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Electrochemical aptasensor for thrombin using co-catalysis of hemin/G-quadruplex DNAzyme and octahedral Cu_2O-Au nanocomposites for signal amplification

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ABSTRACT

In this work, novel octahedral Cu_2O-Au nanocomposites were synthesized and first applied in an electrochemical aptasensor to detect thrombin (TB) with the aid of a DNAzyme for signal amplification. The octahedral Cu_2O-Au nanocomposites have not only simultaneously served as signal amplifying molecules but have also been utilized as an ideal loading platform to immobilize a large number of electroactive substances and recognition probes. Gold nanoparticles (AuNPs) were grown directly on the surface of the octahedral Cu_2O nanocrystals, and the Cu_2O-Au nanocomposites obtained had the advantages of large surface areas and excellent biocompatibilities. The hemin/G-quadruplex, which was formed by intercalating hemin into the amino terminated thrombin binding aptamer (NH_2-TBA), and the electroactive toluidine blue (Tb) were immobilized onto the Cu_2O-Au nanocomposite surfaces through a stable Au-N bond. AuNPs, Cu_2O and hemin/G-quadruplex co-catalyse the H_2O_2 in the working buffer to promote the electron transfer of Tb as a multiple signal amplification strategy in order to improve the performance of the electrochemical aptasensor. Under optimal conditions, the designed aptasensor exhibited sensitive detection of TB from 100 fM to 20 nM with a lower detection limit of 23fM. This proposed aptasensor exhibited good sensitivity, high specificity and acceptable reproducibility and could be widely applied in bioassay analysis.

1. Introduction

Thrombin is a mutated serine protease which Na^+-activated, and is a central protease in the coagulation cascade (Crawley et al., 2007; Di Cera, 2008; Huntington, 2005). In blood vessel injury, thrombin is rapidly produced from a proactive enzyme prothrombin through a series of enzymatic cleavages and its concentration can vary from pM to mM during coagulation (Brummel-Ziedins et al., 2005; Deng et al., 2014; Shuman and Majerus, 1976). Meanwhile, thrombin plays a key role in physiological and pathological coagulation, and is involved in various diseases such as central nervous system injury, thromboembolic disease, Alzheimer's disease and cancer (Franchini and Mannucci, 2012; Nishino et al., 1993). Therefore, a high sensitivity and specificity thrombin detection method for research and clinical diagnosis is very meaningful.

Aptamers have been widely used in disease diagnosis and bioassays since their first report in 1990 (Ellington and Szostak, 1990; Tuerk and Gold, 1990). Compared to traditional recognition element antibodies, the aptamers have many unique properties that make them more advantageous, including low cost, large scale production, and no restrictions on the detection target (Kim et al., 2016; Zhang et al., 2010). In recent years, many aptamer-based sensors have been developed, such as fluorescent aptasensors (Wang et al., 2008), electrochemical aptasensors (Li et al., 2008), colorimetric aptasensors (Chang et al., 2013) and surface plasmon resonance aptasensors (Subramanian et al., 2013). Among these sensors, electrochemical aptasensor was widely used because its characteristics include simple operation, the possibility of miniaturization, fast response time, and relatively low cost (Gao et al., 2016; Zhao et al., 2011). In addition, thrombin-binding aptamer (TBA) can be folded into a G-quadruplex structure and its conformational switch is triggered by binding with thrombin (Deng et al., 2014). Hemin can intercalate into TBA as a cofactor to form a hemin/G-quadruplex, and can catalyse H_2O_2-mediated oxidation reactions (Bai et al., 2011; Kong et al., 2010). Thus, the hemin/G-quadruplex, a known horseradish peroxidase mimicking DNAzyme, can be widely used in electrochemical aptasensor signal amplification strategies (Yang et al., 2015).

