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Electrochemical synthesis of gold nanostructure modified electrode and its development in electrochemical DNA biosensor

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ABSTRACT

In this article, gold nanostructure modified electrodes were achieved by a simple one-step electrodeposition method. The morphologies of modified electrodes could be easily controlled by changing the pH of $HAuCl_4$ solution. The novel nanoflower-like particles with the nanoplates as the building blocks could be interestingly obtained at pH 5.0. The gold nanoflower modified electrodes were then used for the fabrication of electrochemical DNA biosensor. The DNA biosensor fabrication process was characterized by cyclic voltammetry and electrochemical impedance spectroscopy with the use of ferricyanide as an electrochemical redox indicator. The DNA immobilization and hybridization on gold nanoflower modified electrode was studied with the use of $[Ru(NH_3)_6]^{3+}$ as a hybridization indicator. The electrochemical DNA biosensor shows a good selectivity and sensitivity toward the detection of target DNA. A detection limit of 1 pM toward target DNA could be obtained.

1. Introduction

During the past decade, DNA biosensor has received substantial development in gene analysis, detection of genetic disorders, tissue matching, forensic and medical applications (Taton et al., 2000; Gunnarsson et al., 2008). Different techniques have been developed for DNA detection including fluorescence (Yang et al., 2008; Duan et al., 2007; Qiu et al., 2011), electrochemistry (Drummond et al., 2003; Liu et al., 2008; Zhang et al., 2009; Li et al., 2010), electrochemiluminescence (Zhu et al., 2008; Zhang et al., 2008b), chemiluminescence (Weizmann et al., 2003; Li et al., 2007), surface plasmon resonance spectroscopy (Kim et al., 2007; Seefeld et al., 2011) and quartz crystal microbalance (Patolsky et al., 2000; Minunni et al., 2005), etc. Among them, electrochemical technique affords a lot of advantages such as its simplicity, rapid, low-cost and high sensitivity (Munde et al., 2007; Sassolas et al., 2008). Currently, much effort has been devoted to upgrade the detection sensitivity and selectivity of electrochemical DNA biosensor (Kannan et al., 2011).

Recent developments in nanomaterials create many opportunities to advance DNA sensing and gene detection. The nanomaterials have been widely used as a medium of signal amplification to enhance the limit of DNA detection (Wang, 2003; Katz et al., 2004). For example, Chang et al. (2007) constructed a polyaniline nanotube array and realized an ultrasensitive detection of nucleic acid with a detection limit of 1.0 fM. Hu et al. (2008) developed a nanoporous gold electrode and achieved a detection limit of about 28 aM toward target DNA with the use of multifunctional encoded Au nanoparticle as signal amplification medium. Chen et al. (2009) developed a label-free dual sensing strategy toward DNA molecules using GaN nanowires and revealed excellent selectivity and specificity at picomolar concentration of target DNA. Soleymani et al. (2009) fabricated controlled Pd nanostructure modified electrodes and achieved the sensitive detection of DNA with the estimated detection limit of about 1 fM. Even though there are a lot of research works reported for electrode modification by different nanomaterials to improve the DNA biosensor performance, the preparation of nanomaterials or the electrode modification strategy is often relatively complex. Sometimes, the introduction of some organic molecules for example surfactant or polymer matrix in the preparation or assembly of nanomaterials often causes some uncertain effects in DNA detection. Furthermore, some DNA biosensors based on nanomaterial modification are still very limited for the improvement of DNA biosensor performance. Thus, the construction of nanostructure modified electrode by a simple strategy to improve the DNA detection sensitivity is highly desirable.

Among all kinds of nanomaterials, gold-based nanomaterials were the mostly used ones for the fabrication of electrochemical biosensor owing to its easy and rich surface function strategies and good biocompatibility (Liu et al., 2010). The gold nanomaterials could be easily attached on the electrode surface by some different strategies including direct electrostatic assembly, covalent linking, polymer entrapment or co-mixing, and electrodeposition methods.

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The electrodeposition method is the most widely used approach for the substrate modification (Seo et al., 2011; Zhu et al., 2011). Gold nanostructures with different morphologies have been easily obtained by electrodeposition. For example, Guo et al. (2007a) prepared hierarchical flowerlike gold microstructures with gold nanoplates or nanopricks as building blocks by electrochemical deposition in a high concentration of 24.3 mM $HAuCl_4$ without introducing any template or surfactant. Zhang et al. (2008a) fabricated various gold nanostructures on glassy carbon electrodes in a low concentration of 5 mM $HAuCl_4$ solution by a simple one-step electrodeposition method. Li et al. (2011) obtained dendritic gold nanostructure modified electrode by electrodeposition in 2.8 mM $HAuCl_4$ and 0.1 M H_2SO_4 solution under a very negative potential of −1.5 V. Although electrodeposition has been demonstrated as an effective strategy for gold nanostructure modification, the role of nanostructuring in modulating biorecognition performance has been less addressed and needs further explored in detail, especially for these nanostructures with special morphology. The development of hierarchical nanostructures with novel morphology may provide a valuable platform for the applications in DNA biosensor, and even the protein, enzyme biosensor.

