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Target-catalyzed assembly formation of metal-ion dependent DNAzymes for non-enzymatic and label-free amplified ATP detection

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ABSTRACT

In this work, on the basis of a dual recycling amplification means with the involvement of toehold-mediated strand displacement reaction (TSDR) and metal-ion dependent DNAzymes, we describe the establishment of a non-enzymatic and label-free method for sensitively detecting ATP in human serums. The target ATP binds the corresponding aptamer recognition probes and causes structure switching of the aptamers to expose the toehold regions for TSDR-based recycling of ATP and subsequent catalytic assembly formation of many DNAzymes with the assistance of DNA fuel strands. The corresponding metal ions further cyclically catalyze the cleavage of the G-quadruplex containing hairpin substrate strands of the DNAzymes, releasing lots of free G-quadruplex segments, which associate with the thioflavin T dye to result in drastically enhanced fluorescent signals for detecting ATP with high sensitivity. Such an ATP sensing method shows a dynamic range of 50–600 nM and a detection limit of 20 nM. The developed sensing system also has a high discrimination capability between ATP and other interfering analogue molecules, and can realize the detection of human serum ATP molecules, demonstrating its potential for convenient monitoring of low levels of ATP for the diagnosis of different diseases.

1. Introduction

Aptamers, synthetic single-stranded DNA/RNA oligonucleotides, act vital roles in numerous research areas. Aptamers are isolated by means of systematic evolution of ligands by exponential enrichment (SELEX), which is considered to be an essential method for the selection of specific DNA/RNA sequences from the random molecular nucleic acid libraries [1,2]. In addition, aptamers can act as a new class of recognition molecules that bind their targets, including small molecules [3–5], proteins [6], drugs [7], metal ions [8] and even cell surfaces [9] with high specificity and selectivity. Compared with traditional antibodies, aptamers show overwhelming advantages in biochemical analysis with the features of low molecular weight, easy synthesis and modification, high stability, wide range of targets and non-toxicity [10–13]. These unique characteristics of aptamers have attracted a lot of research interests, and many aptamer-based sensors have been designed and constructed for detecting a variety of target molecules, providing important methods for food safety monitoring, environmental testing, and disease treatment [14–16].

ATP, known as the “molecular currency” of intracellular species, plays a primary energy carrier for many living systems [17]. It can be stored as energy in cells or be converted to ADP to release energy to regulate various cell life activities [18]. Accumulating evidences have indicated that the variation of ATP concentration in human plays a very important role in association of different diseases. For instance, low concentrations of ATP indicate cell damage and apoptosis, whereas high levels of ATP suggest the occurrence of cardiovascular diseases and Alzheimer’s disease [19,20]. Therefore, the exploration of accurate and sensitive ATP detection methods is becoming urgent in clinical diagnosis and disease treatment.

Traditional instrumental assays for ATP, such as mass spectrometry and high performance liquid chromatography, require not only expensive experimental instruments but also cumbersome sample preparation processes as well as skilled operators [21]. These limitations has triggered recent development of several signal amplified approaches, such as enzyme-assisted target recycling [22,23] and nanomaterial-mediated signal amplification [24,25], for sensitively detecting ATP. Although featured with high sensitivity, analytical performances of the enzyme amplification approaches are greatly influenced by the environment and may produce false positive signals. Besides, the involvement of nanomaterials requires complicated synthesis and conjugation steps. Therefore, the development of effective and sensitive fluorescent sensors that can achieve simple enzyme- and label-free detection of ATP is urgently needed.

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We, herein, report on a simple enzyme- and label-free signal amplification approach for fluorescent detection of low levels of ATP via target-catalyzed assembly formation of DNAzymes on the basis of toehold-mediated strand displacement reactions (TSDRs). A typical TSDR involves the competition and displacement of a short single stranded complementary strand in a duplex at the toehold site by an invading strand to achieve a more stable duplex [26–28]. TSDR can occur spontaneously at room temperature, avoiding the involvement of enzymes, which has been demonstrated to be useful for the construction of enzyme-free amplification means for the detection of different nucleic acid targets [29–31]. Metal-ion dependent-DNAzyme is known as a class of catalytically functional DNA molecules, like proteins and RNA catalytic enzymes that can catalyze many types of biochemical reactions. Through the interactions with certain specific metal-ion cofactors, the single-stranded DNAzyme sequences fold into three-dimensional structures that bind to the corresponding substrates to exert catalytic functions [32–35]. Metal-ion dependent-DNAzymes exhibit significant advantages over common enzymes with the features of ease of synthesis, stability and cost. In our assay approach, the target ATP binds and causes structure conformation change of the aptamer probes to initiate TSDR assembly formation of Mg^{2+}-dependent DNAzymes. Moreover, with the assistance of the ssDNA fuel strands, the target ATP is recycled to trigger the assembly process to occur in a catalytic fashion, leading to the assembly formation of many DNAzymes. Consequently, catalytic digestion of the substrate sequences of the DNAzymes by Mg^{2+} results in the liberation of numerous G-quadruplex sequences, which associate with the thioflavin T (ThT) organic dye to produce significantly enhanced fluorescent emission for sensitively detecting ATP at the nanomolar level with a label- and enzyme-free convenient way.

