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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.

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.

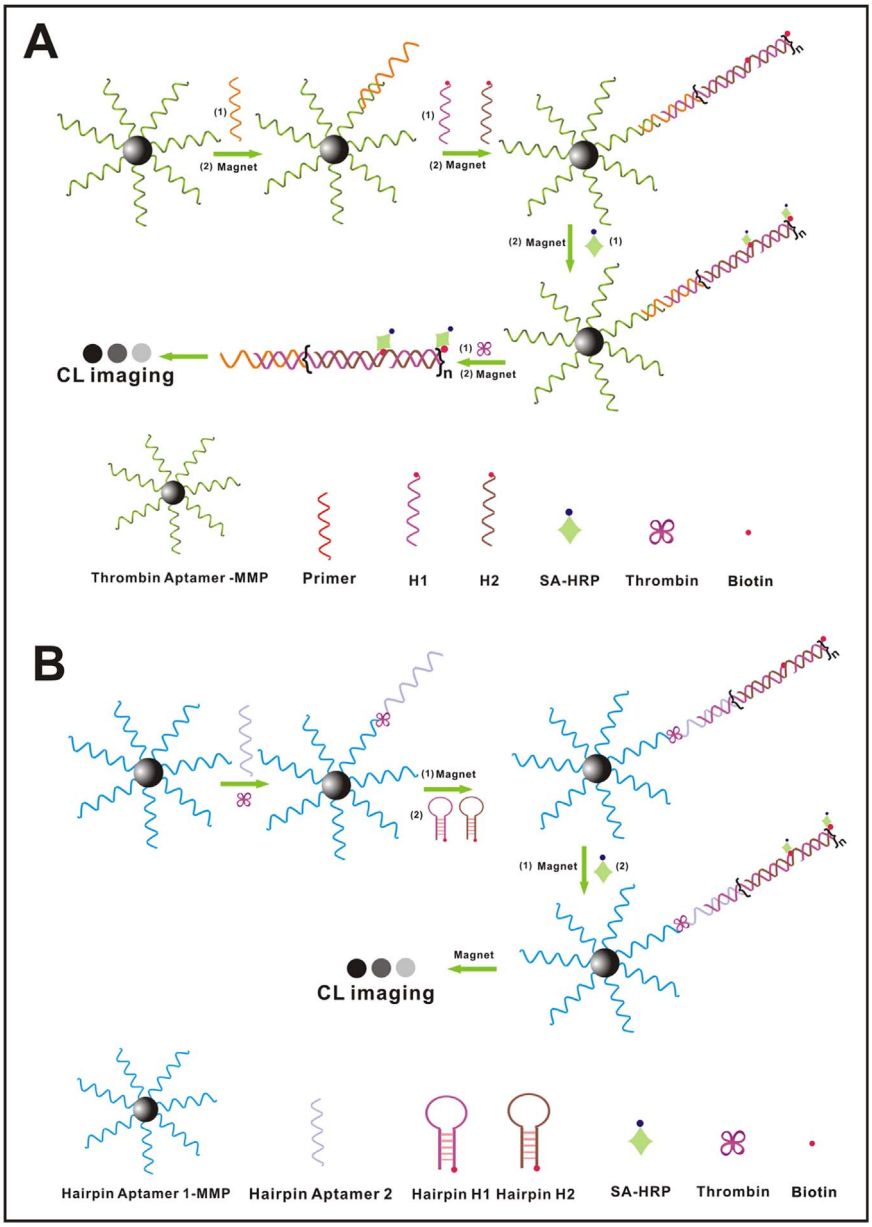
2. Experiment

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 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 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).



Scheme 1. Schematic illustration of the principle for the ultrasensitive detection of thrombin.

