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Target recycling amplification for label-free and sensitive colorimetric detection of adenosine triphosphate based on un-modified aptamers and DNAzymes

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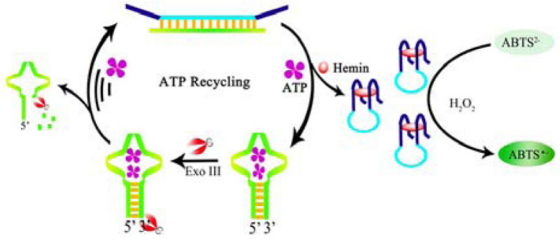
HIGHLIGHTS

- Completely un-modified aptamers and DNAzymes are employed as probes.
- The colorimetric signal output is intensified by Exo III-assisted ATP recycling.
- Selective and sensitive colorimetric detection of ATP at sub-nanomolar is achieved.

GRAPHICAL ABSTRACT



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ABSTRACT

Based on target recycling amplification, the development of a new label-free, simple and sensitive colorimetric detection method for ATP by using un-modified aptamers and DNAzymes is described. The association of the model target molecules (ATP) with the corresponding aptamers of the dsDNA probes leads to the release of the G-quadruplex sequences. The ATP-bound aptamers can be further degraded by Exonuclease III to release ATP, which can again bind the aptamers of the dsDNA probes to initiate the target recycling amplification process. Due to this target recycling amplification, the amount of the released G-quadruplex sequences is significantly enhanced. Subsequently, these G-quadruplex sequences bind hemin to form numerous peroxidase mimicking DNAzymes, which cause substantially intensified color change of the probe solution for highly sensitive colorimetric detection of ATP down to the sub-nanomolar (0.33 nM) level. Our method is highly selective toward ATP against other control molecules and can be performed in one single homogeneous solution, which makes our sensing approach hold great potential for sensitive colorimetric detection of other small molecules and proteins.

1. Introduction

Adenosine triphosphate (ATP) is one of the most important metabolites in all living cells, functioning as a major universal energy currency for the regulation of a variety of essential biological activities, such as biosynthesis, DNA replication and repair [1–3]. ATP has also been widely used as an effective indicator for the diagnosis of Parkinson's disease, hypoglycemia and hypoxia [4–6]. Considering the importance of ATP in biochemical studies and clinical diagnosis, the development of simple, sensitive and selective methods for the monitoring of ATP is therefore highly demanded. Although traditional methods such as mass spectrometry [7], high-performance liquid chromatography (HPLC) [8], can offer sensitive detection of ATP, these approaches generally suffer from tedious sample separation, expensive instruments and the requirement of highly trained personnel, which limit their wide

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applications. These limitations of the traditional methods have led to the development of new alternatives for ATP detection, among which the aptamer-based approaches have gained increasingly interest due to the unique advantages of the aptamer probes.

Aptamers are single-stranded artificial oligonucleotides (DNA or RNA) isolated from random-sequence nucleic acid libraries by an in vitro selection process termed as the systematic evolution of ligands by exponential enrichment (SELEX) [9,10]. These SELEX-isolated aptamers can bind a wide range of targets (e.g., proteins, amino acids, cells, organic and inorganic compounds) with high specificity and affinity [11,12]. Due to their versatile target binding capabilities and superior advantages in terms of stability, low cost, easy synthesis and modification over antibodies, aptamers have been increasingly used as recognition probes for designing different types of biosensors. With the successful selection of the ATP binding aptamer [13], several aptasensors for ATP based on fluorescent [14,15], electrochemical [16] and bioluminescent [17,18] signal transduction means have been suggested. Indeed, these approaches have advanced the detection of ATP with reduced procedures and acceptable sensitivity. However, the fluorescent/bioluminescent approaches for ATP detection commonly require the labeling of the probes with fluorescent/enzyme tags while the electrochemical methods require the attachment of the probes on the electrode sensing surfaces, which basically increase the complexity and cost for ATP monitoring. Therefore, the exploration of new methods without using complicated instruments or labeled probes can substantially facilitate ATP detection. In this regard, Lu and co-workers [19] reported the first example of a colorimetric ATP detection method based on ATP-induced disassembly of the aptamer-linked gold nanoparticle (AuNP) aggregates recently. In their approach, the presence of ATP led to the disassembly of the AuNP aggregates assembled from aptamer-conjugated AuNPs, which resulted in the change of the optical plasmon resonance properties of the AuNPs [20,21] and caused the color change of the probing solution from blue to red for colorimetric detection of ATP. Despite the advantage of avoiding the use of complicated instruments, this AuNP-based colorimetric ATP detection method required cumbersome procedures (~48 h) for AuNP-aptamer/complementary DNA conjugation and the sensitivity was compromised due to the lack of effective signal amplifications.

