Biosensors and Bioelectronics 26 (2010) 834–840

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Quantum dots electrochemical aptasensor based on three-dimensionally ordered macroporous gold film for the detection of ATP

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ARTICLE INFO

Article history:
Received 18 March 2010
Received in revised form 23 April 2010
Accepted 11 May 2010
Available online 19 May 2010

Keywords:
Aptasensor
Quantum dots
Macroporous
ATP
Stripping analysis

ABSTRACT

A sensitive electrochemical aptasensor was successfully fabricated for the detection of adenosine triphosphate (ATP) by combining three-dimensionally ordered macroporous (3DOM) gold film and quantum dots (QDs). The 3DOM gold film was electrochemically fabricated with an inverted opal template, making the active surface area of the electrode up to 9.52 times larger than that of a classical bare flat one. 5′-Thiolated ATP-binding aptamer (ABA) was first assembled onto the 3DOM gold film via sulfur–gold affinity. Then, 5′-biotinated complementary strand (BCS) was immobilized via hybridization reaction to form the DNA/DNA duplex. Since the tertiary structure of the aptamer was stabilized in the presence of target ATP, the duplex can be denatured to liberate BCS. The reaction was monitored by electrochemical stripping analysis of dissolved QDs which were bound to the residual BCS through biotin–streptavidin system. The decrease of peak current was proportional to the amount of ATP. The unique interconnected structure in 3DOM gold film along with the "built-in" preconcentration remarkably improved the sensitivity. ATP detection with high selectivity, wide linear dynamic range of 4 orders of magnitude and high sensitivity down to 0.01 nm were achieved. The results demonstrated that the novel strategy was feasible for sensitive ATP assay and provided a promising model for the detection of small molecules.

1. Introduction

Aptamers are functional oligonucleotides in vitro selected from random-sequence nucleic acid libraries (Ellington and Szostak, 1990; Robertson and Joyce, 1990; Tuerk and Gold, 1990), which possess specific recognition abilities to various targets ranging from small molecules to large proteins and even cells (Hermann and Patel, 2000). The high binding specificity and affinity, target versatility, simplicity of in vitro selection along with the chemical stability, make aptamers promising candidates in bioapplications. Recently, substantial research efforts are exerted to develop aptamer-based sensing interfaces (*i.e.*, aptasensors) with different detection techniques including surface plasmon resonance (SPR) (Minunni et al., 2005; Win et al., 2006), colorimetry (Wang et al., 2007), quartz crystal microbalance (QCM) (Liss et al., 2002), fluorescence (Abérem et al., 2006; Nutiu and Li, 2003, 2005; Shlyahovsky et al., 2007; Wang et al., 2005b), electrophoresis (Andre et al., 2005), electrochemiluminescence (ECL) (Fang et al., 2008; Huang et al., 2009), electrochemistry (Baker et al., 2006; Hansen et al., 2006b; Radi et al., 2005, 2006; Radi and O'Sullivan, 2006; Xiao et al., 2005a,b; Zayats et al., 2006; Zuo et al., 2007, 2009), and so on. Due to their inherent properties of high sensitivity, simplicity, fast response and portability, electrochemical assays are widely used in this field and a series of electrochemical aptasensors have been fabricated for the determination of adenosine triphosphate (ATP), cocaine, potassium and thrombin (Baker et al., 2006; Radi and O'Sullivan, 2006; Radi et al., 2006; Xiao et al., 2005a,b; Zuo et al., 2007, 2009). In those woks, the aptamers were dual-modified with a thiol group at their one end for the immobilization of sensing probe onto Au electrode surface, and a redox moiety such as methylene blue or ferrocene at the other end for electrochemical sensing. The redox moiety approaches the electrode surface and generates measurable electrochemical signals when the aptamer undergoes target binding-induced conformational change. However, the effective exchange of electrons between redox moiety and the electrode can be suppressed since the redox moiety might be distal to the electrode surface, which leads to poor sensitivity.

Signal enlargement associated with the use of nanoparticles is an effective path and has attracted considerable attention for ultrasensitive detection of nucleic acids and proteins (Rosi and Mirkin, 2005; Wang, 2005; Zhang et al., 2006, 2008). Such attractive protocol is also employed to design electrochemical aptamer-based platforms aiming to address the demands of furthering high sensitivity. Chen and Dong developed sensitively amplified electrochemical aptasensors based on gold nanoparticles (Li et al., 2009; Du et al., 2009). In their designs, the gold nanoparticles carried a

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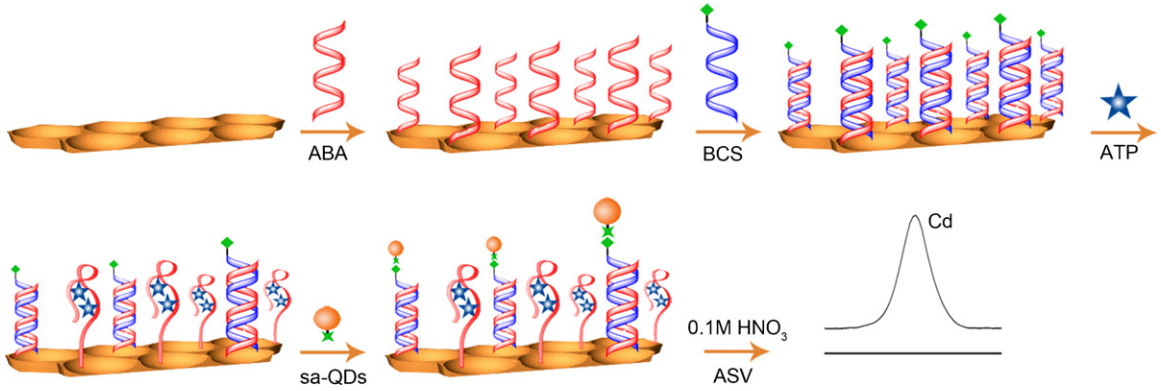
doi:10.1016/j.bios.2010.05.021



