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Electrochemical aptamer-based nanosensor fabricated on single Au nanowire electrodes for adenosine triphosphate assay

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ABSTRACT

In this work, single Au nanowire electrodes (AuNWEs) were fabricated by laser-assisted pulling/hydrofluoric acid (HF) etching process, which then were characterized by transmission electron microscopy (TEM), electrochemical method and finite-element simulation. The as-prepared single AuNWEs were used to construct electrochemical aptamer-based nanosensors (E-AB nanosensors) based on the formation of Au-S bond that duplex DNA tagged with methylene blue (MB) was modified on the surface of electrode. In the presence of adenosine triphosphate (ATP), the MB-labeled aptamer dissociated from the duplex DNA due to the strong specific affinity between aptamer and target, which lead to the reduction of MB electrochemical signals. Moreover, BSA was employed to further passivate electrode surface bonding sites for the stable of the sensor. The as-prepared E-AB nanosensor has been used for ATP assay with excellent sensitivity and selectivity, even in a complex system like cerebrospinal fluid of rat brain. Considering the unique properties of good stability, larger surface area and smaller overall dimensions, this E-AB nanosensor should be an ideal platform for widely sensing applications in living bio-system.

1. Introduction

This paper describes the fabrication of single Au nanowire electrodes (AuNWEs) and their applications to construct electrochemical aptamer-based nanosensors for adenosine triphosphate (ATP) assay. Single AuNWEs were prepared by laser-assisted pulling/hydrofluoric acid (HF) etching process, which then were modified by duplex DNA tagged with methylene blue (MB). The amount of ATP could be monitored through the decrease of MB electrochemical signals based on the dissociation of the duplex DNA due to the strong specific affinity between aptamer and target ATP.

Electrochemical aptamer-based sensors (E-AB sensors) have been widely reported for the quick and sensitive detection of various targets, including proteins (e.g. thrombin, insulin) (Cunningham et al., 2014; He et al., 2014; Ricci et al., 2009), small molecules (e.g. tobramycin, cocaine) (Schoukroun-Barnes et al., 2014; Swensen et al., 2009; Taghdisi et al., 2015; Wen et al., 2011) and even cells (Kashefi-Kheyrabadi et al., 2014) because of their excellent characteristics, such as reagentless, easy labeling, design flexibility (Zayats et al., 2006) and the strong specific affinity to the targets. Most E-AB sensors were fabricated on macro- or microscale electrodes, which were based on conformational/structure changes of redox-tagged aptamer attached on electrode surface induced by target, but the strong binding affinity between aptamer and target probably limited the regeneration of sensors (Radi et al., 2006). Therefore, aptamer-DNA duplexes were considered in sensor architectures. Meanwhile, E-AB sensors fabricated on the nanomaterials-modified (especially gold-based nanomaterials) electrodes with improved sensing performance have been occasionally reported (Liu et al., 2014). However, as we known, the nanomaterials-modified electrodes were inevitably unstable in aqueous solution and the surface area is uncontrollable.

On the other hand, single nanoelectrodes have attracted tremendous focus and efforts recently because of their unique properties such as high diffusive mass transport, small RC time constants and low ohmic drops in basic research and potential applications (Clausmeyer and Schuhmann, 2016; Fan et al., 2016; Yu et al., 2016), including in vivo measurements (Sun et al., 2008; Yang et al., 2016), scanning electrochemical microscopy(SECM) (Bae et al., 2017; Etienne et al., 2006; Gossage et al., 2016; Yu et al., 2015), atomic force microscopy(AFM) (Nogala et al., 2012), and single particle analysis (Kim et al., 2014; Li et al., 2010; Mirkin et al., 2016). Single nanoelectrodes with different geometric shapes, such as nanodisk, nanowire and nanopore, have been developed by our lab (Li et al., 2013; Zhang et al., 2015) and other groups (Clausmeyer et al., 2016; Li et al., 2009; Liu et al., 2015; Sun and Mirkin, 2006). To solve the

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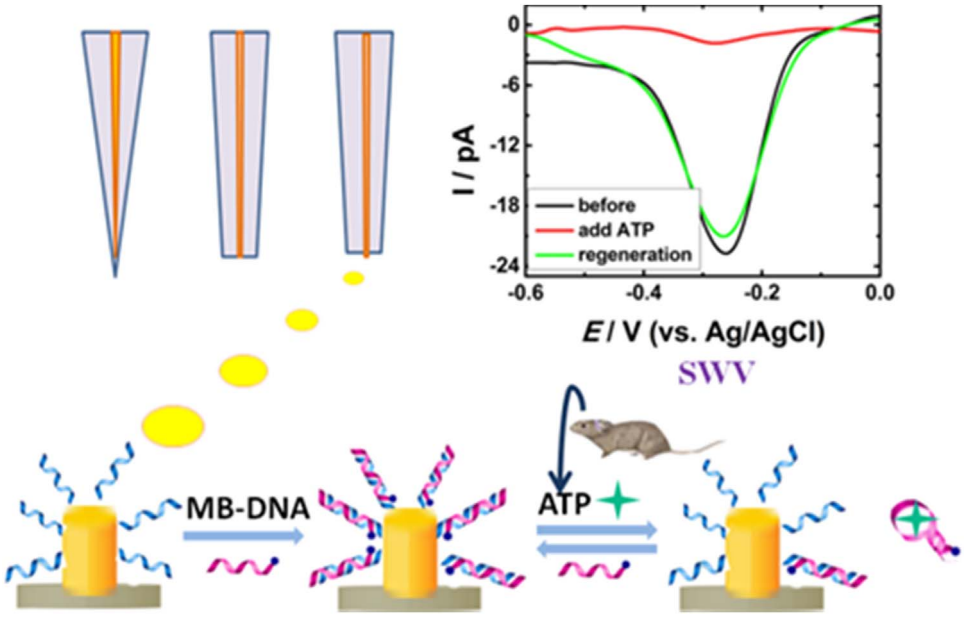

