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Label-free fluorescence dual-amplified detection of adenosine based on exonuclease III-assisted DNA cycling and hybridization chain reaction

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ABSTRACT

In this work, we constructed a label-free and dual-amplified fluorescence aptasensor for sensitive analysis of adenosine based on exonuclease III (Exo III)-assisted DNA cycling and hybridization chain reaction (HCR). Firstly, we fabricated a trifunctional probe that consisting of the catalytic strand, the aptamer sequence and a streptavidin-magnetic nanobead (streptavidin-MNB). The streptavidin-MNB played a role of enrichment and separation to achieve a low background. The aptamer sequence was employed as a recognition element to bind the target adenosine, leading to the releasing of the catalytic stand. Then, the catalytic strand induced the Exo III-assisted DNA cycling reaction and produced a large amount of DNA fragments, which got a primary amplification. Subsequently, the DNA fragments acted as trigger strands to initiate HCR, forming nicked double helices with multiple G-quadruplex structures, which achieved a secondary amplification. Finally, the G-quadruplex structures bonded with the N-nethyl mesopor-phyrin IX (NMM) and yielded an enhanced fluorescence signal, realizing the label-free detection. In the proposed strategy, a small amount of adenosine can be converted to a large amount of DNA triggers, leading to a significant amplification for the target. This method exhibited a high sensitivity toward adenosine with a detection limit of 4.2×10^{-7} mol L^{-1}, which was about 10 times lower than that of the reported label-free strategies. Moreover, this assay can significantly distinguish the content of adenosine in urine samples of cancer patients and normal human, indicating that our method will offer a new strategy for reliable quantification of adenosine in medical research and early clinical diagnosis.

1. Introduction

Adenosine (AD) is an endogenous nucleoside produced by ATP degradation and performs extremely important signaling functions in the peripheral and central nervous system, as well as immune system (Zhang et al., 2008; Wang et al., 2014). Recently, increasing evidences have suggested that adenosine has tumor-promoting activity (Spychala, 2000; Goyal et al., 2008; Giglioni et al., 2008). As a kind of potential tumor markers, the sensitive determination of adenosine is essential for better understanding of their roles in tumor cell proliferation and further illustrating their function in cancer clinical diagnosis and treatment. Conventional methods, including high-performance liquid chromatography (HPLC) (Luippold et al., 1999; Dobolyi et al., 1998), capillary electrophoresis (Lin et al., 1997), and radioimmunoassay (Siragy and Linden, 1996), possessed some limitation such as tedious sample preparation, the large amounts of samples and reagents, low sensitivity, and even the dangers of radioactive elements. As an alternative, fluorescence aptasensor has attracted considerable attention for its simplicity, speediness, good selectivity and high sensitivity. To obtain obvious fluorescent signal, some commonly label-based approaches were developed (Zhang et al., 2010; Dave and Liu 2012; Hu et al., 2012). Dong's group has constructed a fluorescent aptasensor based on graphene oxide and exonuclease III (Exo III) assisted signal amplification (Hu et al., 2012). This aptasensor realized the sensitive detection of adenosine, but the label steps may decrease the performance of the signal probe. Additionally, the complexity and costliness also restricted their universal application.

In order to simplify the experiment and reduce the cost, a few label-free fluorescence aptasensors have been reported for the detection of adenosine (Xu and Lu, 2010; Xiang et al., 2009, 2010). Lu's group has developed a fluorescence aptasensors based on regulation of malachite green fluorescence and realized the label-free

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detection of adenosine (Xu and Lu, 2010). However, due to the ratio of target and signal is 1:1, the sensitivity of this method is relatively low with a detection limit of 2.0×10^{-5} moL L^{-1}. To improve the sensitivity, some signal amplification strategies have been introduced to the construction of label-free fluorescence aptasensors for adenosine analysis (Song et al., 2012; Fu et al., 2013; Liao et al., 2012). Among them, our group has developed a label-free aptasensor for amplified detection of adenosine on the basis of target-catalyzed hairpin self-assembly (Fu et al., 2013). By employing the signal amplification, the sensitivity of this method has a certain improvement with a detection limit of 6.0×10^{-6} moL L^{-1}, but it still cannot meet the requirement of the low level adenosine detection in body fluids (Dolezalova et al., 2005; Kloor et al., 2000; Chen et al., 2008). Therefore, the sensitivity of adenosine detection need to be further enhanced.

In past decade, many signal amplification strategies have been developed as effective tools to increase the sensitivity of target analysis (Peng et al., 2014; Liu et al., 2013; Ma et al., 2014; Zheng et al., 2012). Exo III-assisted DNA cycling method is one of the commonly used strategies. Differing from nicking endonucleases, Exo III does not need a specific recognition site and can selectively catalyze the stepwise hydrolysis of mononucleotides from the recessed or blunt 3′-hydroxyl termini of duplex DNA (Xu et al., 2012; Gao and Li 2014; Liu et al., 2013; Zhang et al., 2013). Through Exo III-assisted cycling reaction, a large amount of single-stranded DNAs can be acquired for signal amplification. However, most of the previous works using Exo III-assisted DNA cycling were involved in labeling procedures, such as tagging fluorophore for optical detection and tagging redox labels for electrochemical analysis (Hu et al., 2012; Gao and Li, 2014; Liu et al., 2014; Xuan et al., 2012).