In this context, the hierarchical gold nanostructure modified electrodes were obtained by a simple electrochemical deposition method in a low concentration of 5 mM $HAuCl_4$ solution. The morphology of the final gold nanostructures could be easily controlled by simply changing the solution pH of gold precursors. Interestingly, a novel flower-like gold nanostructure with the nanoplates as the building blocks was obtained at pH 5.0. This gold nanoflower modified electrode has been demonstrated with an excellent capability for DNA immobilization and hybridization. With the use of $[Ru(NH_3)_6]^{3+}$ as an electroactive complex (Steel et al., 1998), the fabricated electrochemical DNA biosensor shows a very good selectivity and sensitivity toward the detection of target DNA. A detection limit of 1 pM toward target DNA could be achieved. The current electrode modification strategy is also expected to extend for the applications in protein, enzyme biosensor.

2. Experimental

2.1. Materials and chemicals

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

thiolated probe DNA sequence: 5′-SH-GCGCGAACCGTATA-3′;
complementary target DNA sequence: 5′-TATACGGTTCGCGC-3′; and
noncomplementary target DNA: 5′-ACTGATGCTACCAT-3′.

Hexaammineruthenium(III) chloride (RuHex) and 6-mercapto-1-hexanol (MCH) were purchased from Sigma (St. Louis, MO, USA), and tetrachloroaurate(III) tetrahydrate ($HAuCl_4{\cdot}4H_2O$, 47.8% Au) was obtained from National Chemical Reagent Ltd. (Shanghai, China). All other chemicals were all of analytical grade and used without further purification. Double distilled water was used throughout.

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 an Ag/AgCl reference electrode. The EIS measurements were performed in a solution of 1 mM $K_4Fe(CN)_6/K_3Fe(CN)_6$ in phosphate-buffered saline (PBS buffer, 25 mM, pH 7.4, 0.1 M KCl). The scanning electron microscopy (SEM) images were obtained on a Hitachi S-4300 scanning electron microscope (Japan).

2.3. Preparation of gold nanostructure modified electrode

Prior to use, a bare Au disk electrode was polished sequentially with 1 μm, 0.3 μm, and 0.05 μm alumina slurry and ultrasonicated thoroughly in acetone and water. The well-polished electrode was then subjected to electrochemical pretreatment by cycling the potential between −0.2 and 1.5 V in H_2SO_4 (0.5 M) at a scan rate of 100 mV s^{-1} until a stable cyclic voltammogram was obtained, and then the cleaned electrode was allowed to be dried at room temperature. The pH values of 5 mM $HAuCl_4$ were adjusted to a fixed value with NaOH and HCl solution and were aged for about 12 h. Gold nanostructures were electrodeposited on planar gold electrode at a constant potential of 0.5 V at room temperature.

2.4. DNA immobilization and hybridization

The probe DNA immobilization on gold nanostructure modified electrode was performed by dropping 20 μL of 0.1 μM probe DNA solution in 25 mM PBS (pH 7.4, 0.1 M KCl) on the electrode surface for 8 h. The DNA-modified electrode was further treated with 1 mM MCH for 30 min to obtain a well aligned DNA monolayer, following by washing with PBS buffer solution and double distilled water to remove unspecific absorbed DNA. For the execution of DNA hybridization, probe DNA immobilized electrode was immersed into stirred 1 mL PBS buffer solution containing different concentration of target DNA for 1.5 h at 37 °C. Then the electrodes were taken out and rinsed with PBS buffer solution and double distilled water and dried with nitrogen.

2.5. DNA hybridization detection with an electrochemical indicator of RuHex

The probe DNA or hybridized electrodes were firstly immersed into 50 μM RuHex in 25 mM PBS (pH = 7.4) for 20 min, and then DPV measurements were recorded in blank PBS solution. All the electrochemical measures were carried out under the atmosphere of nitrogen.

3. Results and discussion

3.1. Electrodeposition of gold nanostructures on the Au substrate

Schematic representation of the fabrication procedure of DNA biosensor is shown in Fig. 1. The gold nanostructure modified electrode was firstly prepared by an electrochemical deposition strategy and then used as the substrate for DNA immobilization and hybridization.

Fig. 2 shows the SEM images obtained for the gold nanostructures electrodeposited at different pHs on gold substrate. It could be seen that, at pH 2.0, the irregular gold nanoparticles with relatively smooth surface were obtained and closely occupied the whole electrode surface at deposition time of 240 s (Fig. 2A). At pH 3.0, the popcorn-like gold nanostructures composed with aggregated nanocrystals were clearly illustrated (Fig. 2B). Under pH 4 condition, the popcorn-like gold nanostructures became more