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Homogeneously ultrasensitive electrochemical detection of adenosine triphosphate based on multiple signal amplification strategy

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ABSTRACT

An ultrasensitive electrochemical aptasensor was successfully fabricated for the detection of adenosine triphosphate (ATP). For the first time, one detection system combined several elements: magnetic aptamer sequences for target recognition and separation, a DNAzyme assisted cyclic signal amplification strategy, layer-by-layer (LBL) quantum dots (QDs) composites for promoting square wave anodic stripping voltammetric (SWASV) analysis and Bi, Nafion (Nf) and three-dimensional ordered macroporous polyaniline-ionic liquid (Bi/Nf/3DOM PANI-IL) film modified glassy carbon electrode (GCE) for monitoring enhanced SWASV signal. The modification of Nf/3DOM PANI-IL on GCE showed that the preconcentration efficiency was improved by the electrostatic absorption of Cd^{2+} with negative Nf layer with the enhanced analytical sensitivity due to a large active surface area of 3DOM structure. The increased SWASV peak current values of the label $(CdS)_4$@SiO_2 composites were found to be proportional to the logarithmic value of ATP concentrations in the range of 1 pM–10 nM and 10 nM–1 μM, with the detection limit as low as 0.5 pM. The proposed aptasensor has shown an excellent performance such as high sensitivity, good selectivity and analytical application in real samples. The results demonstrated that the multiple signal amplified strategy we developed was feasible for clinical ATP assay and would provide a promising model for the detection of other small molecules.

1. Introduction

Small organic molecules in body fluids or tissues, with a molecular weight of less than 1000 Da, are important analytes of various molecular and cellular researches, as well as clinical diagnostics (D'Orazio, 2011). For example, adenosine-5′-triphosphate (ATP, molecular weight of 507.2 Da) is one of the most important metabolites in biological systems. It is not only the energy source for biological reactions, but also an extracellular signaling agent in biological processes such as photosynthesis, enzyme catalysis, biosynthesis, DNA replication, and cellular respiration (Knowles, 1980; Brandon et al., 2006). Furthermore, ATP also serves as a marker for evaluating micro-fungal contamination in food industry (Davidson et al., 1999). Therefore, the determination of ATP has become very important. Many techniques have been developed to detect ATP, including chromatography (Stratford, Dennis (1994)), bioluminescence and chemiluminescence (Ribeiro et al., 1998). Recently, new designs of ATP detection have emerged based on the advancement of analytical technologies employing aptamers.

Aptamers are *in vitro* selected functional single-stranded DNAs, RNAs or even chemically modified nucleic acids, which could fold into special structures and possess high recognition ability to specific targets ranging from metal ions, organic and inorganic small molecule, proteins and even whole cells (Li et al., 2013). In particularly, aptamer provides excellent advantages of reversible thermal denaturation and unlimited shelf life (Xu et al., 2013). A variety of aptamer sensors (aptasensors) based on electrochemistry (Chen et al., 2013; Wang et al., 2012), fluorescence (Tedsana et al., 2013), luminescence (Lu et al., 2013), surface plasmon resonance (SPR) (Liu et al., 2012), colorimetry (Guo et al., 2011) and quartz crystal microbalance (QCM) (Ruslinda et al., 2012) has been developed for the detection of ATP. Among these aptasensors, electrochemical aptasensors are the most attractive due to their advantages of fast response, portability, high sensitivity, simple instrumentation, low cost (Xiao et al., 2005; Du et al., 2011; Yin et al., 2012).