To achieve the label-free detection of adenosine based on Exo III-assisted DNA cycling, we combined the hybridization chain reaction (HCR) to produce multiple G-quadruplex structures, through which multiple N-nethyl mesopor-phyrin IX (NMM) can be attached for efficient signal output and signal amplification (Shimron et al., 2012; Ren et al., 2011; Dong et al., 2012). By combining the two signal amplification strategies, we constructed a new label-free and dual-amplified fluorescence aptasensor for sensitive detection of adenosine. Firstly, we fabricated a trifunctional probe that consisting of the catalytic strand, the aptamer sequence and a streptavidin-magnetic nanobead (streptavidin-MNB). The streptavidin-MNB played a role of enrichment and separation to achieve a low background. When the target was recognized by the aptamer sequence, the catalytic strand can be released immediately. Then, the catalytic strand induced the Exo III-assisted DNA cycling reaction and produced a large amount of DNA fragments, which got a primary amplification. Subsequently, the DNA fragments acted as trigger strands to initiate the HCR, forming nicked double helices with multiple G-quadruplex structures, which achieved a secondary amplification. Finally, the NMM was inserted into the G-quadruplex structures and yielded an enhanced fluorescence response, realizing the label-free detection. In the proposed strategy, a small amount of adenosine can be converted to a large amount of DNA triggers, leading to a significant amplification for the target. The proposed method exhibited a high sensitivity with a detection limit of 4.2×10^{-7} mol L^{-1}, which attributed to the dual amplification of Exo III-assisted DNA cycling reaction and HCR, as well as the low background of streptavidin-MNBs. More importantly, this assay can significantly distinguish the content of adenosine in urine samples between cancer patients and healthy persons, indicating that our method will offer a new strategy for reliable quantification of adenosine in medical research and early clinical diagnosis.

2. Experimental section

2.1. Materials and reagents

All DNA oligonucleotides used in this work were synthesized and purified by Sangon Inc. (Shanghai, China), and their sequences were listed in Table S1. The DNA stock solutions were prepared in TE buffer (1.0×10^{-2} mol L^{-1} Tris–HCl, pH 8.0, 1.0×10^{-3} mol L^{-1} EDTA) and stored at −20 °C. Adenosine was purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Cytidine, Uridine, Guanosine, Exonuclease III were purchased from Shanghai Sangon Biological Engineering Technology and Services Co. Ltd. (Shanghai, China). Streptavidin-MNBs (350 nm diameter, 0.05% Tween-20, and 1.0×10^{-5} mol L^{-1} EDTA at a concentration of 3.324×10^{11} beads mL^{-1}, 1.343 g mL^{-1}) were purchased from Bangs Laboratories Inc. (Fishers, IN). Tris (> 99.8%) from Amresco Inc. (Solon, OH), and Tween-20 from Sigma (St. Louis, MO) were used in the present work. N-methyl mesoporphyrin IX (NMM) was purchased from J&K Scientific Ltd. (Beijing, China). All other reagents (analytical grade) were acquired from standard reagent suppliers and used without further purification. All solutions were prepared with ultrapure water obtained through a Millipore Milli-Q water purification system (> 18.25 MΩ).

2.2. Preparation of MNB-probe

For the magnetic probe preparation, 1.0 μL streptavidin-MNBs suspension was washed five times with 200 μL TTL buffer (0.10 mol L^{-1} Tris–HCl, pH 8.0, 0.1% (v/v) Tween-20, 1.0 mol L^{-1} LiCl) to remove surfactants before use. Then, the mixtures of Bio-Apt (1.0×10^{-5} mol L^{-1}) and cDNA (1.0×10^{-5} mol L^{-1}) were heated at 95 °C for 5 min and incubated for 2 h at room temperature. After that, the DNA mixtures were mixed with the MNBs and incubated for 2 h at room temperature. At last, the MNB-probe was washed twice with PBS buffer (0.15 mol L^{-1} NaCl, 7.6×10^{-3} mol L^{-1} NaH_2PO_4, 2.4×10^{-3} mol L^{-1} Na_2HPO_4, pH 7.4) to remove the excess DNA. All washing steps in this work were performed under a magnetic field.

2.3. Exo III-assisted DNA cycling and HCR

The experiments were performed in TNaK buffer which consisted of 2.0×10^{-2} mol L^{-1} Tris, 0.60 mol L^{-1} NaCl, and 5.0×10^{-3} mol L^{-1} KCl (pH 7.5). HP, H1, and H2 were heated at 95 °C for 5 min and then allowed to cool slowly to room temperature for 1 h before use to ensure the formation of hairpin structure. The standard solution of adenosine was prepared in Tris–HCl buffer (2.0×10^{-2} mol L^{-1} Tris–HCl, 0.30 mol L^{-1} NaCl, pH 8.0). The following procedure was 5 μL adenosine and 15 μL TNaK buffer added to the vessel and incubated at room temperature for 2 h. Subsequently, the MNBs were magnetically separated under a magnetic field, and retained the reaction solution to the new vessel. Then, 5 μL HP (5.0×10^{-6} mol L^{-1}), Exo III (0.40 U mL^{-1}), and 5 μL 10 × Exo III Buffer (0.50 mol L^{-1} Tris–HCl, pH 8.0, 5.0×10^{-2} mol L^{-1} $MgCl_2$, 1.0×10^{-2} mol L^{-1} DTT) were added respectively. Such digestion reaction was performed at 37 °C for 1 h. After digestion, the reaction was terminated through heating at 75 °C for 20 min. Subsequently, 5 μL H1 (1.0×10^{-6} mol L^{-1}) and 5 μL H2 (1.5×10^{-6} mol L^{-1}) were added for HCR, and carried out at 37 °C for 3.5 h.

2.4. Fluorescence measurements and apparatus

After the HCR, 2 μL NMM (5.0×10^{-5} mol L^{-1}) and 8 μL KCl (1.0 mol L^{-1}) were added to the resulting product and incubated at 37 °C for 30 min. All the fluorescence measurements were