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The nanomaterial has unique properties such as a large surface/volume ratio, excellent biocompatibility, enhanced electrical conductivity, high loading of receptor molecules and catalytic ability (Kurbanoglu et al., 2017; Xu et al., 2015). Nanomaterials have been extensively used as signal amplifying molecules and nanocarriers in order to improve the sensitivity of the electrochemical aptasensor (Chen et al., 2015b; Wang et al., 2016b). Gold nanoparticles (AuNPs) are the most commonly used nanomaterials due to a large surface area, good redox activity and excellent biocompatibility (Meng et al., 2011; Wen et al., 2014). For a long time, cuprous oxide (Cu_2O) nanomaterials have attracted widespread attention from researchers because they have an excellent ability to catalyse H_2O_2 at a low cost (Chu et al., 2016; Liu et al., 2013; Zhang et al., 2009). Wei Chen's group reported the use of graphene wrapped Cu_2O nanocubes to fabricate non-enzymatic electrochemical sensors for the detection of glucose and hydrogen peroxide (Liu et al., 2013). Huimin Wu's group reported Cu_2O-Pt core-shell nanoparticles as a platform for the sensitive detection of dopamine (Jin et al., 2016). The research shows that octahedral Cu_2O nanocrystals exhibited more excellent electrocatalytical properties than other shapes of Cu_2O nanocrystals (Chen et al., 2016b; Won and Stanciu, 2012). Additionally, the octahedral Cu_2O nanocrystals have a large surface area and are versatile building blocks with reversible redox activity (Li et al., 2017). Compared to single metal oxides, nanocomposites doped with noble metals have more obvious unique characteristics than do their monometallic counterparts (Won and Stanciu, 2012). Thus, the Cu_2O-Au nanocomposites were synthesized through a facile in situ method. AuNPs cover the surface of the octahedral Cu_2O nanocrystals to further increase the surface area and immobilize the recognition element and electroactive substances while showing excellent electrocatalytic performance towards the reduction of H_2O_2.

In this work, we report an enzyme-free electrochemical aptasensor for ultrasensitive thrombin detection based on using the AuNPs functionalized octahedral Cu_2O nanocrystals as signal amplifying molecules and nanocarriers. First, AuNPs were grown directly on the surface of the octahedral Cu_2O nanocrystals by a simple in situ reaction. Compared with the octahedral Cu_2O nanocrystals, the resultant Cu_2O-Au nanocomposites possess larger surface area, significant catalytic ability and good stability, which would immobilize more of the electroactive substances and recognition probes , improving the electrochemical signal and contributing to improved sensitivity. Tb was commonly used as an electron transfer medium to provide electrochemical signals. Tb with amino groups and amino-terminated TBA were attached onto the Cu_2O-Au nanocomposite surface through an Au-N bond. With the introduction of hemin, there was a formation of a large number of hemin/G-quadruplex mimicking DNAzymes, further enhancing the sensitivity of the aptasensor. AuNPs were immobilized on the surface of the glassy carbon electrode (GCE) by electrodeposition to capture the TBA. The captured TBA, target protein thrombin, Tb and hemin/G-quadruplex labelled Cu_2O-Au nanocomposites together formed a sandwich-type electrochemical aptasensor. After the H_2O_2 was introduced into the working buffer, the Cu_2O, AuNPs and hemin/G-quadruplex co-catalysed H_2O_2 reduction to promote the electron transfer of Tb, obtaining an excellent electrochemical signal response. The proposed enzyme-free aptasensor showed high sensitivity for the quantitative determination of TB in human serum, which has considerable potential in clinical and diagnostic applications.

2. Experimental methods

2.1. Reagents and chemicals

Thrombin (TB), gold chloride ($HAuCl_4{\cdot}4H_2O$), Hemin, and bovine serum albumin (BSA) were all purchased from Sigma-Aldrich Chemical (St. Louis, USA, www.sigmaaldrich.com). Polyvinylpyrrolidone (PVP K_{29-32}), Hydrazine monohydrate ($N_2H_4{\cdot}H_2O$, wt98%) and toluidine blue (Tb) were all purchased from aladdin reagents Co., Ltd. (Shanghai, China). Anhydrous copper (II) chloride($CuCl_2$) and thrombin binding aptamers (TBA) were purchased from Sangon Biotech Co., Ltd. (Shanghai, China)., and the sequences of the oligonucleotides were as follows:

TBA: 5′-NH_2-$(CH_2)_6$-GGT TGG TGT GGT TGG-3′.