2. Experimental

2.1. Chemicals and reagents

Oligonucleotides with sequences information listed below were all synthesized and purified by Shanghai Sangon Biotech Co., Ltd. (Shanghai, China). Hairpin Aptamer (HA): 5′- ATA CCG ACA GCG ATC TTT ACC ACC TTT CCA TTC ACC TTC CTG GGG GAG TAT TGC GGA GGA AGG T-3′; Blocking Probe (BP): 5′-GAA TGG AAA GGT GGT AAA G-3′; Substrate Sequence (SS): 5′-TGG GTT GGG CGG GAT GGG AAT TAT GCT TGG TTrA GGT CGG TAT CGT GGG TTC CCG CCC-3′; Fuel Strand (FS): 5′-AGG AAG GTG AAT GGA AAG GTG GTA AAG CAC CCA TGT ACC AAG CAT-3′. Guanosine triphosphate (GTP), ATP, uridine triphosphate (UTP) and cytidine triphosphate (CTP) were obtained from Worthington Biochemicals (Lakewood, NJ, USA). ThT was purchased from Sigma-Aldrich and dissolved in ultrapure water to prepare a stock solution (30 mM). It was stored at −20 °C and diluted to the desirable concentration by Tris-HCl buffer before use. Human serums were ordered from Sigma-Aldrich (St. Louis, MO). Other reagents were provided by Sinopharm Chemical Reagents, Co., Ltd. (Shanghai, China). All reagents were analytical grade and used as received. Ultrapure water having specific resistance (18.3 MΩ cm) was used in all the experiments.

2.2. Amplified fluorescent detection of ATP

The sensing probe (SP) for ATP was first prepared by annealing the mixture of HA (1 μM) and BP (1 μM) at 90 °C for 10 min in Tris-HCl buffer (20 mM Tris-HCl, pH 7.4, 100 mM NaCl, 15 mM $MgCl_2$, and 10 mM KCl), followed by cooling down at the rate of 1 °C•min^{-1} to 25 °C. SS was annealed with the same procedure to ensure the formation of stable hairpin structure. For ATP sensing, different concentrations of ATP were mixed with the sensing solution of SP (100 nM), FS (120 nM) and SS (400 nM) in Tris-HCl buffer for 2 h at room temperature. Subsequently, ThT (4 μM) was added to the reaction solution and incubated for 3 min in the dark. This was followed by measuring the fluorescence responses of the mixtures.

2.3. Native polyacrylamide gel electrophoresis (PAGE) experiment

The ATP-catalyzed assembly formation of the DNAzymes and subsequent cyclic cleavage of the DNAzymes were verified by electrophoresis analysis on the 16% native polyacrylamide gel. Electrophoresis was performed in 1 × TBE buffer at 100 V for 90 min. After staining with ethidium bromide, the gel was photographed with a digital camera under ultraviolet light.

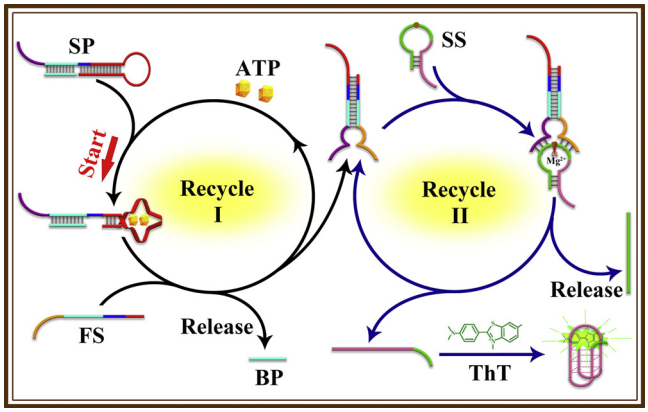
2.4. Fluorescence intensity measurements

Fluorescence spectra of the solutions were recorded by using a Shimadzu spectrophotometer (model No. RF-5301PC) at room temperature with a xenon lamp (150 W, Ushio Inc., Japan). The fluorescence intensity was monitored by exciting the samples at 425 nm and measuring the emission at 485 nm. The instrumental emission and excitation slit widths were both set at 10 nm and the emission spectral range between 460 nm and 570 nm was recorded. The results were reported as mean values of triplicates, and the experimental data were analyzed using the Origin software.

3. Results and discussion

3.1. Principle of the developed fluorescent ATP sensing strategy

The proposed principle of our amplified method for sensitive fluorescent monitoring of ATP on the basis of target-catalyzed assembly formation of Mg^{2+}-dependent DNAzymes is illustrated in Scheme 1. In this protocol, the sensing probe (SP) is composed of a hairpin aptamer (HA) and a blocking probe (BP) sequences. HA contains four functional regions: the ATP binding aptamer (red), toehold (blue), strand displacement sequence (cyan) and the split enzymatic sequence of the Mg^{2+}-dependent DNAzyme (purple). Initially, the toehold segment is designed in the stem of HA while the strand displacement sequence is hybridized with BP to inhibit TSDR-induced spontaneous assembly of FS and HA without the presence of ATP. In addition, part of the G-quadruplex sequence, which can associate with ThT to enhance the fluorescence of ThT, is hybridized in the stem of SS. Therefore, without ATP, the production of the Mg^{2+}-dependent DNAzymes is suppressed and low background fluorescence is expected. However, when ATP is incubated with the mixture of the sensing probes of FS and SS, ATP associates with the aptamer region of HA to result in structure switching of HA, leading to the exposure of the toehold region. FS further binds the exposed toehold region to form the HA/FS duplex and



Scheme 1. Diagram illustration of the target-catalyzed assembly formation of DNAzymes for amplified fluorescent determination of ATP.