

# [Method development for protein detection](https://assignbuster.com/method-development-for-protein-detection/)

Binding-induced and label-free colorimetric method forproteindetectionbasedonbinding-induced DNA hybridization and DNAzyme–based signalamplification

INTRODUCTION

In diagnosing the early stage of a disease or pathological condition, proteins, especially those associated with cancers, are of great importancebecause they are the molecular machinery of life.[BB2013-AM-3, 1, 2]Enzyme-linked immunosorbent assay (ELISA) is the most commonly used method for protein detection.[PD-3, 1, 2] Unfortunately, this antibody-based assay requires a long incubation periods and long assay times with the involvement of multiple washing steps.[PD-5, 1, 2] Additionally, it is faced with the challenges of insufficient sensitivity and limited dynamic range.[PD-4, 5, 6, 7] As an alternative to the antibody-based assay, aptamer-based assays have gained tremendous attention recently.[PD-4, 9-11] Aptamers are single stranded DNA or RNA oligonucleotides selected from random sequence nucleic acid libraries through an in vitro selection process termed systematic evolution of ligands by exponential enrichment (SELEX).[PD-6, 4-6] They possess high affinity and good selectivity for small molecules, proteins or other targets.[BB-11, 20-22] Compared to antibodies, aptamers exhibit obvious advantages including a better stability for long-term storage, a rapider preparation by chemical synthesis in large quantity, and the flexible modification with a variety of functional groups.[PD-4, BB-11, 23, 24] Some aptamer-based amplified detection assays for protein have been developed in the past two decades, such as the polymerase chain reaction (PCR), rolling circle amplification (RCA), strand displacement amplification (SDA) and ligase chain reaction (LCR). [BB-AM-3] Although these amplified assays greatly enhance the sensitivity of protein detection, they are usually time-consuming and too complicated. Therefore, the amplified detection of protein is still challenging in bioanalytical chemistry.[BB2013-AM-3]

Deoxyribozymes (DNAzymes) are artificial nucleic acids, which are isolated from in vitro selection. [DM-5] Similar to traditional protein enzymes, they exhibit high catalytic hydrolytic toward specific substrates, while they possess higher thermal stabilitythat can be denatured and renatured formanycycles without losing catalytic activities.[DM-4] This obvious advantage makes DNAzymes ideal biocatalysts for achieving signal amplification in biological applications.[DM-4]An important development in the DNAzyme field is the discovery of the G-quadruplexDNAzyme.[DM-9, 9] The G-quadruplex sequences can associate with a cofactor, hemin, to form peroxidase-mimicking DNAzymes to catalyze the H 2 O 2 -mediated oxidation of 2, 2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) to a green-colored product ABTS •− or enhance the chemiluminescence of the luminol-H 2 O 2 system.[DM-6, 25, DM-5, 44] With this main advantage, G-quadruplex DNAzyme has been employed to develop many colorimetric, chemiluminescent or fluorescent sensing platforms for the detection of proteins, DNA and other biomolecules.[DM-9, 14, DM-2, 34-37] Recently, Willner’s group reported an enzyme-free amplified detection platform based on the hemin/G-quadruplex horseradish peroxidase (HRP)-mimicking DNAzyme.[AC2012-2] This strategy is quite successful, while the target detection is limited to DNA, and the detection of protein represents another challenge.

In this work, taking the advantages of the high selectivity and affinity of aptamers and the HRP-mimicking DNAzyme amplification strategy, we designed a new binding-induced and label-free ultrasensitive colorimetric method for amplified detection of protein. As a proof of principle, human α-thrombin and its two aptamers, Apt29 and Apt15, are used. In this sensing system, Apt29 and Apt15 are integrated into the proximity probes as recognition elements for the thrombin. These two proximity probes hybridize with each other stably only when both of them bind to the thrombin simultaneously.[BB2013-AM-3] The binding-induced hybridization duplex triggers an autonomous cross-opening of the two functional hairpin structures. And this leads to the formation of a variety ofhemin/G-quadruplex DNAzymes. The DNAzymes catalyze the oxidation of ABTS, generating a green colorimetric signal, which can be monitored simply by a spectrophotometer. [DM-3, 29, 30]This binding-induced and DNAzyme-based signal amplified method has a great potential for protein detection. [BB2013-AM-3] In addition, since various recognition elements might be fused, this method can be further extended to sensitive detection of other proteins.[DM-4]

EXPERIMENTAL SCETION

Materials and Reagents.

