Biosensors and Bioelectronics 57 (2014) 219–225

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

A novel label-free amperometric immunosensor for carcinoembryonic antigen based on Ag nanoparticle decorated infinite coordination polymer fibres

Wenbo Lu [a], Xiaowei Cao [a], Lin Tao [a], Juan Ge [a,b], Jian Dong [a], Weiping Qian [a,*]

[a] State Key Laboratory of Bioelectronics, School of Biological Science and Medical Engineering, Southeast University, Nanjing 210096, PR China
[b] School of Chemistry and Chemical Engineering, Southeast University, PR China

ARTICLE INFO

Article history:
Received 2 December 2013
Received in revised form
9 February 2014
Accepted 10 February 2014
Available online 19 February 2014

Keywords:
Infinite coordination polymer
Fibres
Thionine
Carcinoembryonic antigen
Immunosensor

ABSTRACT

In this article, for the first time, a novel, high-yield and template-free method for the synthesis of Ag nanoparticle decorated thionine/infinite coordination polymer (AgNP/THI/ICP) fibres is proposed. The thionine can be adsorbed to the AgNP/THI/ICP fibres by π-conjugation and act as the redox probe. The AgNP/THI/ICP fibres not only favor the immobilization of antibody but also facilitate the electron transfer. It is found that the AgNP/THI/ICP fibres can be designed to act as a sensitive label-free electrochemical immunosensor for carcinoembryonic antigen (CEA) determination. Under the optimized conditions, the linear range of the proposed immunosensor is estimated to be from 50 fg/mL to 100 ng/mL and the detection limit is estimated to be 0.5 fg/mL at a signal-to-noise ratio of 3, respectively. The prepared immunosensor for detection of CEA shows high sensitivity, reproducibility and stability. Our study demonstrates that the proposed immunosensor has also been used to determine CEA successfully in diluted blood samples.

1. Introduction

Infinite coordination polymer particles (ICPs) are a class of emerging functional materials that are formed by bridging repeating organic ligands with metallic nodes (Oh and Mirkin, 2005). Over the past decades, the ICPs have attracted enormous research interest in chemistry and materials science, because they can be made from readily available and highly tailorable metal and ligand precursors, have chemically adjustable porosities and high internal surface areas, and provide with a network structure that can be deliberately and easily modified for many applications (Spokoyny et al., 2009; Lu et al., 2013; Ballesteros-Rivas et al., 2011). The outstanding properties acquired have promised potential applications across various technological fields including gas sorption and separation, molecular sieving, drug delivery, photonics, heterogeneous catalysis and biosensing (Zhang et al., 2013; Bae et al., 2008; Horcajada et al., 2006; Li and Zeng, 2013; Rowsell and Yaghi, 2005). A new path to design multifunctional ICPs materials has been opened up by the incorporation or integration of other functional materials into ICPs. However, few research involving in different types of metal nanoparticles (Au, Ag, Pt, etc.) incorporated into different ICPs structures has been reported. Accordingly, the key challenge herein is to determine appropriate methods that can help to generate the interaction between the incorporated metal nanoparticles and ICPs materials.

Tumor markers are molecules occurring in blood or tissue, which are associated with cancer and whose measurement or identification is useful in patient diagnosis or clinical management (Miyake et al., 2010; Wang et al., 2006; Yang et al., 2010; Zhong et al., 2010). In the tumor process, changed levels of tumor markers in patients are associated with certain tumor. Carcinoembryonic antigen (CEA) is a glycoprotein most often associated with colorectal carcinomas and commonly used as a clinical tumor marker for clinical diagnosis of breast tumors, colon tumors, ovarian carcinoma and cervical carcinomas (Huang et al., 2010; Iwazawa et al., 2000; Naghibalhossaini and Ebadi, 2006). Levels of CEA are significantly lower in colon tissue of adults about 2.5 μg/L (Zamcheck and Martin, 2006). However, when tumor markers arise in any endodermal tissue including gastrointestinal tract, pancreas, and breast, the levels of CEA correlated with cancer incidents can become elevated (Liu and Jiang, 2006; Li et al., 2008; Jiang et al., 2011; Cai et al., 2012). As a result, determination of CEA plays a key role in clinical research and diagnosis. Numerous immunoassay techniques have been developed for the detection of CEA, including fluoroimmunoassay (Yuan et al., 2001), enzyme immunoassay (Lai et al., 2011), and electrochemical immunoassay

* Corresponding author. Tel./fax: +86 25 83795719.
E-mail address: wqian@seu.edu.cn (W. Qian).

http://dx.doi.org/10.1016/j.bios.2014.02.027
0956-5663

(Kong et al., 2013a, 2013b; Lin et al., 2012; Qu et al., 2007; Lu et al., 2014). Among these methods, electrochemical sensors for immunoassays have attracted increasing attention due to the sensitive and convenient label-free advantage (Chikkaveeraiah et al., 2012; Perfezou et al., 2012; Wan et al., 2013). Recently, amperometric immunosensors utilized many nanomaterials to immobilize CEA antibody, including poly(styrene-co-acrylic acid) microbead carried AuNPs (Lin et al., 2012), nanotubular mesoporous PdCu alloy (Cai et al., 2012), glucose oxidase–conjugated Au–Ag hollow microspheres (Tang et al., 2011), and Ag nanoparticle-decorated the nafion membrane containing SiO_2 nanoparticles (Wang et al., 2013). However, all these above mentioned methods suffer from more or less drawbacks such as the involvement of an extra label signal antibody (Lin et al., 2012), the need for special biological enzyme (Tang et al., 2011), and the requirement of additional immobilized reagent (polyethyleneimine, glutaraldehyde, and nafion) (Wang et al., 2013; Cai et al., 2012). Accordingly, the development of a biocompatible nanomaterial for the effective immobilization and a highly sensitive immunosensor is highly desired for the detection of CEA.

