Highly sensitive fluorescent aptasensor for adenosine detection based on gold nanoparticle tagged molecular beacon

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Abstract. In this contribution, a sensitive fluorescent aptasensing strategy for the detection of adenosine is proposed based on structure switching of gold nanoparticle modified molecular beacon. Hairpin structured molecular beacon exhibits low background fluorescence since the fluorophore and nanoparticle quencher are in close proximity in the absence of adenosine. Binding with target molecules induces structure switching of molecular beacon, resulting in significant fluorescence increase due to separation of fluorophore and quencher. The fluorescence intensity is linear with adenosine concentration in a dynamic range from 90nM -1.5μM with a detection limit of 60 nM. Moreover, good reproducibility and high specificity are achieved.

Introduction

Antibody-antigen specific reaction based immunoassay is traditionally the main analysis technique for protein [1]. However, since the antibodies usually need long synthesis term accompany with weak preserving stability, the application of immunoassay technique is limited to some extent. In the past decades, a new class of recognition element for protein named aptamer has been widely studied. They are artificially selected single stranded DNA or RNA oligonucleotides via SELEX (Systematic Evolution of Ligands by Exponential Enrichment) from random-sequence nucleic acid libraries and exhibit many advantages such as good stability, easy modification, simple synthesis and wide applicability. When binding with target molecules, aptamers generally afford characteristic spatial structures to bind with their target with high specificity and affinity [2]. Therefore, lots of aptamer based biosensing strategies have been developed including colorimetric, electrochemical, fluorescent methods, and so on [3-6]. Among these techniques, the fluorescent analysis method has attracted increasing attention because of its numerous merits such as high sensitivity, low cost, and rapid response.

Molecular beacon is a fluorescent probe which presents a hairpin structure consists of loop and stem. Usually, the 5' and 3' terminal of molecular beacon are labeled with fluorophore and quencher. Recent studies show that gold nanoparticle can act as a efficient quencher in molecular. Therefore, in the presented study, we proposed a fluorescent biosensing strategy for sensitive detection of small molecules adenosine based on structure-switching of gold nanoparticle tagged molecular beacon. The fluorescence is quenched in the absence of adenosine since the fluorophore is in close proximity with quencher. After reaction with adenosine, the molecular beacon opens its hybridized stem and the fluorophore is far away from the gold nanoparticle quencher, resulting in restore of the fluorescence. The change of fluorescence intensity is related to the target concentration. By this way, a fluorescent method for small molecule adenosine is developed. The experimental conditions are optimized, and the performance features such as reproducibility and specificity are also evaluated. Fabrication and operation principle of the biosensor are shown in Figure 1.

Fig. 1. Scheme illustration of operation principle of the developed aptasensor.

Materials and Methods

Chemicals. The signaling molecular beacon probe utilized in this study is synthesized by Sangon Biotechnology Co., Ltd. (Shanghai, China) with the sequence of SH-*GTC ACC T*GG GGG AGT ATT GCG GAG GA*A GGT GAC*-FAM (5'-3'). The stem of molecular beacon possesses 7 pairs of bases and is emphasized in italic. The underlined sequence is the original aptamer for adenosine. Tris is bought from Dingguo Biotechnology Co., Ltd. (Changsha, China). All other chemicals are of analytical reagent grade and used as received. The 10 mM PBS buffer (10 mM PBS, 0.3 M NaCl, pH 7.4) is used to prepare working solutions. Deionized and sterilized water (resistance > 18 MΩ·cm) is used throughout the experiments.

Apparatus. All fluorescence measurements are carried out using a Hitachi F-7000 fluorescence spectrometer (Hitachi, Japan) with a personal computer data processing unit. The light is a 150 W Xe lamp and the detector is a R928F red-sensitive photomultiplier. Excitation and emission slits are all set for 5.0 nm band-pass. The excited wavelength for FAM fluorophore is 498 nm, and the emission spectra from 505 to 650 nm are collected. All measurements are carried out under ambient temperature (25 ˚C) unless stated otherwise.