Scheme 1. Schematic illustration of the QDs electrochemical aptasensor based on 3DOM gold film.

large number of DNA strands and $[Ru(NH_3)_6]^{3+}$ (or methylene blue) probes, and this directly resulted in the significant enhancement of sensitivity. Recently, quantum dots (QDs) have been used as the electroactive labels for the detection of DNA and proteins due to their unique amplification feature (Hansen et al., 2006a,b; Liu et al., 2004; Wang, 2007; Wang et al., 2003; Yang et al., 2009). Hansen reported a metal sulfide QDs-based electrochemical method which could detect down to 100 amol of target DNA (Hansen et al., 2006a). Liu reported an aptamer-based sandwich type sensor by using CdSe QDs as electrochemical labels for the detection of thrombin in human serum (Yang et al., 2009). Therefore, QDs can be used as the ideal candidates for the sensitive detections in electrochemical bioassays.

Another effective way to improve the sensitivity of biosensors is the import of three-dimensionally ordered macroporous (3DOM) films. 3DOM films have highly ordered porous structure and significantly enlarged active surface area. The electrodes modified with 3DOM films have been successfully used to design electrochemical sensors (Walcarius and Kuhn, 2008). Macroporous gold film electrode was used for the direct electron transfer of hemoglobin since it had a high capacity for the loading of protein (Wang et al., 2005a). Macroporous ultramicro-electrodes (UMEs) were also used for electrochemical glucose sensing and the electrochemical signal was amplified up to 2 orders of magnitude (Szamocki et al., 2007). We have recently proposed a 3DOM gold film based label-free immunosensor for the detection of C-reactive protein which had better performance than that of nonporous ones (Chen et al., 2008). However, to our best knowledge, no report was found involving 3DOM gold films as signal enlargement element in the fabrication process of aptasensor.

Herein, a sensitive electrochemical aptasensor combining unique features of both 3DOM gold film and QDs was fabricated for the detection of ATP. It is well-known that ATP is the mediator of energy exchanges that occur in all living cells, both catabolic, degradative processes and anabolic, biosynthesis processes and has been widely used as an index for biomass determinations in clinical microbiology, food quality control and environmental analyses (Yu et al., 2008). Therefore, we chose ATP as a model analyte in this work. The whole fabrication process is shown in Scheme 1. 5′-Thiol-modified ATP-binding aptamer (ABA) strand was immobilized on 3DOM gold film by sulfur–gold affinity and further hybridized with its 5′-biotinated complementary strand (BCS) to form duplex DNA. In the presence of target ATP, the ABA forms stable tertiary structure with ATP, this accordingly denatures the duplex and liberates those complementary DNA (Zuo et al., 2007). Streptavidin modified QDs were then bound to the remaining complementary DNA by the strong biotin–streptavidin affinity and subsequently dissolved with HNO_3 for electrochemical stripping analysis. Thus, the target-induced strand displacement was successfully monitored and ATP detection could be realized through the electrochemical signal. As the active surface area of the 3DOM gold film modified electrode improved significantly, the quantity of immobilized duplex DNA increased. As a result, a wide linear dynamic range of 4 orders of magnitude from 0.01 to 100 nM was reached with the minimum detectable concentration at sub-nanomolar level (0.01 nM). Since it can be readily extended to functionalize other probes at the 3DOM gold film, the aptasensor presented here provides a promising protocol for small molecule assays.

2. Experimental

2.1. Chemicals and materials

Oligonucleotide containing specific sequence (ABA, 5′-HS-$(CH_2)_6$-ACC TGG GGG AGT ATT GCG GAG GAA GGT-3′) and the 5′-biotinated complementary strand (BCS, 5′-biotin-ACC TTC CTC CGC AAT ACT CCC CCA GGT-3′) were synthesized and purified by Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China). Tris(2-carboxyethyl)-phosphine hydrochloride (TCEP) was purchased from Bio Basic Inc. (Markham Ontario, Canada). ATP, cytidine triphosphate (CTP), guanosine triphosphate (GTP), uridine triphosphate (UTP), 6-mercapto-1-hexanol (MCH), Tween-20 and bovine serum albumin (BSA, 96–99%) were obtained from Sigma–Aldrich Chemical Co. (St. Louis, MO). Tris(hydroxylmethyl)amino-methane (Tris) was bought from Shanghai Chemical Reagent Company (Shanghai, China). Streptavidin-QDs (with a CdSe/ZnS core–shell structure about 10 nm) were obtained from Wuhan Jiayuan Quantum Dots Co. Ltd. (Wuhan, China). Monodispersed SiO_2 spheres with a diameter of 500 nm were purchased from Alfa Aesar (Ward Hill, MA). All other chemicals were of analytical grade. Ultrapure fresh water was obtained from a Millipore water purification system (MilliQ, specific resistivity > 18 MΩ cm, S.A. Molsheim, France).

2.2. Instruments

Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were performed on an Autolab PGSTAT12 (Eco chemie, BV, The Netherlands, controlled by GPES and FRA softwares). Square wave anodic stripping voltammetric (SWASV) measurements were carried out on a CHI660B electrochemical workstation (Shanghai CH Instruments Co.). A conventional three-electrode system comprising of a platinum wire auxiliary electrode, a saturated calomel reference electrode (SCE) and a 3DOM gold film modified working electrode was used in the CV and EIS measurements, while in the SWASV measurements, the working electrode was a glassy-carbon disk electrode (3 mm in diameter). All potentials herein are referenced to the SCE. The morphology of the 3DOM