The purpose of this work is to explore a novel nanocomposite (AgNPs decorated thionine/ICP fibres) based biosensor for detecting CEA with low detection limit, and to develop a stable, sensitive, simple, and low-cost label-free immunosensor. We report on the rapid, large-scale formation of infinite coordination polymer (ICP) fibres, carried out by mixing aqueous $HAuCl_4$ solution, $AgNO_3$ solution and ethanol solution of 1,10-phenanthroline monohydrate at room temperature for the first time. The AgNPs decorated thionine/ICP (AgNP/THI/ICP) fibres are prepared by direct adsorption of preformed, negatively charged AgNPs onto the surface of ICP fibres. Thionine is used to adhere the AgNPs to the ICP fibres and acts as the redox probe. The AgNP/THI/ICP fibres not only favor the immobilization of antibody but also facilitate the electron transfer. It is found that the resultant AgNP/THI/ICP fibres can be designed to act as a sensitive label-free electrochemical immunosensor for CEA determination. The immunosensor is prepared by immobilizing capture anti-CEA on AgNP/THI/ICP fibres assembled on glassy carbon electrode (GCE). The prepared immunosensor for detection of CEA shows high sensitivity, low detection limit and long-term stability. To the best of knowledge, it is the first time that the AgNP/THI/ICP fibres have been used as a label-free electrochemical immunosensor, which might be an effective candidate for the detection of CEA. The proposed immunosensor could provide a new approach to detection of CEA in clinical diagnosis with favorable results.

2. Materials and methods

2.1. Materials

1,10-Phenanthroline monohydrate, bovine serum albumin (BSA), Vitamin C, Glycine, glucose, L-cysteine, dopamine and L-glutamate were purchased from Aladin Ltd. (Shanghai, China). Chloroauric acid solution ($HAuCl_4 \cdot 4H_2O$) and silver nitrate ($AgNO_3$) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). NaH_2PO_4, Na_2HPO_4, ethanol were purchased from Beijing Chemical Reagent (Beijing, China). CEA standard grade antigens, alpha fetoprotein (AFP) standard grade antigens, and anti-CEA antibodies were purchased from Linc-Bio Science Co. (Shanghai, China). All chemicals were used as received without further purification. The water used throughout all experiments was purified through a Millipore system. Phosphate buffer solution (PBS) was prepared by mixing stock solutions of NaH_2PO_4 and Na_2HPO_4. Human blood samples were kindly provided by the Hospital of Southeast University (Sipailou 2, Nanjing, China). The human serum was separated from the blood samples by centrifugation. Human serum samples were diluted to different concentrations with a PBS solution of pH 6.5, and each sample was analyzed for three times.

2.2. Instruments

Transmission electron microscopy (TEM) measurements were made on a HITACHI H-8100 EM (Hitachi, Tokyo, Japan) with an accelerating applied potential of 200 kV. The sample for TEM characterization was prepared by placing a drop of the dispersion on carbon-coated copper grid and dried at room temperature. Fourier Transform Infrared spectroscopy (FT-IR) measurements were made on a FT-IR Spectrometer TENSOR 27 (Bruker Optik GmbH, Ettlingen, Germany). Scanning electron microscopy (SEM) measurements were made on a XL30 ESEM FEG scanning electron microscope at an accelerating applied potential of 20 kV. The sample for SEM characterization was prepared by placing a drop of the dispersion on a bare Si substrate and air-dried at room temperature. Electrochemical measurements were performed with a CHI 660D electrochemical analyzer (CH Instruments, Inc., Shanghai). A conventional three electrode cell was used, including a GCE (geometric area$=0.07\ cm^2$) as the working electrode, a Ag/AgCl (3 M KCl) electrode as the reference electrode, and platinum foil as the counter electrode. All potentials given in this work were referred to the Ag/AgCl electrode. All the experiments were carried out at ambient temperature.

2.3. Synthesis of ICP fibres

In a typical synthesis, 200 μL of 24.3 mM $HAuCl_4$ aqueous solution was added into 200 μL of 1,10-phenanthroline monohydrate in ethanol to form a homogeneous solution for a few seconds, followed by adding 100 μL of 0.1 M $AgNO_3$ aqueous solution, resulting in the formation of a large amount of yellow precipitate immediately. The precipitate thus formed was washed with water several times and then redispersed in 2 mL water for characterization and further use.

2.4. Preparation of AgNPs

AgNPs were prepared via reduction of $AgNO_3$ by sodium citrate, according to established method (Lee and Meisel, 1982). In brief, 16 μL of 0.5 M $AgNO_3$ aqueous solution was introduced into 10 mL of 1% sodium citrate solution under stirring. Then the resulting mixture was heated to 100 °C and kept at this temperature for 60 min. As-formed AgNPs dispersion was stored at 4 °C for further use.

2.5. Synthesis of AgNPs/THI/ICP fibres

The AgNPs/THI/ICP fibres were prepared by adsorption of citrate-stabilized AgNPs onto the ICP fibres using thionine as a cross-linking agent. In a typical synthesis, 2 mL of ICP fibres dispersion aqueous solution into 6 mL of 1 mg/mL thionine solution under vigorous stirring for 10 min, followed by adding 2 mL of AgNPs aqueous solutions. And then the mixture was sonicated for 30 min. Finally, the precipitate was collected by centrifugation and washed with water twice and then redispersed in 0.2 mL of water for characterization and further use.

2.6. Fabrication of the immunosensor

The modified electrodes were prepared by a simple casting method. Prior to the surface coating, the GCE was polished with 1.0 and 0.3 μm alumina powder, respectively, and rinsed with doubly distilled water, followed by sonication in ethanol solution