Preparation of gold nanoparticle modified molecular beacon Gold nanoparticle modified molecular was prepared according to the procedure described in the previous studies [6] with slight modifications: First, the gold nanoparticle was synthesized according to reference 6. Then, 2 OD of thiol tagged oligonucleotide was dissolved in 1 ml of sterilized distilled water, and 50 μL of the resulting solution was added into 400 μL of fresh prepared nanoparticle solution. After standing for 24 h at 4 ˚C, the conjugate solution was buffered at pH 7.4 with 10 mM phosphate. During the salt aging process, the oligonucleotide funtionalized gold nanoparticle solution was gradually brought to the 0.1 M PBS (10 mM phosphate buffer, pH 7.4, 0.1 M NaCl) over a 24 h period. After standing for an additional 24 h at 4 ˚C, the excess of reagents was removed by centrifugation at 12000 rpm for 30 min. The supernatant was discarded, and the red pellet was redispersed in 0.3 M PBS (10 mM phosphate buffer, pH 7.4, 0.3 M NaCl). The oligonucleotide funtionalized gold nanoparticle solution was stored in refrigeratory until use.

Fluorescent measurements. All fluorescent measurements are carried out in a quartz cuvette with 1mm slit. To evaluate the analytical performances of the aptasensor, 20 μL of molecular beacon solution is mixed with 20 μL of adenosine with a certain concentration and allowed to react for different periods at different temperature, followed by diluting with working buffer solution to a final volume of 100 μL. Then, fluorescence spectra of the hybridization solution are recorded and the peak intensity at 520nm is used to evaluate the analytical performance of the proposed sensing system.

Results and discussion

Sensing mechanism. We developed a quantitative aptasensing system based on target-binding induced structure-switching of the molecular beacon in the proposed work,. As with traditional molecular beacon, a 7 bases stem and a 19 bases loop are involved in the molecular beacon molecule with a fluorophore of FAM at 5' end and a quencher at 3' end, respectively. The difference is that a gold nanoparticle is used as the quencher to replace with organic dye due to its significant quenching efficiency. As shown in Fig. 1, the fluorophore and the gold nanoparticle quencher are in close

proximity in the absence of target molecules, which induces fluorescence resonance energy transfer between fluorophore and gold nanoparticle. As a result, the fluorescence of the FAM fluorophore is sufficiently quenched by gold nanoparticle and only low background fluorescence can be observed. On the contrary, introduction of adenosine induces the signaling molecular beacon changing its spatial structure to specifically bind with the target molecules, which triggers the deconstruction of stem and separation of fluorophore and the gold nanoparticle. Consequently, the fluorescence of FAM restores and significant fluorescence enhancement is obtained. The change of fluorescence intensity before and after target binding is related to the concentration of adenosine.

Investigation of reaction temperature. The reaction temperature influences the melting of molecular beacon and reaction rate between adenosine and its aptamer probe. In order investigate the dependence of reaction temperature on the analytical performance of the proposed sensor, 20 μL of molecular beacon solution is mixed with 20 μL of 1.5 μM adenosine. The resulting solution is diluted with working buffer solution to a final volume of 100 μL and is allowed to react for 60 min at different temperature. The fluorescence change before and after target binding increases rapidly and reaches peak at about 25 ˚Cas shown in Fig. 2A. However, sharply decrease of the fluorescence change is found when the temperature rises continue since the unreacted molecular beacon would open the stem and restores the fluorescence when the reaction temperature closes to the melting temperature of the molecular beacon. In order to get the highest specific fluorescence response, all of the experiments are carried out at 25 ˚C except stated otherwise.

