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Highly sensitive chemiluminescence biosensor for protein detection based on the functionalized magnetic microparticles and the hybridization chain reaction

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ABSTRACT

An ultrasensitive chemiluminescence (CL) biosensor for the detection of protein is developed in this study based on the functionalized magnetic microparticles (MMPs) and the hybridization chain reaction (HCR). First, the primer hybridized with the thrombin aptamer conjugated on the surface of MMPs. Then the HCR was triggered by part of the primer and its products were assembled on the surface of the MMPs. Through the interaction between streptavidin and biotin, the streptavidin-horseradish peroxidase (SA-HRP) was coupled with the HCR products. In the presence of thrombin, the HCR products conjugating with SA-HRP were released from the surface of MMPs after the aptamer recognized and bound to its target molecule. So the released SA-HRP in the supernatant produced a significant chemiluminescence imaging signal after the addition of H_2O_2-luminol. The detection limit of thrombin with this method could be as low as 9.7 fM. Besides, the sensing strategy was modified by changing the adding order of reagents that was then successfully applied in the detection of thrombin in complex sample. What's more, the DNA detection also could be carried out with this method, which demonstrated the universality of the proposed sensing strategy.

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1. Introduction

Protein, related to a variety of life activities, is a key to the metabolism (Vaart et al., 2000). Due to its critical roles, highly sensitive and selective protein detection methods are of great importance in clinical medicine, biology (Wang et al., 2015a, 2015b; Deng et al., 2014), food safety (Garber et al., 2010; Yamada et al., 2004), and disease diagnosis (Chikkaveeraiah et al., 2012; Li et al., 2012a, 2012b; Zhang et al., 2015). Thrombin, one kind of important protein, plays a key role in the cascade of blood coagulation proteases (Fuglestad et al., 2013), and is a major mediator of cellular processes, such as nerve cells, activation of platelets, and erythrocyte, which are related to tumor metastasis and cardiovascular disease (CVD) (Desai et al., 2011). Over the past two decades, the detection of thrombin has attracted many research interests. In order to improve the sensitivity of the methods, a great many new materials, such as quantum dots (Choi et al., 2006; Liu et al., 2013a, 2013b), Au nanowires (Huang et al., 2008), and silver nanoparticles (Tung et al., 2012; Li et al., 2012a, 2012b), have been applied to the detection of thrombin. In addition, the functionalized magnetic microparticles (MMPs) were also used for the thrombin detection (Ding et al., 2015). Owing to the flexibility and simplicity of MMPs functionalization, the antibody or DNA aptamer can be easily conjugated to the surface of MMPs with the effect of chemical coupling or physical adsorption. What's more, the magnetic separation will effectively reduce or eliminate the interferences from complex matrix (Tram et al., 2014). As a result, a higher sensitivity could be provided by magnetic separation.

Recently, various signal amplification strategies based on nucleases, such as rolling circle amplification (RCA) (Liu et al., 2014a, 2014b), isothermal strand-displacement polymerase reaction (ISDPR) (Guo et al., 2009), and the target DNA recycling amplification with endonuclease or exonuclease were employed in the biosensing to achieve stronger signals. Hybridization chain reaction (HCR), as a potential DNA amplification technique, can offer great signal amplification through alternating copolymers from a cascade of hybridization events without the involvement of nucleases (Xuan et al., 2014). There are two auxiliary probes, H1 and H2, which is triggered by an initiator and can be assembled into long nicked double-stranded DNA (dsDNA) structures (Wu et al., 2015). The amplification technique has been applied for DNA

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detection effectively, such as Itamar's group used the amplified optical detection of DNA by Mg^{2+}-dependent DNAzyme subunits with a sensitivity corresponding to 10 fM (Wang et al., 2011). Compared with the nuclease-involved signal amplification, the HCR can be employed under harder conditions without fear of nuclease inactivation (Liu et al., 2013a, 2013b). These advantages make HCR an excellent isothermal signal amplification technique which was widely applied in the various detections, especially in the complex biological environment (Yao et al., 2015; Wang et al., 2015a, 2015b), for instance, combined with the horseradish peroxidase (HRP)-mimicking DNAzyme acted as catalytic label for chemiluminescent readout, people could detection as low as 100 fM DNA under real sample preparation conditions (Shimron et al., 2012).

To reach more sensitive detection, the detection methods with lower background were employed. Chemiluminescence (CL), whose light emission from a chemical reaction, is an effective analytical technology with low background (Jilani et al., 2011; Zong et al., 2014). Among the common CL reactions, the horseradish peroxidase (HRP)-catalyzed luminol-PIP-H_2O_2 reaction with PIP as the enhancer, allowing a higher sensitivity and steady-state light signal, is suitable for CL imaging detection (Luo et al., 2014). A strong catalytic effect on the system is produced by the metal ions, metal complexes and DNAzyme (Liu et al., 2014a, 2014b; Cheglakov et al., 2007). For example, the analysis of DNA with a detection limit corresponding to 1 aM based on CL imaging and RCA (Wang et al., 2014). Besides, protein detection can also reach a sensitive level conjugated HCR and CL, such as the detection of carcinoembryonic antigen with a sensitivity corresponding to 0.5 fg/mL (Zhou et al., 2013). However, the horseradish peroxidase (HRP) with biological activity was used more widely in biosensing during the past years. Hence, the system of HRP-catalyzed luminol-p-iodophenol (PIP)-H_2O_2 reaction was applied in the protein detection frequently.

