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Conductive hydrogel composed of 1,3,5-benzenetricarboxylic acid and Fe^{3+} used as enhanced electrochemical immunosensing substrate for tumor biomarker

Huiqiang Wang, Hongliang Han, Zhanfang Ma *

Department of Chemistry, Capital Normal University, Beijing 100048, China

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ABSTRACT

In this work, a new conductive hydrogel was prepared by a simple cross-linking coordination method using 1,3,5-benzenetricarboxylic acid as the ligand and Fe^{3+} as the metal ion. The hydrogel film was formed on a glassy carbon electrode (GCE) by a drop coating method, which can dramatically facilitate the transport of electrons. A sensitive label-free electrochemical immunosensor was fabricated following electrodeposition of gold nanoparticles (AuNPs) on a hydrogel film and immobilization of an antibody. Neuron-specific enolase (NSE), a lung cancer biomarker, was used as the model analyte to be detected. The proposed immunosensor exhibited a wide linear detection range of 1 pg mL^{-1} to 200 ng mL^{-1} and a limit of detection of 0.26 pg mL^{-1} (the ratio of signal to noise (S/N) = 3). Moreover, the detection of NSE in human serum samples showed satisfactory accuracy compared with the data determined by enzyme-linked immunosorbent assay (ELISA), indicating good analytical performance of the immunoassay.

1. Introduction

The early diagnosis of cancer is a crucial factor in enhancing the successful treatment of the disease, leading to an increase in patient survival rate [1–5]. To date, great effort has been made in advancing the methods for early diagnosis, which include enzyme-linked immunosorbent assay (ELISA), fluorescent immunoassay, and chemiluminescence enzyme immunoassay [6–10]. Although these methods offer numerous advantages, their inevitable drawbacks include time-consuming processes and sophisticated manipulation. Thus, development of an immunoassay that is simple to prepare, offers fast detection, and displays high sensitivity is desirable. Electrochemical immunesensors are widely used, particularly for label-free electrochemical immunoassays, due to their high sensitivity and efficiency, low cost, and user-friendly instrumentation [11–14]. Generally, the construction of a label-free electrochemical immunosensor is performed on a sensing substrate with good conductivity, high hydrophilicity, and large specific surface area [15–23].

At present, the substrate materials used for fabricating a label-free immunosensing interface are usually divided into three categories. The first category concerns nanocomposites, such as noble metal, magnetic, and organic dye nanocomposites, which display excellent conductivity, good stability, and great biocompatibility. However, the drawbacks of noble metal, magnetic, and organic dye nanocomposites are high cost, a complex fabrication process, and a tedious modification process to enhance the electrochemical signal, respectively [24–28]. The second category contains carbon materials, such as carbon nanotubes and grapheme, which exhibit advantageous properties of good conductivity and large specific surface areas. Yet, carbon nanotubes require strict conditions for the activation process, and graphene has poor solubility and a tedious post processing [29–32]. Conductive polymers comprise the third category, which commonly includes polyaniline, polypyrrole, and polythiophene. Although these materials possess good conductivity and can act as "molecular wires" to promote electron transfer between redox species and an electrode, they usually cannot provide an electrochemical signal [33–34]. Given the above situation, it is of great significance to develop a new type of nanomaterial-based substrate with excellent conductivity, great hydrophilicity, and large specific surface area for fabricating a label-free electrochemical immunosensor.

Herein, the fabricated novel hydrogel exhibited outstanding conductivity and was achieved using a simple cross-linking coordination method with 1,3,5-benzenetricarboxylate as the ligand and Fe^{3+} as the metal ion. The as-prepared hydrogel was implemented as the immunosensing substrate in a label-free electrochemical immunosensor. To obtain the sensitive label-free amperometric immunosensor, gold nanoparticles (AuNPs) were electrochemically deposited onto the surface of the hydrogel. Neuron-specific enolase (NSE) was chosen as the target analyte to be detected. The proposed immunosensor exhibited superior

* Corresponding author.
E-mail address: mazhanfang@cnu.edu.cn (Z. Ma).

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performance for NSE detection, and the detection results are consistent with those of ELISA.

2. Materials and methods

2.1. Chemicals

Ascorbic acid (AA), and hydrogen tetrachloroaurate hydrate ($HAuCl_4 \cdot xH_2O$, 99.9%) were purchased from Alfa Aesar China (Tianjin). Mouse anti human monoclonal antibody to neuron-specific enolase (anti-NSE), neuron-specific enolase (NSE), carcinoembryonic antigen (CEA), alpha fetoprotein (AFP), prostate specific antigen (PSA), were obtained from Shanghai Linc-Bio Science Co., Human immunoglobulin G (IgG), was obtained from Chengwen Biological Company (Beijing, China). Bovine serum albumin (BSA), urea acid (UA), dopamine (DA), potassium ferricyanide ($K_3Fe(CN)_6$), potassium ferrocyanide ($K_4Fe(CN)_6$), ferric nitrate, 1,3,5-benzenetricarboxylic acid, hydrochloric acid (HCl, 36.0–38.0%), KCl, NaH_2PO_4 and Na_2HPO_4, were purchased from Beijing Chemical Reagents Company (Beijing, China). Clinical human serum samples were obtained from Capital Normal University Hospital (Beijing, China). All other reagents were of analytical grade and used without further purification. Ultrapure water was used in all experiments (resistivity > 18 MΩ cm).

