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Cascaded signal amplification via target-triggered formation of aptazyme for sensitive electrochemical detection of ATP

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ABSTRACT

The construction of reliable sensors for adenosine triphosphate (ATP) detection gains increasing interest because of its important roles in various enzymatic activities and biological processes. Based on a cascaded, significant signal amplification approach by the integration of the aptazymes and catalytic hairpin assembly (CHA), we have developed a sensitive electrochemical sensor for the detection of ATP. The target ATP leads to the conformational change of the aptazyme sequences and their association with the hairpin substrates to form active aptazymes, in which the hairpin substrates are cyclically cleaved by the metal ion cofactors in buffer to release the enzymatic sequences that can also bind the hairpin substrates to generate active DNAzymes. The catalytic cleavage of the hairpin substrates in the aptazymes/DNAzymes thus results in the generation of a large number of intermediate sequences. Subsequently, these intermediate sequences trigger catalytic capture of many methylene blue-tagged signal sequences on the electrode surface through CHA, producing significantly amplified current response for sensitive detection of ATP at 0.6 nM. Besides, the developed sensor can discriminate ATP from analogous interference molecules and be applied to human serum samples, making the sensor a useful addition to the arena for sensitive detection of small molecules.

1. Introduction

Adenosine triphosphate (ATP), a significant small molecule in living species, plays crucial roles in various enzymatic activities and biological processes, including DNA biosynthesis, cardiac function, platelet function, muscle contraction and neurotransmission (Chen et al., 2016; Fu et al., 2016; Zhang et al., 2012). Research evidences have also shown that ATP can be an indicator of animate beings for cell viability and cell injury, and various concentration of ATP has a close relationship with angiocardiopathy, parkinsonism, and Alzheimer's diseases (Li et al., 2016b; Lu et al., 2015). Considering the important biological functions of ATP in biochemical studies, clinical diagnosis, and environmental analysis, the development of sensitive and specific methods that can recognize and detect ATP is of essential significance. In the past few decades, many methods have been reported for the detection of ATP with good performances, such as mass spectrometry (Huang and Chang, 2007), high performance liquid Chromatography (Zhou et al., 2012), fluorescence (Wang et al., 2016; Zhu et al., 2016), electrochemiluminescence (Liu et al., 2014). Although these methods can achieve sensitive detection of ATP, yet the shortcomings such as complex sample preparations, low-sensitivity and high-cost have limited their wide applications (Ma et al., 2016, 2017; Xu et al., 2016). Therefore, the establishment of simple and sensitive signal amplification approaches will potentially facilitate ATP detection.

Aptamers, DNA/RNA oligonucleotides, are derived from random-sequence nucleic acid libraries through an in vitro selection process referred to systematic evolution of ligands by exponential enrichment (SELEX) (Lu et al., 2012; Rohrbach et al., 2013; Zhou et al., 2010). Because these functional oligonucleotides can form unique and exclusive tertiary structures (Yang et al., 2011; Zhang et al., 2010), aptamers are capable of specifically recognizing and binding the corresponding targets from metal ions and small molecules to large proteins and even cells (Hermann and Patel, 2000; Tan et al., 2013; Zhu et al., 2012). In addition, aptamers exhibit several advantages including high thermo-stability, low-cost, ease of synthesis and reversibility over protein antibodies as recognition probes (Li et al., 2012). In recent years, numerous aptamer-based sensors have been reported for the detection of a variety of target analytes (Deng et al., 2014; Dhiman et al., 2017; Zhan et al., 2016).

One of the key factors in designing these aptasensors is the

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integration of effective signal amplification means to achieve high sensitivity. Signal amplifications based on ion-dependent DNAzymes have attracted increasing attention recently due to their simplicity and amplification capability. Ion-dependent DNAzymes possess high catalytic cleavage activities and their function of cleaving substrate sequences is triggered by specific metal-ions. These unique properties have enabled the ion-dependent DNAzymes a convenient signal amplification approach for the detection of metal ions (Hwang et al., 2014; Zhou et al., 2016), DNA (Liu et al., 2015; Wang et al., 2014) and proteins (Li et al., 2016a; Wei et al., 2016). Analogous to DNAzymes, aptazymes, which are composed of aptamers with binding sequences responsive to the target and DNAzyme/RNAzyme sequences, show similar cleavage activities. Besides, aptazymes have the recognition capability of aptamers and the catalytic cleavage activity of the DNAzymes that can only be regulated by the allosteric cofactors (some small molecules) (Liu and Lu, 2004). In other words, the enzymatic function of the aptazymes can only be activated when the allosteric cofactor binds with the corresponding aptamer and causes an adaptive structural folding. Such a unique property has demonstrated to be a useful strategy for the construction of different sensors for the detection of specific small molecule targets (Song et al., 2012; Yang et al., 2016).

By combining both the unique catalytic activities of the aptazymes/DNAzymes with the catalytic hairpin assembly (CHA), we report a new cascaded signal amplification approach for sensitive electrochemical detection of ATP. The target ATP triggers the formation of the aptazymes, in which the hairpin substrates are cyclically cleaved by the metal ions present in buffer to release many intermediate sequences. Subsequent unfolding and attachment of the methylene blue (MB)-tagged hairpins on the electrode surface through the intermediate sequence-induced CHA thus generates cascaded amplification of the current response from MB, leading to highly sensitive detection of ATP. By integrating the aptazymes/DNAzymes with CHA into the sensor design, our ATP sensing platform exhibits three main advantages. First, cascaded and significant signal amplification can be achieved without the involvement of any enzyme or nanomaterial. Second, ATP-responsive allosteric regulation of the activity of the aptazymes makes our sensor highly selective. Third, our ATP detection approach is simple because of the distinct feature of the electrochemical techniques in terms of simple instrumentation, speed, high sensitivity and ease of miniaturization. With these advantages, the method developed herein can potentially advance the monitoring of ATP in a simple and sensitive fashion.