2.2. Modification of MMPs

The carboxyl modified MMPs were conjugated with thrombin aptamer DNA or hairpin aptamer DNA according to the improved protocol suggested by the manufacturer, respectively. Briefly, 400 μL of 10 mg mL^{-1} carboxyl-modified MMPs suspension were washed three times with the same volume PBS buffer (Na_2HPO_4 10 mM, NaH_2PO_4 10 mM, NaCl 15 mM, pH 7.4). 7.2 nmol thrombin aptamer DNA was dissolved in 340 μL of PBS buffer, and mixed with the washed MMPs. After incubated at room temperature under stirring for 15 min, 20 μL of 0.1 M EDC and 40 μL of 0.05 M NHS were added into the MMPs suspension, and then continued for over night. The conjugated MMPs were washed three times with Tris-Tween buffer (Tris 0.25 M, Tween-20 0.01% (w/v), and pH 8.0) for 30 min to quench the unreacted activated carboxylic acid groups at room temperature under shaking. Later, the MMPs closured by 1% BSA were dispersed in TE buffer and stored at 4 °C.

2.3. Procedures for thrombin detection

First of all, 10 μL of thrombin aptamer modified MMPs and 5 μL of primers were added into 185 μL of hybridization buffer (Na_2HPO_4 10 mM, NaH_2PO_4 10 mM, NaCl 150 mM, pH 7.4), followed by incubating for 60 min at room temperature with gentle shaking. After washing MMPs for three times, the HCR was performed by mixing 100 μL of buffer containing 1 μM H_1 and 1 μM H_2,with the above hybridized MMPs for 120 min under stirring. 50 μL of SA-HRP was added into PE pipe included MMPs-HCR which had been washed three times again, and incubated for 30 min. After the complexes were rinsed three times to remove the redundant SA-HRP, they were mixed with different concentrations of target in 100 μL buffer. Then, the supernatant was transferred into the polystyrene microplates while the MMPs were separated by a magnet. 50 μL of CL reaction solution, was added into each well of the microplate, and commixed the supernatant. The CL imaging detection was implemented by the ChemiDoc XRD system and the obtained images were processed by the Quantity

One analysis software. The Chemi Hi Sensitivity application model was used to perform this experiment. The CL imaging signal of the polystyrene microplate was accurately analyzed and the intensity was recorded. The other method is similar to the above one except the adding order of the target.

2.4. Different models comparisons

In this paper, three kinds of measurements models for thrombin detection, which one is no HCR and PSM, the second is only included HCR, and the third is included HCR and PSM, were compared. PSM was conjugated with A DNA and B DNA through the MMPs coupling method mentioned above. In simple terms, 200 μL of PSM washed three times was mixed with PBS buffer which contains 1.8 nmol A DNA and 1.8 nmol B DNA to incubate at room temperature under stirring for 15 min. The mixture of 20 μL EDC (0.4 M), 40 μL NHS (0.2 M) were added into the above solution with gentle shaking for over night. After three times washing with TT buffer for 30 min, the conjugated PSM was washed three times again by using TE buffer. It was dispersed in 200 μL of TE buffer containing 1% BSA. Finally, the PSM was stored at 4 °C for latter usage. The procedure for thrombin detection was the same as above. Also, CL imaging of each measurement was recorded by the ChemiDoc XRD system.

2.5. Thrombin detection in complex biological environment

The detection of thrombin in human serum is realized by the model in Scheme 1B. First, different concentrations of thrombin and 5 nM hairpin probe DNA were added into 100 μL buffer (Tris 10 mM, NaCl 150 mM, $MgCl_2$ 5 mM, KCl 10 mM, human serum 25% (v/v), pH 7.4) including with 10 μg of MMPs, and then the mixture was shaking for 2 h to form sandwich structure. After washing the complexes three times, they were added into 100 μL buffer including 1 μM hairpin H_1 and 1 μM hairpin H_2 for 2 h. 50 μL of SA-HRP was mixed with the MMPs-HCR which had been washed three times again. After the complexes were rinsed three times to remove the redundant SA-HRP, they were removed into the polystyrene microplates. Finally, 50 μL of CL reaction solution was added into each well of the microplate to mix with the complexes. The CL signal of each well was individually analyzed and the intensity was recorded.

