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Electrochemical DNA biosensor fabrication with hollow gold nanospheres modified electrode and its enhancement in DNA immobilization and hybridization

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ABSTRACT

In this article, hollow gold nanospheres (HGN) were prepared by using Co nanoparticles as sacrificial templates and varying the stoichiometric ratio of $HAuCl_4$ over the reductants. The HGN was then modified on the electrode surface via a 1,6-hexanedithiol linking agent to fabricate a novel electrochemical DNA biosensor. The whole DNA biosensor fabrication process was characterized by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) methods with the use of ferricyanide as an electrochemical redox indicator. The probe DNA immobilization and hybridization on the modified electrode was further studied with CV and differential pulse voltammetry (DPV) methods by using $Co(phen)_3^{3+}$ as an electrochemical hybridization indicator. Results revealed that the HGN modified electrode, especially for the HGN with the outer surface surrounded by densely spike-like nanocrystallites, could largely enhance the DNA hybridization ability. The fabricated DNA biosensor was proved to have a low detection limit (1 pM) and a wide dynamic range (from 1 pM to 10 nM) with a high stability and reusability.

1. Introduction

The development of DNA biosensors has recently attracted substantial attentions in connection with research efforts directed at gene analysis, the detection of genetic disorders, tissue matching, and forensic applications (Taton et al., 2000; Gunnarsson et al., 2008). Many techniques including fluorescence (Yang et al., 2008; Duan et al., 2007), electrochemiluminescence (Zhu et al., 2008; Zhang et al., 2008a,b), electrochemistry (Kara et al., 2002; Drummond et al., 2003), surface plasmon resonance spectroscopy (Kim et al., 2007) and quartz crystal microbalance (Patolsky et al., 2000; Minunni et al., 2005) have been developed for the DNA detection. Among them electrochemical techniques offer a lot of advantages such as simplicity, rapidness, low-cost and high sensitivity (Zhang et al., 2009a,b; March et al., 2008). Many protocols have been proposed for electrochemical monitoring of DNA hybridization (Munde et al., 2007; Gorodetsky et al., 2008).

A key issue with any DNA hybridization biosensor is how to enhance the probe DNA immobilization amount, and moreover maintain the accessibility of probe DNA for hybridization detection (Kjällman et al., 2008; Liu et al., 2005; Cederquist et al., 2008). In order to enhance the immobilization amount of probe DNA and optimize the DNA hybridization efficiency, different kinds of strategies have been developed (Liu et al., 2008; Gasparac et al., 2004). Including which, nanomaterials have been paid a great deal of attention in electrode surface modification for DNA biosensor fabrication owing to their increased surface area, improved electrochemical properties and possible beneficial orientation effect in DNA immobilization and hybridization. These nanomaterials used for electrode surface modification includes metal nanoparticles (Li et al., 2007; Liu et al., 2005), semiconductor nanoparticles (Du et al., 2009), nanowires (Zhang et al., 2008a,b), carbon nanotube (Basuray et al., 2009; Zhang et al., 2009a,b), nanopores (Hu et al., 2008), etc. Among all the nanomaterials, gold nanoparticles are the most frequently used for electrode surface modification in the fabrication of biosensor (Yamada et al., 2003; Zhao et al., 2007; Jena and Raj, 2007; Cai et al., 2001; Liu et al., 2002a,b).

Recently, hollow metallic particles have received a lot of attentions because of their use as drug carriers, electrocatalytic materials, etc. owing to the distinctive advantages of low density, high specific surface area, and reduction of costs compared with their solid counterparts (Sun and Xia, 2002a, 2002b; Liang et al., 2004; Guo et al., 2007). Various templates, including polystyrene spheres, silica spheres, vesicles, microemulsion and metal nanoparticles, etc., have been used to fabricate hollow spheres (Kim et al., 2002; Schmidt and Ostafin, 2002; Wong et al., 2002; Sun and Xia, 2002a,b; Lu et al., 2005; Lu et al., 2007). Whereas the application of hollow metal spheres in biosensor fabrication was still very less

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reported to date. In the field of DNA biosensor fabrication, Lu et al. (2005, 2007) have originally prepared a hollow gold ball with the use of vesicle as a template and further studied its ability in enhancing DNA immobilization and hybridization. Usually, these prepared hollow spheres are in the micro or pseudo micro-meter scale and also not readily operated in the preparation and electrode surface modification. Therefore, it is of great importance to develop the simple preparation method of hollow gold nanospheres (HGN) and explore this kind of special nanomaterials for the application in DNA biosensor fabrication.

In this research, the HGN were simply fabricated based on the displacement reaction between the Co nanoparticles and $HAuCl_4$. The prepared HGN showed a rough hollow ball structure surrounded with dense spike-like gold nanocrystallites. It is specially profit for DNA immobilization and hybridization. The current research focuses on the DNA immobilization and hybridization events on the HGN modified electrode and provides a suitable platform for the DNA biosensor fabrication.

2. Experimental

2.1. Chemicals and materials

Cobalt (II) chloride hexahydrate ($CoCl_2{\cdot}6H_2O$, 99.0%), tetrachloroaurate(III) tetrahydrate ($HAuCl_4{\cdot}4H_2O$, 47.8% Au) were obtained from National Chemical Reagent Ltd., Shanghai, China. Sodium borohydride ($NaBH_4$, 99%) was purchased from Acros Organics (USA). 1,6-hexanedithiol was purchased from Sigma (USA). The tris(1,10-phenanthroline) Cobalt (III) perchlorate, $Co(phen)_3(ClO_4)_3$, was synthesized as that reported in the literature (Gillard et al., 1983). $K_3Fe(CN)_6$, $K_4Fe(CN)_6$ were purchased from Tianjin Ruijinte Chemical Co. Ltd. (China). All other chemicals were all of analytical grade and used without further purification. Doubly distilled water was used throughout.