2. Experimental section

2.1. Materials and reagents

ATP, cytidine triphosphate (CTP), uridine triphosphate (UTP) and guanosine triphosphate (GTP) were purchased from Worthington Biochemicals (Lakewood, NJ, USA). Tris-HCl, tris(2-carboxyethyl) phosphine hydrochloride (TCEP) and 6-mercapto-1-hexanol (MCH) were supplied by Sigma (St. Louis, MO). 4-(2-Hydroxyethyl) piperazine-1 ethanesulfonic acid sodium salt (HEPES) and all synthetic oligonucleotides were obtained from Sangon Biological Engineering Technology and Services Co., Ltd. (Shanghai, China). The sequences of the oligonucleotides were listed as follows. Aptazyme sequence: 5′-AGA TAT CAG CGA TCT GGG GGA GTA TTG CGG AGG AAG CAC CCA TGT TAC GTA ACA-3′; Hairpin substrate (HP1): 5′-GAT ATC AGC GAT CCG GAA CGG CAC CCA TGT TAC GTA TrAG GAT ATC TGC CCT TGG TGC CGT-3′ (rA indicated adenosine ribonucleotide); Hairpin immobilization probe (HP2): 5′-SH-$(CH_2)_6$-TTTTTT ACG GCA CCA AGG GCA GAT ATC CCC ATG TGT AGA GGA TAT CTG CCC TT-3′; Hairpin signal probe (HP3): 5′-GAT ATC CTC TAC ACA TGG GGA TAT CTG CCC TTC CAT GTG TAG A-MB-3′. Reagents were of analytical grade and aqueous solutions were prepared using ultrapure water (specific resistance of 18 MΩ-cm).

2.2. Preparation of the sensors

Prior to use, the gold electrode (AuE, 3 mm in diameter) was first cleaned by immersing in the freshly prepared piranha solution (mixture of 98% H_2SO_4 and 30% H_2O_2 at a volume ratio of 3:1) for at least 30 min. After rinsing thoroughly with ultrapure water, the AuE was polished with 0.3 and 0.05 μm aluminum slurry on a flat pad for 5 min separately. This was followed by sonicating treatments in ultrapure water, ethanol and ultrapure water for 5 min, respectively, to remove the impurities on the AuE surface. Then, the AuE was further electrochemically cleaned in a fresh H_2SO_4 solution (0.5 M) with successive scans from − 0.3 to 1.55 V until a reproducible voltammetric peak was observed. Finally, the AuE was thoroughly rinsed with ultrapure water and dried with nitrogen to obtain the pretreated electrode.

All hairpins were separately annealed at 95 °C for 10 min, followed by cooling down to room temperature at a rate of 1 °C min^{-1} prior to use. A droplet of 10 μL of HP2 (0.5 μM, treated with 10 mM TCEP for 60 min) in Tris-HCl buffer (20 mM, 140 mM NaCl, 5 mM KCl, pH 7.5) was placed onto the pretreated AuE and incubated in humid environment for 120 min at room temperature. After washing with ultrapure water, the resulting HP2-modified AuE was carefully dried under a stream of N_2 and then dipped into 10 μL of 1.0 mM freshly prepared MCH for 120 min to block the surface to obtain the HP2/MCH/AuE.

2.3. Amplified detection of ATP

In the ATP assay, HP1 and the aptazyme sequence were mixed in 25 mM HEPES buffer (100 mM NaCl, 5 mM $MgCl_2$, pH 7.2) for 10 min. Subsequently, different concentrations of the target ATP in HEPES buffer were added to the above mixture to initiate the enzymatic cleavage reaction for 90 min. The resultant mixture with HP3 (the final concentrations of aptazyme sequence, HP1 and HP3 were 1 μM each) were transferred onto the HP2/MCH/AuE and incubated for 120 min at 37 °C. Finally, the electrodes were thoroughly rinsed with Tris-HCl buffer and SWV measurements were performed in Tris-HCl buffer.

2.4. Electrochemical measurements

All measurements including cyclic voltammetry (CV) and square wave voltammetry (SWV), were done using a CHI 852C electrochemical workstation (CH Instruments, Shanghai, China), which equipped with typical three-electrode system comprised of the modified AuE working electrode, a Ag/AgCl electrode (saturated with KCl), and a platinum wire counter electrode. Before SWV measurements, the detection buffer was thoroughly degassed under nitrogen-gas flow for 30 min. SWV measurements were conducted by scanning the potential of the proposed sensor from − 0.5 to 0 V in Tris-HCl buffer with a step potential of 4 mV, a frequency of 25 Hz and an amplitude of 25 mV.

3. Results and discussion

3.1. Design principle of the sensor

In our sensor design, the target-induced aptazyme recycling amplification strategy is combined with CHA to achieve significant signal enhancement for highly sensitive electrochemical detection of ATP, and the detection principle is conceptually depicted in Scheme 1. The aptazyme sequence, containing the ATP binding aptamer sequence and the Mg^{2+}-dependent 10–23 DNAzyme catalytic sequence (Mei et al., 2003), has the double functions of specifically identifying ATP and cleaving the substrate sequences. However, the cleavage function of the aptazyme sequence is inhibited with the absence of the target ATP. On the contrary, when ATP is present, the aptazyme sequences can bind the target ATP and fold into the active aptazyme sequences, which hybridize with the hairpin substrate (HP1) to form the aptazymes. Subsequently, the Mg^{2+} ions in buffer specifically cleave the HP1 into two