2.6. Gel electrophoresis

Different HCR sequence was added into polyethylene pipe respectively to hybrid for 2 h. After this, bromophenol blue and SYBR Green I were mixed with the above products for 15 min. In the end, a 3% agarose gel elelectrophoresis analysis of the products via the HCR was carried out in 1 × Tris-borate-EDTA (pH 8.3) at 110 V constant voltages for about 30 min, and imaged by the ChemiDoc XRD system.

2.7. Atomic force microscope imaging

For atomic force microscope (AFM) characterization of the DNA products that were produced by H1 and H2, the concentration of both DNA strands was 0.5 μM and that of the trigger was 10 nM. For this reaction, samples were prepared in 1 mM $MgCl_2$ solution.

3. Results and discussions

3.1. Principle of thrombin detection

The thrombin detection principle based on the functionalized MMPs and HCR is shown in Scheme 1. The MMPs, modified with the thrombin aptamer, were hybridized with a segment of the primer which the remaining segment can trigger HCR. Like the alternate copolymers were formed through a cascade of hybridization events, biotinylated H_1 and H_2 were assembled as long-range double-stranded DNA structures with nicks on the surface of the MMPs. SA-HRP was therefore loaded into the HCR products as the result of SA-biotin interaction. In the presence of thrombin, the aptamer modified on MMPs would combine with thrombin because the intermolecular force between them is stronger than that between oligonucleotides. Therefore, the HCR products rich in supernatant were obtained after the magnetic separation, and finally CL substrate was added to produce strong CL signal. While in the absence of thrombin, the HCR products still linked to the MMPs, and the supernatant just catalyzed CL substrate very slowly to present weak CL signal. More easily to trigger HCR as a result of linear structure of H_1 and H_2 which lead to less reaction time, and suitable for protein that has only one aptamer, are the advantages of the above model. To demonstrate its generality, this method was also broadened to the detection of DNA, and we investigated the feasibility as shown in Fig. S1. The strategy is so feasible that another detection scheme is also presented just by changing the order of reagents addition (Scheme 1B). Because H_1 and H_2 could be adsorbed by MMPs, the hairpin structure DNA instead of linear DNA was used in this strategy to solve this problem. The DNA modified on MMPs and the hairpin aptamer 2 were two different aptamers of thrombin. So sandwich structure was formed through the binding of thrombin with two aptamers. Then the remaining segment of hairpin aptamer 2 triggered the HCR which is similar to Scheme 1A. However, as in the strategy of Scheme 1B, the SA-HRP complexes were existed on the surface of MMPs instead of the supernatant, so the interfaces from the supernatant could be avoided, which is favor to the detection in complex biological environment (Fig. S2).

3.2. Amplification comparison for thrombin detection

In order to compare the effect of amplification, three models were evaluated. As presented in Scheme S1, the principles of these models are nearly the same as that in Scheme 1A. Fig. 1 depicts that under the same concentration of thrombin, the relative CL intensity of no HCR and PSM is almost like the background. And the signal in the condition of only HCR is much higher than that of no HCR, but is relative weaker than the one including both of HCR and PSM. It is indicated that HCR improves CL signal effectively, even the PSM can also enrich DNA on its surface to amplify signal. But the modification of PSM is easy to coagulation, which