Scheme 1. Illustration of fabrication of an electrochemical aptamer-based nanosensor on a single Au nanowire electrode and its recognition towards adenosine triphosphate (ATP) by square wave voltammetry (SWV).

unstable issue by use of nanomaterials-modified electrodes mentioned above and strong binding between aptamer and macro-sized electrode, single nanodisk electrodes exhibited their advantages for sensing applications. For example, Zhang's group (Jena et al., 2010) and Lai's group (Salamifar and Lai, 2014) fabricated single Au nanodisk electrodes for ferrocene counting and DNA sensing through electrodepositing gold wire into single nanopore; Schuhmann's group (Clausmeyer et al., 2014; Marquitan et al., 2016) and Mirkin's group (Wang et al., 2012) developed Prussian blue and Pt modified nanoelectrodes for monitoring reactive oxygen and nitrogen species with high sensitivity. Compared to nanodisk electrodes mentioned above, single nanowire electrodes have the unique properties of good stability, larger surface area and smaller overall dimensions, which should be an ideal platform to fabricate nanosensor for in-vivo bio-sensing in living system.

In this work, adenosine triphosphate (ATP) was chosen as the model and a novel E-AB nanosensor for analysis of ATP was established using single AuNWE with reproducible and high-sensitive performance. Scheme 1 gives the process of preparing single AuNWE and the signaling mechanism of the sensors. The single AuNWEs were fabricated by a laser-assisted pulling technique and HF etching process. After that, the as-prepared single AuNWEs were used to construct electrochemical aptamer-based nanosensors (E-AB nanosensors) based on the formation of Au-S bond that duplex DNA tagged with methylene blue (MB) was modified on the surface of electrode. In the presence of adenosine triphosphate (ATP), the MB-labeled aptamer dissociated from the duplex DNA due to the strong specific affinity between aptamer and target which lead to the reduction of MB signals. This E-AB nanosensor exhibits excellent sensitivity and selectivity, even in a complex system like cerebrospinal fluid of rat brain, which is important for real applications in living bio-system.

2. Materials and methods

2.1. Chemicals and materials

Potassium ferricyanide ($K_3Fe(CN)_6$, Acros Organics), 1-ferrocenylethanol (FcMeOH, 98%, Perfemiker), Ferrocene (Fc, Aldrich), hexaammineruthenium(III) chloride ($Ru(NH_3)_6Cl_3$, Aldrich), sodium chloride (NaCl, Sangon Biotech), acetonitrile (ACN, Sangon Biotech) were of analytical reagent grade and used as received. The silver-filled epoxy glue was bought from DuPont Lt. Co. 25 μm diameter Au microwires (99.95%, hard) were obtained from Alfa-Aesar. Glass capillaries (*i.d.* = 80 μm; *o.d.* = 300 μm) were purchased from Sutter Instrument Co. (Novato, CA). The electrodes were polished with finer grit sandpaper (400 W, 800 W, and 1200 W grits; Sandpaper Inc., Rockland, MA).

The adenosine triphosphate (ATP) aptamer and other deoxyribonucleic acids were obtained from Sangon Biotech Co. Ltd (Shanghai, China). The sequence of SH-DNA is 5′-SH-(CH_2)6-TTT CCT CCG CAA TAC TCC CCC AG. The sequence of ATP aptamer (MB-DNA) is CTG GGG GAG TAT TGC GGA GGA AA-MB-3′. Adenosine triphosphate (ATP), guanosine triphosphate (GTP), cytidine triphosphate (CTP), uridine triphosphate (UTP), Dopamine (DA), Uric Acid(UA) and ascorbic acid (AA) were all purchased from Sangon Biotech Co. Ltd. (Shanghai, China). Tris-(2-carboxyethyl) phosphine hydrochloride (TCEP), 6-mercaptohexanol (MCH) and bovine serum albumin (BSA) were purchased from Sigma. The cerebrospinal fluid (CSF) of rat brain was purchased from Wannan Medical College. The used aqueous solutions throughout were collected from a Millipore system with ultra-pure water (> 18 MΩ cm).

2.2. Instrumentation

The electrochemical experiments were performed with a CHI 900 C Electrochemical Workstation (Chenhua Instrument Company, Shanghai, China). Transmission Electron Microscopy (TEM) images of the electrode tips were recorded on a JEM-2100F microscopy (Tokyo, Japan).

2.3. Fabrication of single Au nanowire electrodes

First, four steps were involved in the preparation of Au nanodisk electrode by the method of laser-assisted pulling as previously described (Zhang et al., 2015). Briefly, a 25 μm diameter Au wire sealed into a quartz glass capillary (i. d. 80 μm, o. d. 300 μm) was pulled into two ultra-sharp Au nanowire tips (< 10 nm) with laser-assisted pulling method (a laser puller, P-2000, Sutter). The ultra-sharp was inserted carefully into borosilicate glass tube (o.d. = 1.0 mm; i.d. = 0.64 mm) using a hydrogen flame under in-house vacuum, and then polished with sandpaper in order to make an Au nanodisk electrode. In the