All of synthetic oligonucleotides were purchased from SBS Genetech. Co. Ltd. (China). Their base sequences are listed as follows:

Probe DNA: 5′-SH-GCG CGA ACC GTA TA-3′
Complementary target DNA: 5′-TAT ACG GTT CGC GC-3′
Non-complementary target DNA: 5′-ACT GAT GCT ACC AT-3′

2.2. Apparatus

Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) measurements were performed with a CHI832B electrochemical analyzer (Shanghai CH Instrument Company, China). Electrochemical impedance spectroscopy (EIS) measurements were carried out on a CHI660C electrochemical workstation (Shanghai CH Instrument Company, China). All electrochemical experiments were performed with a conventional three-electrode system comprising a gold working electrode, a platinum wire auxiliary electrode, and a Ag/AgCl reference electrode. The EIS measurements were performed in 1.0 mM $K_3[Fe(CN)_6]$ containing 0.10 M KCl with the frequency range from 10^4 to 0.01 Hz.

2.3. Fabrication of hollow gold nanospheres (HGN)

The HGN were prepared according to the reference (Liang et al., 2005) with a slight modification. For the synthesis of HGN, the Co nanoparticles were first prepared. The synthesis of Co nanoparticles was carried out based on the reported method (Kobayashi et al., 2003). The Co nanoparticles could be easily obtained with the addition of 0.2 mL of 0.4 M $CoCl_2$ solution into 200 mL of deaerated aqueous solution containing 8 mM $NaBH_4$ and 0.8 mM citric acid. The nitrogen gas was bubbled before mixing for 20 min and continued during the reaction in order to avoid the oxidation of obtained Co nanoparticles by existed atmospheric oxygen in the solution. The hydrogen gas was observed to be evolved during the reaction and continued for several minutes. Following with the end of gas evolution, 30 mL of Co nanoparticle colloidal solutions were extracted and transferred to different volumes (5, 8, 12, 18 and 40 mL, respectively) of stirred 1 mM $HAuCl_4$ aqueous solutions to achieve the preparation of the HGN. The obtained suspension solutions were centrifuged at 10,000 rps and the precipitates were designated as samples A–E.

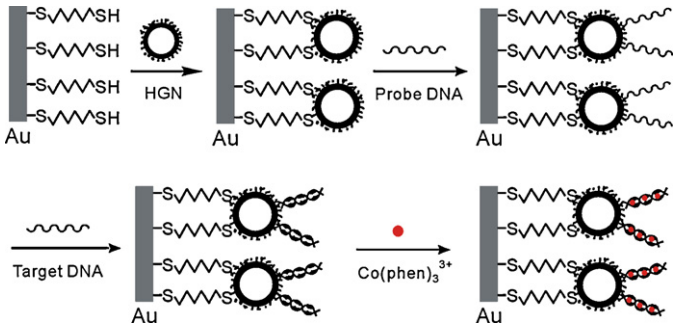

Fig. 1. Schematic illustration of the fabrication steps of the electrochemical DNA biosensor.

2.4. DNA biosensor fabrication

The gold working electrode surface was freshly polished prior to use with 0.05 μm alumina powder and then cleaned ultrasonically sequentially in acetone and water for 3 min. The whole DNA biosensor fabrication process was schematically demonstrated in Fig. 1. The detailed procedures for DNA biosensor fabrication are listed in the AppendixBSupplementary Information.

2.5. DNA hybridization detection with an electrochemical indicator of $Co(phen)_3^{3+}$

The probe DNA or hybridized electrodes were first immersed into 100 μM $Co(phen)_3^{3+}$ in 0.01 M PBS (pH 7.3) for 20 min, and then the cyclic voltammograms and differential pulse voltammograms were recorded in blank PBS solution.

3. Results and discussions

3.1. Characterization of hollow gold nanospheres (HGN)

In current work, the Co nanoparticles were synthesized with the reduction of Co^{2+} by $NaBH_4$. After the formation of Co nanoparticles, the solution was kept for several minutes to allow excessive $NaBH_4$ to react with water completely (Liang et al., 2004). Because the reduction potential of the $AuCl_4^-$/Au redox couple (0.935 V vs SHE) is much higher than that of the Co^{2+}/Co redox couple (−0.377 V vs SHE), the galvanic replacement reaction as presented in Eq. (1) will occur spontaneously as soon as $AuCl_4^-$ contacts with Co nanoparticles. Fig. 2 shows typical TEM images of samples A–E, where there is a strong contrast difference in all of the spheres with a bright center surrounded by a much darker edge, confirming their hollow architecture. The calculation of the maximum quantities of employed Co nanoparticles and $HAuCl_4$ in the preparation of sample B (See the AppendixBSupplementary Information) indicated that the amount of $HAuCl_4$ in sample B could be just sufficient to react with the added Co nanoparticles completely according to Eq. (1), while the ratio of $HAuCl_4$ in sample A and samples C–E is lower and higher than that in sample B, respectively.

$$3Co + 2AuCl_4^- = 2Au + 3Co^{2+} + 8Cl^- \quad (1)$$