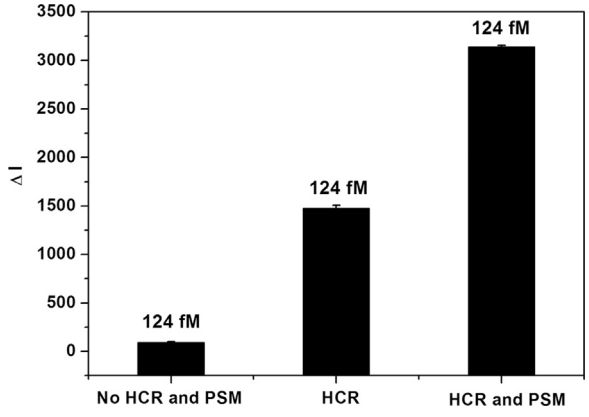


Fig. 1. The relative CL intensities histogram of the sensing platform in the presence of 124 fM of thrombin for different models. Experimental conditions: 10 μg MMPs, 1 μM H_1 and H_2, 12.5 nM primer and 12.5 ng SA-HRP.

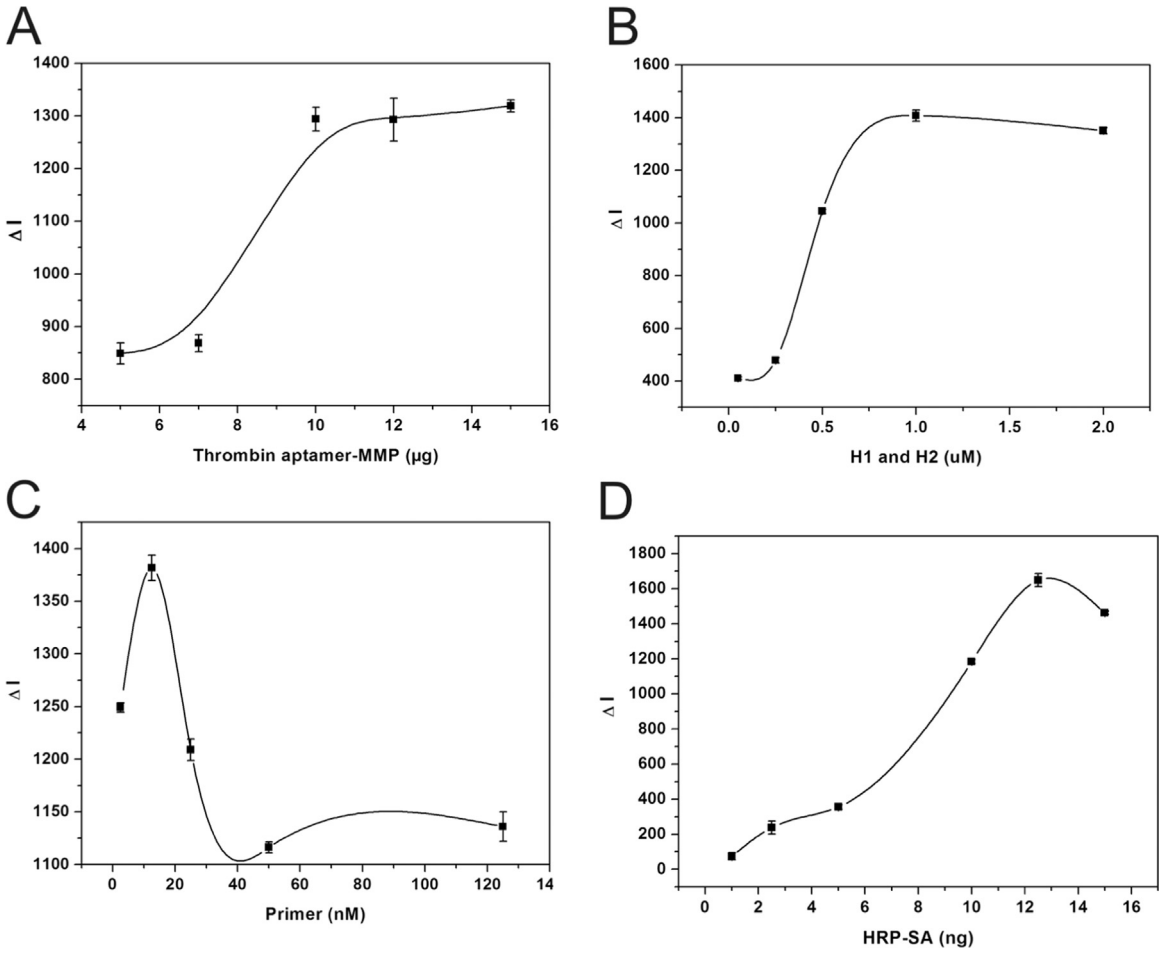


Fig. 2. Effects of (A) the amount of MMPs, (B) the concentration of H_1 and H_2, (C) the concentration of primer and (D) the amount of SA-HRP on the relative CL intensity of the sensing system.

weakened the detection performance of the method. So the strategy with only HCR included was employed in this work.

3.3. Optimization of detection conditions

The parameters of thrombin detection which are key points to the model in Scheme 1A were investigated. As shown in Fig. 2A, 5 different amounts of thrombin aptamer-MMPs in the assay were chosen to obtain the optimal one. With the increase of MMPs, the relative CL intensity rises rapidly, and then it levels off after the amounts of MMPs more than 10 μg. Therefore, 10 μg of MMPs was chosen in this assay. The concentrations of H_1 and H_2 were examined from 0.05 to 2 μM, and the results are presented in Fig. 2B. The relative CL intensity increases at first with the variation of the concentration, and reaches a plateau after 1 μM. So, 1 μM of H_1 and H_2 was selected as the best one. The concentration of primer also influences the density of HCR products on the surface of MMPs. Fig. 2C presents the relationship between primer and the relative CL intensity. When the concentration of primer increases, the relative CL intensity increases quickly at first, and decreases after the peak. So the inflection point 12.5 nM of primer was selected. Besides, the variation between the relative CL intensity and the amounts of SA-HRP is similar to above. The relative CL intensity increases