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Terminal protection of a small molecule-linked loop DNA probe for turn-on label-free fluorescence detection of proteins

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ABSTRACT

A novel label-free turn-on fluorescence biosensor for the determination of streptavidin (SA) was proposed. Using terminal protection of small molecule-linked DNA chimeras, which can protect DNA from degradation by various exonucleases when the small molecule moieties are bound to their protein target, we designed a loop probe, where the 3′-end was modified with biotin to resist digestion by exonucleases in the presence of target SA. Coupled with an intercalating dye, SYBR Green I, strong enhancement of the fluorescence signals was obtained compared with that in the absence of SA. A linear correlation equation was obtained for SA from 0 to 200 nM with a limit detection of 0.4 nM. This strategy holds great promise for practical applications with good specificity and sensitivity.

1. Introduction

Investigations of small molecule–protein interaction are of great importance in chemistry, biology (Stockwell, 2004) and medicine (Howitz et al., 2003), which can not only help to reveal the mechanisms of many important physiological processes, but also provide the opportunities for molecular diagnostics, anticancer therapies (Vassilev et al., 2004) and biomedical research. Numerous analytical methods are proposed for the detection of proteins (Wang et al., 2005) and small molecules (Kanoh et al., 2003). Monoclonal antibodies and aptamers are typically used for protein detection (Wu et al., 2010), but generally, aptamers appears to have more advantages such as low molecular weight, simple and reproducible synthesis, thermal and long-term stability, reusability, and signal transduction (Famulok et al., 2007). However, the limited number of aptamers and proteins with RNA aptamers restricts their applications. In addition, RNA aptamers are unstable and easily degraded by ribonucleases. The capability of signal transduction of many aptamer assays is not enormous (Iliuk et al., 2011; Lubin and Plaxco, 2010). Oligonucleotides terminally tethered with protein binding small molecules may represent an ideal option for the detection of these proteins. (Wu et al., 2009) developed a novel method using the binding of small molecules linked to DNA by protein, called terminal protection of small molecule linked DNA (TPSMLD). The small organic molecules that bind to target protein receptors with reasonable affinity can resist degradation by the 3′ single-strand-specific exonuclease I. This interesting finding led to the detection of DNA (Wu et al., 2011) and proteins (Cao et al., 2012; He et al., 2013; Zhao et al., 2014).

Several biosensors are constructed based on TPSMLD, including electrochemical (Cao et al., 2012; Wu et al., 2009), fluorescence (Chen et al., 2014; He et al., 2013; Wu et al., 2011) and colorimetric methods (Yang and Gao, 2014; Zhao et al., 2014). In the fluorescence DNA analysis, some signal amplification strategy such as rolling circle amplification (Wang et al., 2013a) and other signal amplification technology (Wang et al., 2014; Wang et al., 2013a) can achieve a lower detection limit, but they usually need fluorescent reporters, which cause complex procedures, high cost and lower reproducibility. The development of a simple, inexpensive and label-free biosensor should be more attractive.

In this work, with a combination of Exo I and Exo III, we proposed a universal strategy for constructing a label-free fluorescence dsDNA biosensor for the sensitive detection of proteins. Owing to the specific properties of Exo I and Exo III, the DNA probe could be digested completely in the absence of target proteins, thus avoiding the incomplete digestion influence, and the sensor

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generating only a low background signal. Compared to other structures, like DNA labeled with fluorophore and quencher (Ou et al., 2013; Zhou et al., 2013), two single stranded DNA (Yang and Gao, 2014) and reorganized G-quadruplex structure (Wei et al., 2012; Zhao et al., 2014), the single-stranded DNA probe with two stem-loop structures could reduce the difficulty of design about DNA, avoid non-specific hybridization interference and effectively reduce the experiment costs. In the experimental process, this method has simple steps to operate and need less reaction time. In our design, streptavidin (SA) was chosen as a model target to conduct this experiment.

2. Materials and methods

2.1. Reagents and materials

Exonuclease I (Exo I) and exonuclease III (Exo III) were purchased from TaKaRa Biotechnology Co., Ltd. (Dalian, China.); and SA, and SYGB Green I (SG I) from the Sangon Biotechnology Co., Ltd. (Shanghai, China). The original SG I solution was 10,000-fold concentrated and diluted to 100-fold with ultrapure water before use. All other chemicals were of analytical grade and used without additional purification. All solutions in the experiments were prepared using ultrapure water which was obtained through a Millipore Autopure WR600A system (Millipore, Ltd., U.S.A.) and had an electric resistance $\geq 18\ M\Omega$.

The oligonucleotides used in this work were synthesized by the Sangon Biotechnology Co., Ltd. (Shanghai, China) and the sequences of the synthesized oligonucleotides(5′→3′) are as follows:

CGTCGAACGAGACTTTTTTTTTTGTCTCGTTCGACGCGGCA-CATTGCGTTTTTTTTTTCGCAATGTGCCG-biotin.