Herein, we constructed a sensitive CL biosensor for the detection of thrombin based on the functionalized MMPs and HCR. As shown in Scheme 1A, the aptamer of thrombin on the surface of MMPs hybridized with a segment of primer whose remaining segment can trigger HCR. Through a cascade of hybridization events, the biotinylated H_1 and H_2 were assembled into long-range double-stranded DNA structures with nicks on the surface of the MMPs. Then, the streptavidin-horseradish peroxidase was loaded into the HCR products as a result of the interaction between SA and biotin. In the presence of thrombin, the aptamer on the surface of MMPs would combine with thrombin because the intermolecular force between aptamer and thrombin is stronger than that between aptamer and primer. Therefore, the HCR products would release to supernatant, and finally a strong CL signal produced in the supernatant. While in the absence of thrombin, the HCR products still linked on the surface of MMPs, and the supernatant just catalyzed CL substrate very slowly to present weak CL signal. Our present work shows that the linear H_1 and H_2 could be adsorbed to the surface of MMPs, which will increase the background signal. In order to solve this problem, the hairpin structure DNA instead of the linear DNA was used in this strategy (Scheme 1B). Two different aptamer of thrombin were used in this model, and one of them was modified on the surface of MMPs. In the presence of thrombin, a sandwich structure was formed through the binding between thrombin and two aptamers. Then the remaining segment of hairpin aptamer 2 triggered the HCR which was similar to Scheme 1A. As the SA-HRP complexes were existed on the surface of MMPs instead of the supernatant, so the interferences from the supernatant could be avoided, this was favor to detect thrombin in complex biological environment. Compared with previous studies (Shimron et al., 2012; Zhou et al., 2013), we used HRP instead of DNAzyme to reduce experiment step by avoiding the addition of Hemin. And two different models have been developed by changing the adding order of reagents. Also, the introduced magnet beads made an easy separation from complex biological environment. By changing the sequence of aptamers, this biosensor was extended to detect DNA sequence (Fig. S1). Also, other proteins could be detected by adding their aptamers.

2. Experiment

2.1. Materials and reagents

The carboxylated MMPs (1.02 μm, 10 mg mL^{-1}) were purchased from Invitrogen (Norway). The carboxylate-modified polystyrene (PSM) (0.05–0.1 μm, 2.5% w/v) was obtained from Aladdin (Shanghai, China). Trihydroxymethyl aminomethane (Tris), sodium phosphate dibasic, sodium phosphate monobasic dihydrate, potassium chloride, magnesium chloride, sodium chloride, Tween-20, ethylenediaminetetraacetic acid disodium salt (EDTA), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), streptavidin- horseradish peroxidase (SA-HRP), bovine serum albumin (BSA), and N-hydroxysuccinimide (NHS) were obtained from Sigma-Aldrich (St. Louis, MO, USA). All above are of analytical-reagent grade or better. Hydrochloric acid was purchased from Sinopharm Chemical Reagent Co., Ltd. Thrombin was obtained from Shanghai Linc-Bio Co., Ltd. And the HRP substrate kit was purchased from Millipore. Functionalized magnetic microparticles were stored in TE buffer (Tris 20 mM, EDTA 1 mM, pH 7.4). The composition of SA-HRP stock solution was 10 mM Na_2HPO_4, 10 mM NaH_2PO_4, and 15 mM NaCl (pH 7.4). Then the washing buffer is 10 mM Na_2HPO_4 and 10 mM NaH_2PO_4 (0.1% (w/v) Tween-20, pH 7.4). All oligonucleotides with different sequences were synthesized and high-performance liquid chromatography (HPLC) purified by Sangon Biotechnology Co. Ltd. (Shanghai, China). The sequences of the oligonucleotides are as follows:

Thrombin aptamer: 5′-NH_2-TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTA GTC CGT GGT AGG GCA GGT TGG GGT GAC T-3′
Primer: 5′-ACC TGG GGG AGT ATT TTT TTT TAG TCA CCC CA-3′
H_1: 5′-biotin-TAC TCC CCC AGG TGC CCC TCA GAC C-3′
H_2: 3′-biotin-CGG GGA GTC TGG ATG AGG GGG TCC A-5′
Completely complementary DNA: 3′-TCA GGC ACC ATC CCG TCC AAC CCC ACT GA-5′
A DNA: 3′-NH_2-TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTG GTT GGT GTG GTT GG-5′;
B DNA: 3′-NH_2-TTT TTT TTT TAT GAG GGG GTC CA-5′
Hairpin aptamer1: 5′-AGT CCG TGG TAG GGC AGG TTG GGG TGA CTT T-NH2-3′
Hairpin aptamer2: 5′-GGT TGG TGT GGT TGG TTT TTT TTT TCA GCG GGG AGG AAG-3′
Hairpin H_1: 5′-CTT CCT CCC CGC TGA CAA AGT TCA GCG GGG-biotin-3′
Hairpin H_2: 3′-biotin-GTT TCA AGT CGC CCC GAA GGA GGG GCG ACT-5′

Ultrapure water was produced by a Milli-Q Academic purification set (Millipore, Bedford, MA, USA). Polystyrene microplates (Costar) were used as carriers of CL imaging. The CL images were recorded by a ChemiDoc XRD system (Bio-Rad). The pH of solutions was measured by a pB-10 potentiometer (Sartorius). The AFM images were finished by Tapping Mode AFM (Nanoscope IIIa Multimode, Veeco Co., USA).

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