with the amount of SA-HRP in the range from 1 to 12.5 ng, while it decreases slowly after 12.5 ng. Thus, 12.5 ng of SA-HRP was used for the study. The phenomenon of appearing a peak maybe is the density of HCR products leading to different steric hindrance. Similar to Fig. 2, Fig. S3 presented that 10 μg hairpin aptamer 1-MMPs, 1 μM hairpin H_1 and hairpin H_2, 5 nM hairpin aptamer 2 and 10 ng SA-HRP were optimal conditions for the model in Scheme 1B.

3.4. Sensitivity and selectivity of thrombin detection

The thrombin detection is based on enrichment with magnetic beads and the amplification strategy of HCR. In addition, luminol-PIP-H_2O_2 reaction with PIP as the enhancer could also increase the sensitivity. Hence, under the optimal condition, we investigated the sensitivity of the strategy by performing in different models. In the Figure, we define the relative CL intensity as ΔI, and $\Delta I = I_0 - I$, where I_0 means the signal with the presence of target and I means background signal. As described in Fig. 3A, the relative CL intensity increases gradually with the increase of the concentration of thrombin, and a good linear relationship between them are presented. In Fig. 3A, in the linear ranged from 12.3 to 247.7 fM, the regression equation is $\Delta I = 12.452C + 109.335$, where ΔI is the relative CL intensity and C is the concentration of thrombin ($R^2 = 0.9952$). The limit of detection (LOD) for thrombin is calculated to be 9.7 fM (0.66 pg/mL) according to by the equation $C_{LOD} = 3S/k$, where C_{LOD} is the limit of detection, S is standard deviation of the blank (N=6), and k is the slope of regression equation. The comparison of this strategy with other methods reported is shown in Table 1. It is indicated that the LOD of this method is superior to those of some previously reported methods.