2.2. Fluorescence measurement

The fluorescence spectra were recorded at room temperature in a quartz cuvette on an F-4500 spectrophotometer (Hitachi, Japan). The excitation wavelength was 497 nm, and the emission wavelengths were in the range 510–600 nm with both excitation and emission slits of 5 nm.

2.3. Terminal protection based fluorescence assays for the detection of streptavidin

The DNA concentration was estimated on a Nanodrop ND-1000 (Thermo Scientific, USA) with the DNA absorbance at 260 nm. First, a 10 μL aliquot of buffer solution (100 mM Tris–HCl, 10 mM $MgCl_2$, 20 mM DTT, pH 8.0) containing 1 μM biotin-linked loop DNA was denatured at 90 °C for 5 min and cooled to room temperature for further experiments. Then, 10 μL SA (final concentration range from 0 to 800 nM) was added to the DNA solution and the mixture was incubated for 30 min at room temperature to allow the complete interaction between SA and biotin. After that, Exo I and Exo III (final concentration, 0.5 U/μL) were added to the mixture to perform the digestion reaction for 60 min Then, SG I (final concentration, 2.5-fold) was added and well-mixed. Finally, the mixture was diluted to 200 μL and its fluorescence intensity measured.

2.4. The verification of terminal protection based SA-biotin reaction using polyacrylamide gel electrophoresis (PAGE)

An 8% native polyacrylamide gel was prepared for gel electrophoresis analysis using $1 \times TAE/Mg^{2+}$ buffer (tris 40 mM, acetic acid 20 mM, Na_2EDTA 2 mM, pH 8.0, Mg^{2+} 12.5 mM). The sample solutions were prepared as follows: 4 μM DNA and 800 nM SA in 20 μL buffer solution was incubated for 30 min, and then 20 U Exo I and 20 U Exo III were added, followed by another one hour digestion. The blank sample solution was prepared in the same way but without SA, and the control sample was the DNA probe solution (4 μM) without any enzyme treatment. The gel ran at 100 V for 90 min in $1 \times TAE/Mg^{2+}$ buffer. Then, it was stained in stains-all solution (0.1 g stains-all, 450 mL formamide, and 550 mL H_2O) for 30 min After this procedure, the gel was illuminated under sunlight for 5–10 min to obtain the stained bands. Finally, the PAGE results were photographed using a digital camera.

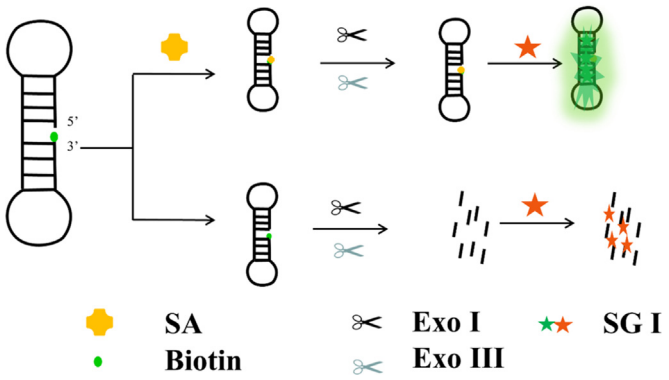
3. Results and discussion

3.1. Design principle of the strategy

In this work, we designed a label-free, fluorescence assay for SA detection based on the terminal protection of SA-biotin-DNA against exonucleases digestion. The sensing mechanism is illustrated in Scheme 1. In this sensing design, the DNA probe was designed as a single-stranded molecule that formed a self-complementary structure at both ends with two stem–loop structures, and biotin was covalently conjugated to the 3′-end. In the absence of SA, DNA-small molecule chimeras could be completely digested to mononucleotides after the addition of Exo I and Exo III. Because of the properties of Exo I and Exo III, the DNA probe section of double strands was degraded stepwise from the blunt 3′-end to 5′-end direction by Exo III at first, and then Exo I catalyzed the removal of 3′-end mononucleotides from the single-stranded DNA fragments. Both Exo I and Exo III were applied to digest the DNA probe to minimize the fluorescence background to an extremely low level. In the presence of SA, the biotin conjugated DNA probe was bound to SA with high affinity, the complex of SA-biotin DNA have steric hindrance effect to protect the DNA probe from degradation by Exo I and Exo III, and maintained the double-strand structure. A strong fluorescence signal was obtained after staining with SG I, and the fluorescence intensity was positively related to the concentration of the protein target SA.

3.2. Assay validation

To demonstrate the feasibility of this proposed approach, the fluorescence intensities of the SA-biotin-DNA probe solution in the absence/presence of the target SA, and with the addition of Exo I and Exo III, were first measured. As shown in Fig. 1(A), in the presence of SA, bright fluorescence of the DNA probe solution was observed (curve a) upon the addition of SG I. This result indicated the effective binding of SA to the ds-DNA probe, which resisted the



Scheme 1. Schematic illustration of the label-free fluorescence strategy for the selective detection of SA based on terminal protection of small molecule-linked loop DNA.