All DNAoligonucleotides were purchased from Genscript (Jiangsu, China). The oligonucleotides were PAGE-purified and diluted in pH 7. 4, 20 mM Tris-HCl buffer solution (containing 100 mM NaCl, 20 mM KCl, and 2 mM MgCl 2 ) to give stock solutions of 100 μM. Before use, two hairpin structures were heated to 95â„ ƒ for 5 min, and slowly cooled down to room temperature. Human α-thrombin (Tb), bovine serum albumin (BSA), and human serum albumin (HSA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Hemin, [tris(hydroxymethy-l)aminomethane] (Tris), 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid sodium salt (HEPES), 2, 2-azino-bis(3-ethylbenzothiozoline-6-sulfonic acid) (ABTS), and H 2 O 2 were purchased from Aladdin Reagents (Shanghai, China). A hemin stock solution (1 mM) was prepared in dimethyl sulfoxide (DMSO) and stored in the dark at −20 °C. All other chemicals were of analytical grade and were used without further purification. All solutions were prepared using double-distilled water, which was obtained through a Milli-Q purification system (Billerica, MA, USA).

Absorbance Measurements.

Absorbance measurements were performed under room temperature using a TU-1901 UV−visible spectrophotometer (Beijing Purkinje General Instrument Co, Ltd., China). Kinetic data were recorded at the wavelength of 420 nm every 5 s during the first 5 min of the reaction. The absorption spectra of the solution was measured in the wavelength range from 390 to 490 nm.

Procedure for Thrombin Assay.

The experiments were performed in 50 μL of Tris-HCl buffer (20 mM Tris-HCl, pH = 7. 4, 100 mM NaCl, 20 mM KCl, 1 mM MgCl 2 ) containing 200 nM P1, 200 nM P2 and varying concentrations of Tb. The mixture was first incubated for 30 min at room temperature to allow complete binding. Next, 25 μL of 2 μM H1 and 25 μL of 2 μM H2 were added and incubated for 6 h at room temperature. Then, 20 μL of 2 μM hemin and 240 μL of HEPES buffer (25 mM HEPES, pH= 7. 4, 200 mM NaCl, 20 mM KCl, 0. 05% Triton X-100, 1% DMSO) were added, and allowed to incubate for 1 h at room temperature. Finally, 30 μL of ABTS and 10 μL of H 2 O 2 were added to the mixture to give the final concentrations of 2 mM and 2 mM, respectively. The resulting samples were tested with a UV−vis spectrometer.

RESULT AND DISCUSSION

Design strategy for human α-thrombin detection

The sequences of the oligonucleotides used in this work were listed in Table 1. It consists of two proximity probes (P1 and P2) and two hairpin structures (H1 and H2). Both proximity probes P1 and P2 consist of four domains. Domain I includes two different thrombin aptamers, Apt29 (29 mer) and Apt15 (15 mer). The Apt29, orange domain of P1, binds to the heparin-binding site and the Apt15, skyblue domain of P2, binds to the fibrinogen-binding site of thrombin, resulting in proximity. Domain II (black) consists of a poly-T sequence that is designed to reduce the effect of steric hindrance induced by thrombin. Domain III (pink) is designed to have only 6 complementary bases, so that two proximity probes P1 and P2 cannot form a stable duplex without the target protein at room temperature. Domain IV (blue) is the key domain for binding-induced DNAzyme-assised signal amplification. By using two functional hairpin structures, the recognition of domain IV could trigger-on the hybridization chain reaction that led to DNAzyme chains consists of the hemin/G-quadruplex HRP-mimicking DNAzyme. Hairpin structure H1 is functionalized at its 5′ end with three-fourths of the G-quadruplex sequence, domain V (green), which is linked to the programmed sequences VIII (red) and VII (blue). One-fourth of the G-quadruplex sequence, domain VI (green), is extended at the 3′ end of the hairpin H1. Hairpin structure H2 is functionalized at its 5′ and 3′ ends with one-fourth of the G-quadruplex (domain VI) and three-fourths of the G-quadruplex (domain V) sequence, respectively. Programmed sequences of domains VII’ and VIII’ in hairpin H2 are complementary to domains VII and VIII in hairpin H1, respectively. Both four domains in hairpin H1 and H2 are incorporated into a stable hairpin configuration in an initially locked format by hybridizing with their partially complementary sequences. It is noteworthy that sequence V is partially hybridized with domain VII in hairpin H1 or VIII’ in hairpin H2, which prevents the self-assembly of the active hemin/G-quadruplex DNAzyme.