In order to improve the sensitivity of ATP electrochemical aptasensors, many signal amplification strategies generally rely on the transduction technology and the signal-reporting pattern (Lubin and Plaxco, 2010). Nanomaterial assisted amplification is an effective route and has attracted considerable attention for the construction of ultrasensitive aptasensors. Among various nanomaterials, three dimensional ordered macroporous (3DOM) Au is

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flavored in new generation of aptasensors, due to its good biocompatibility with immobilized biomolecules and large specific surface area for charge and mass transportation in electrochemistry (Walcarius, 2012). Zhu constructed an electrochemical aptasensor based on 3DOM Au for the detection of ATP, with the detection limit of 0.01 nM (Zhou et al., 2010). Quantum dots (QDs) are nanostructured semiconductor materials with a size typically between 1 and 12 nm that provides unique optical and electronic properties due to the quantum confinement effect (Weller, 1993). Since the first studies applying QDs as electrochemical label were published by Wang et al. (2002), QDs have gained interests for the sensitive detections in electrochemical bioassays (Wang et al., 2003a, 2003b). Typically, this procedure consists of dissolving the QDs by acid attack for releasing metal ions, which can be easily determined by square wave anodic stripping voltammetry (SWASV) with a mercury film glassy carbon electrode. Significantly, acid dissolution of QDs tags releases numerous metal ions, indicating the built-in amplification nature of the QDs labels (Hansen et al., 2006a, 2006b).

However, the conventional SWASV method has a major limitation for wider application because of the requirement to use, manipulate and dispose metallic mercury or mercury salts. Since the year 2000, bismuth-film electrode (BiFE) has become an attractive subject in heavy metal analysis replacing mercury-film electrode (HgFE), due to its environmental friendly nature (Wang et al., 2000). Recently, electroanalysis of trace heavy metal ions at the conducting polymer modified electrodes has received considerable attention, as the polymer can enrich the target heavy metals and improve the analytical performance (Imisides et al., 1991). In this study, Bi/Nafion (Nf)/3DOM polyanline (PANI)-ionic liquid (IL) composite electrode with enlarged surface area, good stability and conductivity was achieved to enhance SWASV electrochemical signal of CdS QDs for the first time.

Further, most electrochemical aptasensors are based on heterogeneous assays involving the immobilization of the aptamers on the electrode surface prior to collecting the target recognition induced electrochemical signal. In this way, the recognition event of aptamer to analyte occurs on the interface between the solution and electrode, and the configurational freedom of the immobilized aptamer is often restricted owing to the steric hindrance of the electrode surface, accompanied by the relatively low binding efficiency and rate of reaction of aptamer toward the substrate compared with homogeneous assays. Until very recently, Li's group first demonstrated an electrochemical aptamer-based strategy for ATP detection in a homogeneous solution phase, with a detection limit of 1 nM (Lu et al., 2013).

Herein, we proposed another homogeneous electrochemical bioassay for the detection of ATP combining target-induced recycling DNAzyme catalysis and SWASV signal amplification. As shown in Scheme 1, the novel bioassay involves three steps (1) the duplex of S1 and 5′-thiol (SH) modified ATP aptamer was chemi-absorbed onto the surface of Au–SiO_2@Fe_3O_4. In the presence of target ATP, the aptamer formed stable tertiary structure with ATP, which accordingly denatured the duplex and liberated the complementary S1; (2) molecule beacon (MB) molecules were immobilized onto the surface of Au–SiO_2@Fe_3O_4 by S–Au interaction. The MB–Au–SiO_2@Fe_3O_4 composites were added into the S1 solution of step 1, and then double stranded DNA modified Au–SiO_2@Fe_3O_4 particles were obtained by hybridization of MB with its partially complementary S1. After Zn^{2+} ions triggered the catalytic reaction with MB at the scissile rA, T1 fragments of MB dissociated from S1/MB–Au–SiO_2@Fe_3O_4. The released S1 hybridized to another MB to trigger a series of DNAzyme catalyzed scission reactions under the cofactor Zn^{2+}, and another batch of T1 fragments was released, which then recycled to the headstream; (3) The multi-layered $(CdS)_4$@SiO_2 nanocomposite was synthesized by firstly the covalent combination between amino-terminated SiO_2 particles and carboxyl-stabilized CdS QDs, and then polyelectrolyte-attended electrostatic self-assembling between neighboring QDs. Single stranded S2 could be combined with $(CdS)_4$@SiO_2 via the acylamide binding in the presence of EDC as the activator, forming S2-$(CdS)_4$@SiO_2. Further, 5′–SH terminal S3 was immobilized onto the surface of 3DOM Au film modified electrode. In the presence of T1, S2-$(CdS)_4$@SiO_2 gained the chance of hybridization with S3 on the electrode surface. Subsequently, $(CdS)_4$@SiO_2 was dissolved with HNO_3 for SWASV analysis. Therefore, the target ATP induced strand displacement was successfully monitored and ATP detection could be realized through the electrochemical signal using electrodeposited Bi/Nf/3DOM PANI-IL modified electrode.