2.2. Apparatus

All electrochemical measurements were carried out on a CHI832 electrochemical workstation (Chenhua Instruments Co., Shanghai, China). Scanning electron microscope (SEM) images and energy dispersive X-ray spectroscopy (EDS) were determined with a Hitachi SU8010 SEM. Thermogravimetry was analysed on TGA/SDTA851 from METTLER TOLEDO. Ultrapure water used in all procedures was purified through an Olst ultrapure K8 apparatus (Olst, Ltd.). A three electrochemical system in the experiment was composed of a glassy carbon electrode (GCE) (4 mm in diameter) as the working electrode, a platinum wire and an Ag/AgCl electrode as counter electrode and reference electrode, respectively.

2.3. Synthesis of the hydrogel

The conductive hydrogel was achieved by a simple cross-linking coordination method using 1,3,5-benzenetricarboxylic acid (20 mg mL^{-1}) as the ligand and ferric nitrate (40 mg mL^{-1}) as the gelatiniser and dopant to synthesise a conducting network. The mixed solution containing 1,3,5-benzenetricarboxylic acid and ferric nitrate was gelated to form the hydrogel, which was ultimately obtained hydrogel by freeze-drying.

2.4. Fabrication of the immunosensor

Prior to the functionalisation procedure, the glassy carbon electrode (GCE) with a diameter of 4 mm was polished using 0.05 μm alumina slurry and was cleaned ultrasonically three times in alternating baths of absolute alcohol and deionized-distilled water. A dispersion solution containing the hydrogel (15 μL) was slowly dripped onto the surface of the pre-treated GCE and allowed to form a thin homogeneous film for 30 min. Subsequently, the electrode was immersed in ultrapure water for 15 min to remove excess ions and organic compounds. AuNPs were electrochemically deposited onto the hydrogel/GCE film by cyclic voltammetry (CV) scanning from −1.0 V (vs Ag/AgCl) to 0.2 V (vs Ag/AgCl) in 0.5 mM $HAuCl_4$ solution containing 0.1 M KCl at a scan rate of 50 mV s^{-1} for 10 cycles. The modified electrode was rinsed with ultrapure water then dipped into anti-NSE solution and incubated in a moisture-saturated environment overnight at 4 °C. Finally, the resulting modified electrode was further incubated with a solution containing 1% BSA (m:m) for 1 h at room temperature to block the remaining active sites against non-specific absorption. After thoroughly rinsing the electrode with ultrapure water once more, the desired immunosensor was obtained and stored at 4 °C prior to use.

3. Results and discussion

3.1. Characterization of the hydrogel

A brown hydrogel was formed immediately after mixing 1,3,5-benzenetricarboxylic acid (the ligand) and ferric nitrate (the gelatiniser and dopant) solutions (Fig. S1). Meanwhile, the electrodeposited AuNPs uniformly distributed over the surface of the hydrogel/GCE film (Fig. S2).

After polymerisation and purification through extensive rinsing with ultrapure water, the hydrogel was subsequently swollen with water content of 96.6% (m:m), which was determined by thermogravimetry (Fig. 1A). The specific surface area of the dehydrated hydrogel was 17.6 m^2 g^{-1}, which was measured using Brunauer-Emmett-Teller (BET) surface area analysis. The chemical composition of the hydrogel was analysed with energy dispersive X-ray spectroscopy (EDS) before and after deposition with AuNPs, which was found to contain Si, C, O, Fe, and Au elements (Fig. 2). Si was present due to the silicon slice that was used as the sample platform, and Au was observed after the electrochemical deposition of AuNPs.

3.2. Characterization of the electrode modification

The stepwise fabrication process of the immunosensor was monitored by square wave voltammetry (SWV) (Fig. 3A) and electrochemical impedance spectroscopy (EIS) (Fig. 3B) in 5 mM $[Fe(CN)_6]^{3-/4-}$ containing 0.1 M KCl. In Fig. 3A, the current response of hydrogel-modified GCE (curve b) was higher than that of bare GCE (curve a), which

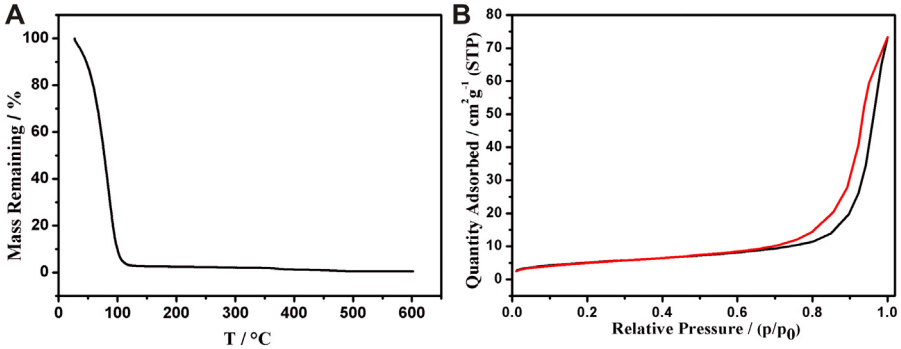

Fig. 1. Thermal gravimetric analysis of the hydrogel (A) and nitrogen adsorption-desorption isotherm of the dehydrated hydrogel (B).