Herein, based on DNAzymes and exonuclease-catalyzed target recycling amplifications, we report on the development of a label-free and sensitive strategy for colorimetric detection of ATP by using completely un-modified probes. DNAzymes are functional catalytic nucleic acids, which can catalyze chemical reactions upon association with specific cofactors [22–24]. For example, the G-quadruplex/hemin (cofactor) DNAzyme exhibits peroxidase mimicking activity to catalyze H_2O_2-meidated oxidization of colorless 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{2-}$) to the green colored product $ABTS^{\bullet-}$, which has been successfully employed for colorimetric detection of nucleic acid and protein targets [25–27]. In our sensing strategy, the G-quadruplex sequences are incorporated into the aptamer dsDNA probes. The presence of ATP leads to the production of the G-quadruplex/hemin DNAzymes for subsequent colored signal output. Besides, an exonuclease-catalyzed signal amplification approach is used to intensify the color change to achieve high sensitivity. With the use of the un-modified probes and the incorporation of the signal amplification approach, sensitive colorimetric detection of ATP can be realized in a true label-free and homogenous fashion.

2. Experimental

2.1. Materials and reagents

Exonuclease III (Exo III) was obtained from Takara Biotechnology Co., Ltd. (Dalian, China). ATP, uridine triphosphate (UTP), guanidine triphosphate (GTP) and cytosine triphosphate (CTP) were ordered from Worthington Biochemical (Lakewood, NJ, USA). Thrombin (TB) and bovine serum albumin (BSA) were from Sigma–Aldrich (St. Louis, MO, USA). Hemin, $ABTS^{2-}$ and H_2O_2 were purchased from Aladdin Reagents (Shanghai, China). The stock solution of hemin (1 μM) was prepared in dimethylsulfoxide (DMSO) and stored in the dark at −20 °C. The G-quadruplex containing sequence (5′-GGG ATG GGT TCC GCA ATA CTC CCT GGG TTG GG-3′) and the ATP binding aptamer (5′-TAC CTG GGG GAG TAT TGC GGA GGA AGG TA-3′) were purchased from Shanghai Sangon Biological Engineering Technology and Services Co., Ltd. (Shanghai, China). All reagents were of analytical grade and used without further purification.

2.2. Apparatus

A canon EOS 550D camera was used to take all photographs. A Shimadzu UV-2450 spectrophotometer (Shimadzu, Japan) was used to obtain the absorption spectra at room temperature in all experiments.

2.3. Amplified colorimetric detection of ATP

The G-quadruplex containing sequence (2 μM) and the ATP binding aptamer (2 μM) were first mixed in Tris-buffer (25 mM Tris–HCl, 100 mM NaCl, 5 mM $MgCl_2$, pH 7.4) at the total volume of 50 μL. The mixture was incubated at 90 °C for 10 min and gradually cooled at room temperature within 2 h. Next, appropriate concentrations of ATP and Exo III were added into the above probe solution and incubated at 37 °C for 60 min. Then, 50 μL 2 × HEPES buffer (50 μM HEPES, 40 mM KCl, 400 mM NaCl, 0.1% TritonX-100, 2% DMSO, pH 7.4) and 10 μL (1.0 μM) hemin were added to the solutions and incubated at room temperature for 60 min. Finally, $ABTS^{2-}$ and H_2O_2 were added to the mixture to reach final concentrations of 6 mM and 2 mM, respectively, with a total volume of 125 μL. Photographs of the solutions were taken after 5 min of color development.

3. Results and discussion

Our Exo III-catalyzed, sensitive and label-free colorimetric ATP sensing principle is illustrated in Scheme 1. The probes involve the partially complementary dsDNA strands containing the G-quadruplex sequences and the ATP binding aptamers. The dsDNA probes are designed in such a way that the middle parts of the two sequences are hybridized to inhibit the formation of the G-quadruplex/hemin DNAzymes in the absence of ATP. Besides these, partially complementary dsDNA probes with four single stranded termini are resistant to Exo III digestion in the absence of ATP due to the fact that Exo III only shows activity to dsDNA strands with blunt or 3′-recessed termini. However, when ATP is added to the probe solution, it associates with the ATP-binding aptamers and causes conformational changes of aptamers to form ternary hairpin structures with blunt termini, which create catalytic sites for Exo III. Exo III then digests the ATP associated aptamers to release ATP, which again binds the aptamers of the dsDNA probes to initiate the ATP recycling process. This ATP recycling process thus results in effective digestion of the ATP-binding aptamers of the dsDNA probes and the formation of many active G-quadruplex sequences, which subsequently bind hemin to generate numerous DNAzymes and cause intensified color change of the solution (with the presence of $ABTS^{2-}$ and H_2O_2) for sensitive colorimetric detection of ATP.

The feasibility of our proposed assay method for label-free and amplified colorimetric ATP detection was first verified with/without the addition of Exo III to the sensing system for