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Simultaneous electrochemical immunoassay using CdS/DNA and PbS/DNA nanochains as labels

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ABSTRACT

An electrochemical method for the simultaneous detection of two different tumor markers, carcinoembryonic antigen (CEA) and α-fetoprotein (AFP), in one-pot, using CdS/DNA and PbS/DNA nanochains as labels was developed. Herein, magnetic beads (MBs) as bimolecule immobilizing carriers, were used for co-immobilization of primary anti-CEA and anti-AFP antibodies. The distinguishable signal labels were synthesized by in situ growth of CdS and PbS nanoparticles on DNA chains, respectively, which were further employed to label the corresponding secondary antibodies. A sandwich-type immunoassay format was formed by the biorecognition of the antigens and corresponding antibodies. The assay was based on the peak currents of Cd^{2+} and Pb^{2+} dissolved from CdS and PbS nanoparticles by HNO_3 using square wave stripping voltammetry. Experimental results show that the multiplexed electrochemical immunoassay has enabled the simultaneous monitoring of CEA and AFP in a single run with wide working ranges of 0.1– 100 ng mL^{-1} for CEA and 0.5–200 ng mL^{-1} for AFP. The detection limits reach to 3.3 pg mL^{-1} for CEA and 7.8 pg mL^{-1} for AFP.

1. Introduction

Cancer is one of the leading causes of mortality, to which early clinical diagnosis is the key to a higher survival rate. Till now, numerous immunosensors and immunoassays have been developed for the determination of tumor markers. However most researches focused on single tumor marker analysis (Soper et al., 2006; Rasooly and Jacobson, 2006; Wang, 2006; Wu et al., 2007a, 2007b), which have ignored the complexity of biological system, especially the human body. They are usually with poor predictive value because most markers are not specific and can sometimes have elevated levels in patients without cancer, giving false positive results. Recently, researches show that the combination of multiple tumor markers can provide improved diagnostic specificity instead of a single tumor marker (Oikonomopoulou et al., 2008; Kozak et al., 2005). Thus, simultaneous multianalyte determination of panels of tumor markers is of great significance (Wu et al., 2012; Wu et al., 2007a, 2007b; Wilson and Nie, 2006). In addition, they may offer higher test throughput while require a shorter analytical time, lower sampling volume and detection cost per assay compared with traditional single-analyte assay (Brecht and Abuknesha, 1995; Zhang et al., 2004).

Among the different techniques for tumor marker analysis, electrochemical immunoassay technique has its special merit: high sensitivity, inherent simplicity, and low cost (Hansen et al., 2006; Wang et al., 2006a, 2006b). To improve the sensitivity for trace target detection, a large number of labels (Kricka, 1992; Song et al., 2010; Hayes et al., 1994; Liu et al., 2004) have been developed in immunoassay systems. Among them, semiconductor nanoparticles are proved to be promising because they are with comparable size to proteins and thus are especially suitable for surface modification with various biomolecules (peptides, oligonucleotides, and proteins) (Wang et al., 2008; Wu et al., 2007a, 2007b; Yang et al., 2009). In particular, when semiconductor nanoparticles composed of different metals are applied in electrochemical immunoassays, they can provide an elegant way to simultaneously detect multiple protein analytes in connection with highly sensitive stripping voltammetric measurement of the corresponding metals (Liu et al., 2006a; Liu et al., 2006b).

Liu et al. has introduced a multitarget electrochemical immunoassay based on the use of magnetic beads (MBs) as antibody immobilizer and semiconductor nanoparticles as labels for the second antibodies (Liu et al., 2004). Besides the efficient magnetic separation, MBs can provide larger immobilization area than the planer structures, leading to higher loading of biomolecules. Recently, Qian et al. has also reported simultaneous detection of dual proteins using CdSe and PbS coated silica nanoparticles as labels and Au as substrates for primary antibody's immobilization (Qian et al., 2011). By applying silica nanoparticles as carriers,

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load of nanoparticles can be increased significantly, giving larger signals than merely nanoparticles. However, since applying silica nanoparticles would also increased the steric hindrance at the same time, which might lead to narrow linear range, improvement is still required.

To meet this requirement, we prepared CdS/DNA and PbS/DNA nanochains using DNA as carriers to load different nanoparticles. The linear shape of DNA can help to reduce the steric hindrance compared to that of the silica/carbon nanoparticles. In addition, CdS/DNA and PbS/DNA nanochain can be fabricated with good controllability and high flexibility. DNA, as biological macromolecule, provides definite nucleic sites and natural linear shape on which assembly of nanoparticles produces essentially one-dimensional nanochains or nanowires (Coffer et al., 1996). Moreover, DNA can be synthesized arbitrarily with desired nucleotide sequences and length. To the best of our knowledge, this is the first time that CdS/DNA and PbS/DNA nanochains are used as labels in immunoassay. To demonstrate the workability for simultaneous detection of multiple tumor markers, the colorectal cancer marker carcinoembryonic antigen (CEA) and the liver and germ cell cancer marker α-fetoprotein (AFP), were chosen as target antigens. The target antigens were captured using MBs conjugated with the corresponding antibodies. Upon the completion of sandwich immunoreaction, CdS/DNA and PbS/DNA labels were attached to the MBs surface through the antibody–antigen immunocomplex. After an acid-dissolution step, the electrochemical signals were simultaneously obtained at different peak potentials. The peak currents and the peak positions were dependent on the concentration and type of the corresponding antigen, respectively.