20 mM Tris-HCL buffer (pH = 7.4) containing 1 mM $MgCl_2$, 1 mM $CaCl_2$, 5 mM KCl, and 140 mM NaCl was used as the aptamer buffer. Phosphate buffered saline (PBS, pH = 6.8) containing 0.1 M Na_2HPO_4, 0.1 M KH_2PO_4 and 0.1 M KCl was used as an electrolyte for all electrochemistry measurements. All chemicals were of analytical-reagent grade. Ultrapure distilled and deionized water (18.2 MΩ) was used for all solution preparations.

2.2. Apparatus

Electrochemical measurements, including chronoamperometry (i-t), cyclic voltammetry (CV),electrochemical impedance spectroscopy (EIS), were all performed on an AUTOLAB PGSTAT302 N electrochemical workstation (Metrohm Technology Co. Ltd., Switzerland). Field emission scanning electron microscopy (FE-SEM) images were obtained using a Hitachi S4800 (Hitachi Limited, Japan). X-ray photoelectron spectroscopy (XPS) was implemented using a VG Scientific ESCALAB 250 spectrometer (Thermoelectricity Instruments, USA) with an Al Ka X-ray (1486.6 eV) as the light source. Atomic force microscopy (AFM) images were monitored by Bruker Dimension icon (USA). Energy dispersive X-ray spectroscopy (EDS) was scaled using a JEOL JSM-6700F microscope (Japan). A conventional three-electrode system was used for all electrochemical measurements, consisting of a platinum wire electrode as the counter electrode, an Ag/AgCl (with 3 M KCl) as the reference electrode, and a modified glassy carbon electrode (GCE, 4 mm in diameter) as the working electrode. PBS was used as the working buffer for all electrochemistry measurement, which was purged with nitrogen gas for 30 min to remove the dissolved oxygen. All experiments were performed at room temperature (25 ± 1 °C) under ambient conditions.

2.3. Synthesis of the octahedral Cu_2O nanocrystals

The octahedral Cu_2O nanocrystals were synthesized according to a previously reported method with a little modification (Zhu et al., 2012). First, 1 mL of 0.1 M $CuCl_2$ solution and 28 mL of deionized water were added to a beaker with vigorous stirring at room temperature. Then, 1 mL of 1.0 M NaOH solution was added. The resulting solution turned light blue immediately, indicating the formation of a $Cu(OH)_2$. After the complete formation of the $Cu(OH)_2$ precipitate, 120 μL of 1 M $N_2H_4{\cdot}H_2O$ was quickly injected in 3 s into the beaker by pipette. The total volume of the solution in the beaker was 30 mL. The solution in the beaker was kept at room temperature for 10 min and then centrifuged at 5000 rpm for 5 min. The centrifuged Cu_2O was redispersed in 30 mL of deionized water followed by the addition of 10 μL of 1 M $N_2H_4{\cdot}H_2O$ and was stirred for 60 min for nanocrystal growth. The prepared Cu_2O nanocrystals were centrifuged at 5000 rpm for 5 min and washed 3 times with 10 mL deionized water to remove unreacted chemicals. The final washing step used 10 mL of ethanol, and the precipitate was dispersed in 4 mL ethanol for storage and analysis.

2.4. Synthesis of the Cu_2O-Au nanocomposites

To synthesize the Cu_2O-Au nanocomposites, 7.2 mg of the Cu_2O octahedral nanocrystals were first dissolved in 26 mL deionized water. 3 mL 1% PVP solution were dropped into the Cu_2O octahedral solution, and then 1 mL of 5 mM $HAuCl_4{\cdot}4H_2O$ aqueous solution was added to the samples. The typical yellow colour of the $HAuCl_4$ aqueous solution and the brick-red colour of Cu_2O disappeared immediately, and a black product was formed. The solutions were placed on a magnetic stirrer at