To evaluate the selectivity of this method, thrombin and other proteins, such as BSA, lysozyme, and trypsin were tested. As

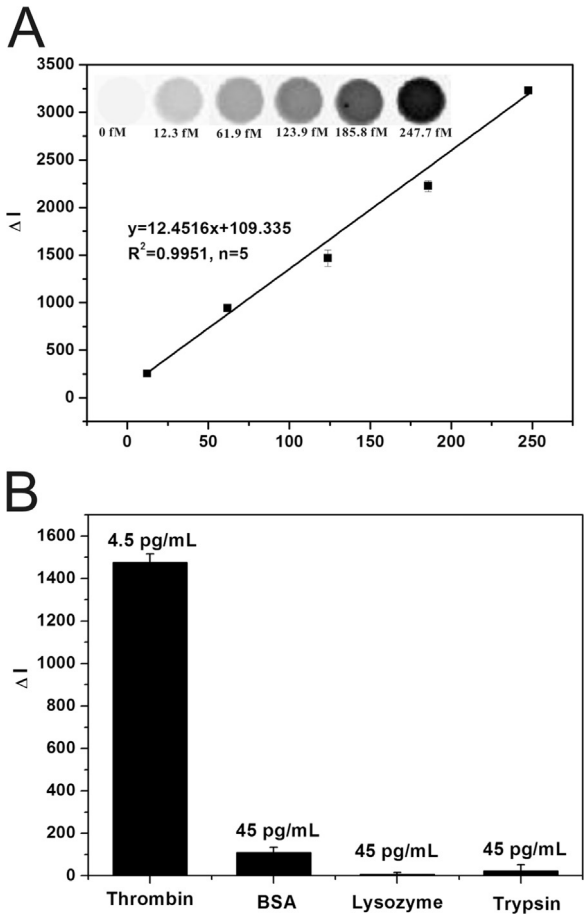


Fig. 3. (A) Relationship between the relative CL intensity and thrombin concentration. Inset: CL images of thrombin at different concentrations for Scheme 1A. (B) Specificity of the assay for thrombin detection with different target protein for Scheme 1A. Experimental conditions: 10 μg MMPs, 1 μM H_1 and H_2, 12.5 nM primer and 12.5 ng SA-HRP.

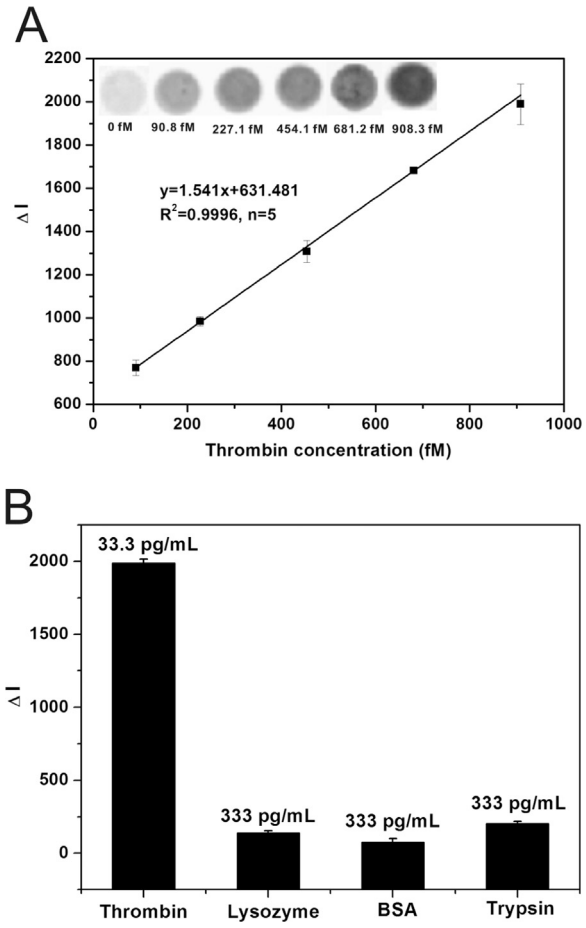


Fig. 4. (A) Relationship between the relative CL intensity and thrombin concentration. Inset: CL images of thrombin at different concentrations for Scheme 1B. (B) Specificity of the assay for thrombin detection with different target protein for Scheme 1B. Experimental conditions: 10 μg MMPs, 1 μM hairpin H_1 and hairpin H_2, 5 nM hairpin aptamer2 and 200 ng/mL SA-HRP.

