer, M.; Lotteau, V.; Paranhos-Baccalà, G.; André, P. *J. Virol. Methods* **2004**, *116*, 103.
5. Yao, G.; Fang, X.; Yokota, H.; Yanagida, T.; Tan, W. *Chem. Eur. J.* **2003**, *9*, 5686.
6. Du, H.; Disney, M. D.; Miller, B. L.; Krauss, T. D. *J. Am. Chem. Soc.* **2003**, *125*, 4012.
7. Cao, Z.; Suljak, S. W.; Tan, W. *Current Proteomics* **2005**, *2*, 31.
8. Frauendorf, C.; Jäschke, A. *Bio. Med. Chem.* **2001**, *9*, 2521.
9. Li, J. J.; Geyer, R.; Tan, W. *Nucleic Acids Research* **2000**, *28*, e52.
10. Fang, X.; Li, J. J.; Tan, W. *Anal. Chem.* **2000**, *72*, 3280.
11. Heyduk, T.; Heyduk, E. *Nature* **2002**, *20*, 171.
12. Dummitt, B.; Chang, Y. H. *Assay and Drug Development Technologies* **2006**, *4*, 343.
13. Molecular beacon probes were custom synthesized from Operon Biotechnologies Inc.; 2211 Seminole Drive, Huntsville, Alabama 35805, USA; http://www.operon.com; The structures of fluorescein, dabsyl and biotin-dT are shown on the Operon's website.
14. The measuring of melting temperature is carried out following the literature on the Bio-Rad Laboratories, Inc. website; http://www.bio-rad.com/cmc_upload/Literature/ 38493/4006200E.pdf