2. Experimental

2.1. Reagents

CEA and AFP standard grade antigens, primary anti-CEA and secondary anti-CEA, anti-AFP antibodies were purchased from Linc-Bio Science Co. (Shanghai, China). The ds-DNA oligonucleotides with terminal $-NH_2$ were synthesized by Shanghai Biochemical Reagent Company (Shanghai, China) with the following sequences: NH_2–$(CH_2)_{12}$ TAA TGT CAT ATA ATG TCA TAT AAT GTC ATA TAA TGT CAT ATA ATG TCA TAT AAT GTC ATA and TAT GAC ATT ATA TGA CAT TAT ATG ACA TTA TAT GAC ATT ATA TGA CAT TAT ATG ACA TTA. Carboxyl-modified MBs were purchased from BaseLine Chromtech Research Centre (Tianjin, China). N-(3-dimethylaminopropyl)-N′-ethyl-carbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), bovine serum albumin (BSA) were of commercial source (Sigma-Aldrich, St. Louis, MO). Tween-20, $Cd(NO_3)_2$, $Pb(NO_3)_2$, Na_2S, glutaraldehyde (GA) were obtained from Shanghai Reagent Company (Shanghai, China). All other reagents were of analytical grade and used as received. Ultrapure water obtained from a Millipore water purification system (≥ 18 MΩ, Milli-Q, Millipore) was used in all runs. HAc–NaAc buffer (0.1 mol L^{-1}, pH 4.5) served as the supporting electrolyte. Serum specimens provided by Gulou Hospital (Nanjing, China) were stored at 4 °C.

2.2. Apparatus

The electrochemical experiments were carried out with CHI 760C electrochemical workstation (Shanghai, China). A glassy carbon electrode with a diameter of 3 mm was used as working electrode, with Ag/AgCl and platinum wire acting as the reference electrode and counter electrode, respectively. The transmission electron microscopic (TEM) image was acquired with a JEOL mode 2000 instrument operated at 200 kV (JEOL Ltd., Japan). The UV–vis spectra were obtained by a Shimadzu UV-3600 spectroscopy (Shimadzu, Japan).

2.3. Preparation of the CdS/DNA and PbS/DNA nanochains

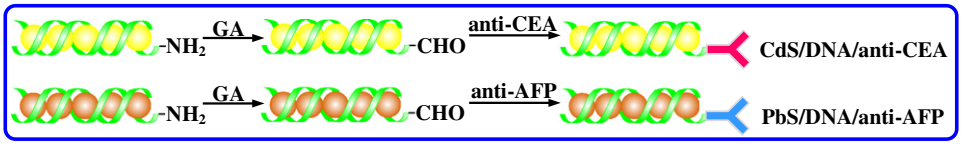
CdS/DNA nanochains were prepared by in situ growth of CdS on DNA chains according to the method reported previously (Dong et al., 2007) with some modification. Briefly, 100 μL of 0.2 mmol L^{-1} $Cd(NO_3)_2$ was added to the ds-DNA solution. The solution was mixed thoroughly and kept for 24 h at 4 °C. Then, 100 μL of 0.2 mmol L^{-1} Na_2S in water/ethanol (1:1, v/v) was added to this solution. The mixture was stirred thoroughly and incubated for a further 24 h at 4 °C. Then, 10 μL of 20 mmol L^{-1} aqueous $Cd(NO_3)_2$ was added and allowed to react for 24 h at 4 °C before introduction of 10 μL Na_2S (20 mmol L^{-1}) in water/ethanol (1:1, v/v). The solution was again incubated for 24 h at 4 °C and then allowed to stand at room temperature. PbS/DNA nanochains were synthesized based on the same procedure except for adding $Pb(NO_3)_2$ for reaction instead of the $Cd(NO_3)_2$. The as-obtained CdS/DNA and PbS/DNA nanochains were fairly stable and could be stored for more than 6 months at 4 °C without agglomeration.

2.4. Preparation of distinguishable signal labels

CdS/DNA and PbS/DNA nanochains were used for the labeling of second anti-CEA antibodies and anti-AFP antibodies through the GA cross-linking between the terminal $-NH_2$ of DNA and the $-NH_2$ of the protein. The entire process for preparation of CdS/DNA/anti-CEA and PbS/DNA/anti-AFP labels was shown in Scheme 1. Firstly, 100 μL CdS/DNA or PbS/DNA was mixed with 20 μL 5% GA for 18 h at room temperature. After dialysis, 500 μL of secondary anti-CEA antibodies or secondary anti-AFP antibodies (0.1 mg mL^{-1}) was added to the mixture and incubated for 24 h at 4 °C. The excess aldehyde-groups were removed by addition of lysine. The resultant CdS/DNA/anti-CEA or PbS/DNA/anti-AFP labels gave a final volume of 1 mL and stored in refrigerator at 4 °C for further use.

2.5. MBs-based immunoassay procedure

The sandwich-type immunoassay protocol was shown in Scheme 2. In a typical experiment, about 10 μL of carboxyl-modified MBs were transferred into a 0.2 ml centrifuge tube and were then washed twice with 100 μL phosphate buffer solution (PBS). This was followed by adding 50 μL 20 mg mL^{-1} EDC and 50 μL 10 mg mL^{-1} NHS and mixing for 1 h. Here, EDC and NHS were employed to activate the carboxyl group of the MBs. Following a magnetic separation, the activated MBs were washed



Scheme 1. Preparation of CdS/DNA/anti-CEA and PbS/DNA/anti-AFP conjugate (Firstly, CdS/DNA and PbS/DNA nanochains reacted with glutaraldehyde. Secondly, CdS/DNA and PbS/DNA nanochain containing terminal –CHO reacted with the anti-CEA and anti-AFP antibodies, respectively).