2. Experimental

2.1. Chemicals and materials

N-(3-dimethylamminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC), N-hydroxysulfosuccinimid sodium salt (NHS), sodium oleate (SO) was purchased from Aladdin chemistry Co. Ltd. (Shanghai, China). $CdCl_2 \cdot 2.5H_2O$, $Bi(NO_3)_3 \cdot 5H_2O$, $Zn(NO_3)_2 \cdot 6H_2O$, $FeSO_4 \cdot 7H_2O$, $FeCl_3 \cdot 6H_2O$, sodium dodecyl benzene sulfonate (SDBS), tetraethoxysilane (TEOS, 98%) and $Na_2S \cdot 9H_2O$ were all obtained from Shanghai Lingfeng chemical reagent Co. LTD (Shanghai, China). Thioglycolic acid (TGA) and poly(diallyldimethylammonium chloride) (PDDA, 20 wt%) were purchased from Aldrich. 1-Butyl-3-methylimidazolium tetrafluoroborate ([BMIm][BF_4], purity $>99\%$) was purchased from Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences. Nafion (Nf) was obtained from DuPont as 5 wt% solution and diluted to 0.5% in water. Aniline (AN) was distilled twice under reduced pressure and stored in dark at low temperature before use. 0.1 M phosphate buffer saline (PBS) was prepared by mixing the stock solutions of KH_2PO_4 and K_2HPO_4, and adjusted to appropriate pH by addition of 0.1 M KOH or H_3PO_4 solution. Adenosine triphosphate (ATP), cytidine triphosphate (CTP), guanosine triphosphate (GTP), uridine triphosphate (UTP), diethyl pyrocarbonate (DEPC) and 6-Mercaptohexanol (MCH) were obtained from Shanghai Baoman Biotech. The SiO_2 spheres with the diameter of 500 nm were obtained from Alfa Asear. The amino functionalized SiO_2 spheres with the diameter of 20 nm were purchased from Haitai NANO Co. Ltd. (Nanjing, China). Au coated glass substrate was provided by Shanghai Institute of Microsystem and Information Technology (SIMIT), Chinese Academy of Sciences (CAS).

The DNA sequences were ordered from Shanghai Sangon Engineering Technology and Services Co., Ltd., as follows:

DNA	Sequence (from 5′ to 3′)
ATP aptamer	SH-$(CH_2)_6$-AAAAAATGGAAGGAGGCG-TTATGAGGGGTCCACGCCAACTATTTCG
S1	CATCTCTTCTCCGAGCCGGTCGAAATAGTGGGTG
S2	NH_2-$(CH_2)_6$-T_{10}-CCACCACCGCCTC
S3	SH-$(CH_2)_6$-T_{10}-CCTTCTCTACAATG
MB	SH-$(CH_2)_6$-CCACCACATTCAAATTCACCAA-CTATrAGGAAGAGATGTTACGAGGCGGTGGTG

S1, S2, S3 and MB stock solutions were all prepared in 25 mM pH 8.0 Tris–HAc solution containing 300 mM NaCl. ATP aptamer and ATP stock solution were prepared in 25 mM pH 8.0 Tris–HAc solution containing 300 mM NaCl and 5 mM $MgCl_2$. The DNA strands hybridization and aging solution was 25 mM pH 8.0 Tris–HAc solution containing 300 mM NaCl. 5 mM MCH solution was prepared in 10 mM pH 7.4 Tris–HAc solution. In order to