Principle of binding-induced DNAzyme-assisted amplification strategy for human α-thrombin detection

The working principle of human α-thrombin detection is illustrated in Scheme 1. In the absence of thrombin, domain III in P1 and domain III’ in P2 will not associate since the complementary sequences (6 nt) are too short to promote efficient hybridization. When the target thrombin is introduced into the system, domain I in P1 and P2 bind to the protein simultaneously, resulting in domain III and III’ sufficiently close and to hybridize to each other to form a stable P1-Tb-P2 duplex, step 1. [BB2013-AM-3] Once the P1-Tb-P2 duplex forms, it associates with the stem region of H1, domain VII, leading to an opening of H1. This opening of H1 results in the release of the single-stranded domain VIII and the conserved three-fourths of the G-quadruplex (domain V), step 2. The released domain VIII then hybridizes with domain VIII’ of the stem in H2, and opens H2 using the strand displacement principle, step3. Subsequently, the liberated domain VII’ in H2 cross-hybridizes with H1 by hybridization of domain VII’ to domain VII in H1, resulting in two G-quadruplex subunits (domain V and VI) sufficiently close and to self-assemble into a G-quadruplex structure, step 4. [AC2012-4] In the process of this autonomous cross-opening of H2 and H1, strand displacement can be repeated continuously, generating numerous G-quadruplex structures. In the presence of hemin, the resulting catalytic hemin/G-quadruplex peroxidase-mimicking DNAzymes catalyze the H 2 O 2 -mediated oxidation of the colorless ABTS 2− to green-colored ABTS •− .

Detection of thrombin in human serum

To further demonstrate the feasibility of the proposed method in real bioenviroments, we performed the detection of thrombin in human serum. [AC2013-4] Three concentrations of thrombin (10 pM, 100 pM, and 1000 pM) were spiked into 10-fold diluted human serum. [ZK-CC-1] Figure 4 shows the time-dependent absorbance changes of ABTS •− in response to different concentrations of thrombin. [AC2014-6] In logarithmic scales, the absorbance value exhibits a linear correlation with thrombin concentration over a range of 3 orders of magnitude from 10 pM to 1000 pM (inset of Figure 4B). [AC2012-12] The result indicated the potentiality of the proposed method for protein detection in real biological samples. [AC2013-4]

Conclusions

In conclusion, we have developed a binding-induced and label-free colorimetric method for protein detection based on binding-induced DNA hybridization and DNAzyme-assisted signal amplification. This method does not require any modification of DNA and involve any protein enzyme, which makes it technically label-free, enzyme-free and very cost-effective. Furthermore, the presentapproach usesa simple separation-free procedure in which the assay is conducted in a homogeneous solution.[AC2014-3] In addition, due to the excellent specificity of two proximity probes to the thrombin and the ingenious design of two hairpin structures,[AC-EA-2] this method exhibits a high sensitivity for thrombin detection, with a low detection limit of 2. 5 pM. More importantly, this method can be extended to sensitive detection of other proteins by simple changing the aptamer sequences of the two proximity probes. To sum up, this simple and cost-effective colorimetric signal amplified method has great potential to be used as a universal tool for ultrasensitive analysis of thrombin or other proteins in serum and supply valuable information for biomedical research and clinical diagnosis.[ZK-CC-1]