Table 1
The limits of thrombin detection in recent references.

Strategy	LOD	Ref.
Surface-Enhanced Raman Scattering	18 pM	Zheng et al., 2014
Fluorescence	10 ng/mL	Tennico et al., 2010
Electroanalysis	0.14 pM	Xie et al., 2014
Electroanalysis	0.05 pM	Li et al., 2015
Electroanalysis	0.19 ng/mL	Cao et al., 2012
Electroanalysis	5 pg/mL	Rahman et al., 2009
Chemiluminescence Imaging	0.49 pM	Zong et al., 2014
Chemiluminescence Imaging	0.92 pM	Zou et al., 2016
Chemiluminescence Imaging	Scheme 1A: 9.7 fM (0.66 pg/mL)	**This method**

shown in Fig. 3B, the relative CL intensity of thrombin is much higher than the others, although their concentrations are ten times as that of thrombin. This high selectivity is mainly owing to the special recognition ability of the aptamer of thrombin. Therefore, the proposed method possesses a desired selectivity for thrombin detection.

Similarly, the sensitivity and selectivity of the strategy illustrated in Scheme 1B were also evaluated. As shown in Fig. 4A, there is a good linear relationship between the relative CL intensity and the concentrations of thrombin (R^2=0.9996). The limit of detection (LOD) for thrombin is calculated to be 57.9 fM (3.9 pg/mL). What's more, as shown in Fig. 3B, the method has good selectivity.

3.5. Thrombin detection in complex biological environment

In order to avoid impurity interference in human serum, the method as shown in Scheme 1B has been developed to analyze thrombin in human serum. The buffer was introduced to mix with human serum for helping the sandwich structure formed. As shown in Fig. 4, the relative CL intensities detected in the diluted human serum had slightly differences with those obtained in buffer, and the relative standard error of CL intensities detected between in buffer and in human serum is presented within the range of 5%. So it is indicated that the method is capable of thrombin sensing in complex biological environment (Fig. 5).

4. Conclusion

In conclusion, an ultrasensitive chemiluminescence biosensor for thrombin was constructed based on the functionalized MMPs and HCR. It is proved this biosensor for thrombin is of high sensitivity and selectivity. And the results show that the detection limit of thrombin is as low as 9.7 fM, which is mainly attributed to HCR amplification and enrichment of MMPs. The strategy was applied to detect thrombin in human serum with good result. Moreover, the proposed method was also successfully extended to

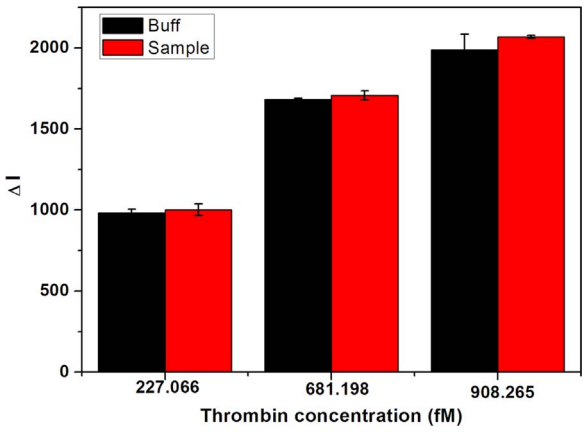


Fig. 5. The relative CL intensities of the sensing system for thrombin detection in buffer and 25% diluted human serum samples, respectively.

DNA detection. Hence, this biosensor will provide a broad platform to detect various proteins and DNA at a high sensitive level.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2016.08.067.

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