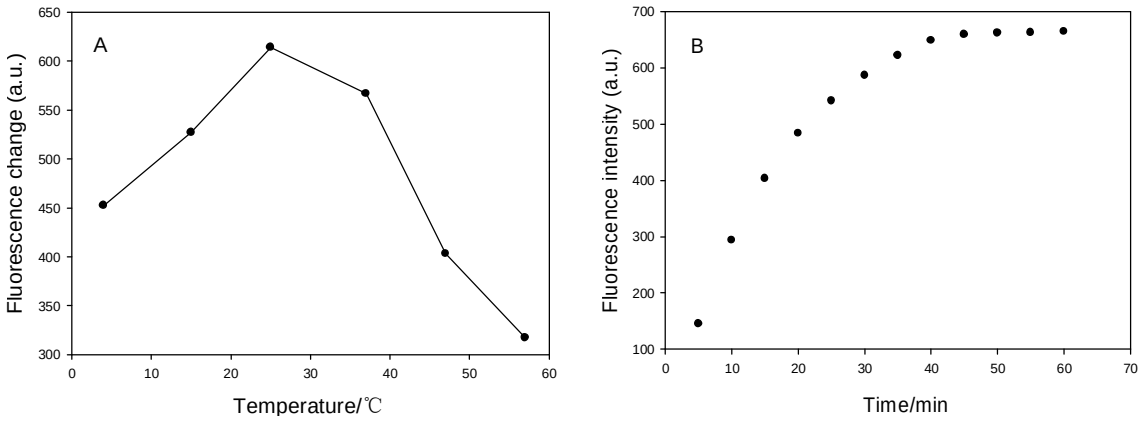

Fig. 2. (A) Effect of reaction temperature. The concentration of adenosine is 1.5 μM, six typical temperatures (4, 15, 25, 37, 47 and 57 ˚C) are investigated. (B) Effect of incubation time. The concentration of adenosine is 1.5 μM, different periods of time (5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 and 60 min) is investigated.

Effect of incubation time. The incubation time directly influences the reaction degree between signaling molecular beacon and adenosine. In order to optimize the incubation time, 20 μL of molecular beacon solution is mixed with 20 μL of 1.5 μM adenosine followed by diluting with working buffer solution to a final volume of 100 μL. The fluorescence spectra are immediately collected after the resulting mixture maintaining at 25 ˚C for different period of time as shown in Fig. 2B. The fluorescence intensity increases gradually with the augment of incubation time and then tends constant after about 45 min, indicating that reaction reaches equilibrium. To ensure the completeness of the reaction, 45 min is chosen as the optimum incubation time in following experiments.

Detection of adenosine. In order to obtain the calibration curve, a series of samples with different concentrations are detected under the optimized experimental conditions. The fluorescence spectra are shown in Fig. 3A. The fluorescence intensity increases with the augment of the concentration of adenosine. A linear relationship is achieved between fluorescence intensity and log concentration of target molecules in the concentration range from 90 nM to 1.5 μM as shown in Fig. 3B, and the detection limit is 60 nM according to a signal-to-noise ratio of three. The calibration equation is F = 430.1 log$C$ - 707.7 with a correlation coefficient of 0.9918 (n=3, R.S.D. =4.3%).

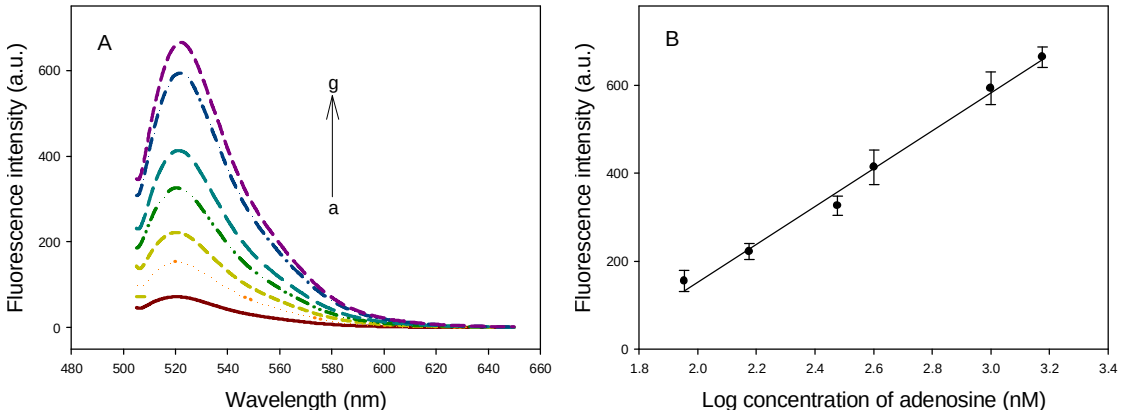

Fig. 3. (A) Fluorescent response of the proposed aptasensor to adenosine of different concentrations (a: blank sample, b-g: the concentration of adenosine increases). (B) The linear relationship between fluorescent intensity and log concentration of adenosine.

Specificity. To validate the selectivity of the aptasensor, the fluorescence intensity corresponding to cytidine, uridine and guanosine are evaluated, the fluorescence intensity upon the interferents is not more than 5% of the fluorescence triggered by target adenosine even though their concentration is at least 3333-fold higher (namely 5 mM) than that of adenosine (data not shown). That's to say, the presented sensing system exhibits excellent specificity to the target adenosine molecules.

Conclusions

In this work, we proposed a sensitive sensing strategy for adenosine based on structure switching of gold nanoparticle tagged molecular beacon. Under the optimized experimental conditions, the adenosine could be sensitively detected with a relative wide linear detection range and a low detection limit. Moreover, the presented biosensor exhibits high selectivity and fast analysis speed.

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