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Short communication

Electrochemical immunosensor for competitive detection of neuron specific enolase using functional carbon nanotubes and gold nanoprobe

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ABSTRACT

An electrochemical immunosensor for detection of neuron specific enolase (NSE) was designed by immobilizing NSE covalently functionalized single-walled carbon nanotubes (NSE-SWNTs) on a glassy carbon electrode. The NSE-SWNTs not only enhanced electrochemical signal but also presented abundant antigen domains for competitive immunological recognition to anti-NSE primary antibody and then gold nanoprobes labeled with alkaline phosphatase conjugated secondary antibody (AP-anti-IgG/AuNPs). The AP-anti-IgG/AuNPs exhibited highly catalytic activity toward enzyme substrate and significantly amplified the amperometric signal for target molecule detection. Based on the dual signal amplification of SWNTs and gold nanoprobe, the immunosensor could response down to 0.033 ng mL^{-1} NSE with a linear range from 0.1 ng mL^{-1} to 2 μg mL^{-1}, and showed acceptable precision and reproducibility. The designed immunosensor was amenable to direct quantification of target protein with a wide range of concentration in complex clinical serum specimens. The assay results were in a good agreement with the reference values. The proposed electrochemical immunosensor provided a pragmatic platform for convenient detection of tumor markers in clinical diagnosis.

1. Introduction

The quantitative detection of tumor markers in biological specimens plays important roles in screening and diagnosis of cancers [1–3]. Various immunoassay methods have been developed to detect tumor markers in clinical laboratory, including enzyme-linked immunosorbent assay [4,5], radio immunoassay [6], fluorescence immunoassay [7] and chemoluminescence immunoassay [8]. In comparison with these immunological methods, electrochemical immunosensor has attracted considerable interest for its intrinsic advantages such as simple, low cost, good portability, and high sensitivity [9].

To achieve highly sensitive electrochemical immunosensing, various nanomaterials have recently been applied for signal amplification, including gold nanoparticles [10], carbon nanostructures [11–13] and quantum dots [14,15]. All these nanomaterials are excellent carriers in the amplification of recognition events and enhancement of signal transduction [16,17]. As one of the most popular reporter labels, enzymes, including alkaline phosphatase (AP), horseradish peroxidase (HRP) and glucose oxidase (GOD) have been immobilized with primary antibodies on nanomaterials for enhancing the enzymatically catalytical signal [11,18,19]. However, most researches pay primary attention to achieving high sensitivity of the immunosensors, take little account of the ability to detect high-concentration practical samples which are frequent in clinical laboratory diagnosis. For example, neuron specific enolase (NSE), a widely used biomarker for small-cell lung cancer [20], neuroblastoma [21], and neuroendocrine cancers [22], often expresses up to nearly 1 μg mL^{-1} in serum of serious cancer patients [23]. Clinical samples with high-concentration target proteins cannot be directly detected using these electrochemical immunosensors [24,25], and the sample dilution process will result in more complex procedure and an inaccurate result, which limits their practical application in clinical laboratory diagnosis of cancers. Therefore, it remains a challenge to develop a simple, pragmatic, sensitive immunosensor that can also directly detect high concentration cancer markers in clinical specimens.

Herein, using NSE as a model, a novel electrochemical immunosensor was prepared by modifying a glassy carbon

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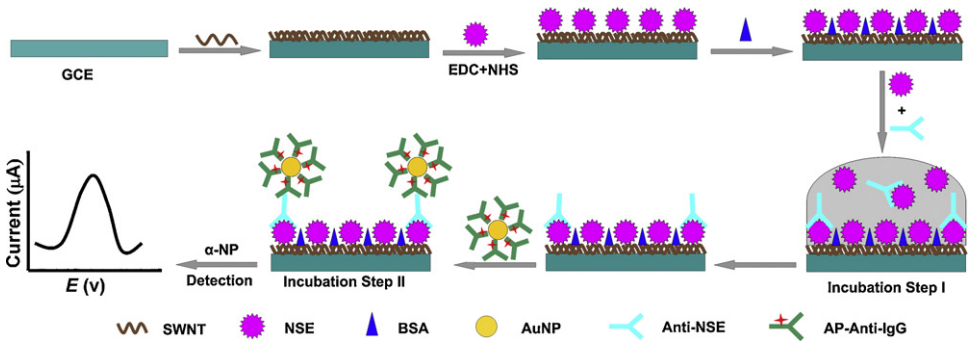

Scheme 1. Schematic representation of the designed electrochemical immunosensor for NSE detection.

electrode (GCE) with NSE covalently functionalized single-walled carbon nanotubes (NSE-SWNTs). The excellent electrical conductivity of SWNTs and the high loading of NSE on SWNTs not only enhanced electrochemical signal but also presented abundant antigen domains for competitive recognition to anti-NSE primary antibody, which greatly extended the up-limit of the detectable range. Using alkaline phosphatase conjugated secondary antibody to label gold nanoparticle, a gold nanoprobe, AP-anti-IgG/AuNP, was designed for immunoassay. The AP-anti-IgG/AuNPs exhibited highly catalytic activity toward hydrolysis of α-naphthyl phosphate (α-NP), leading to a dual signal amplification of SWNTs and gold nanoprobe for detection of low-concentration target (Scheme 1). Meanwhile, in the competitive format the high loading of NSE on SWNTs was sufficiently utilized to greatly extend the up-limit of the detectable range of the designed immunosensor. The proposed method showed low cost, acceptable precision and reproducibility, and could be successfully applied in direct detection of NSE in clinical serum specimens. The designed immunosensor provided a pragmatic tool for convenient detection of tumor markers in clinical diagnosis.

2. Experimental

2.1. Reagents and apparatus

Neuron specific enolase, rabbit anti-NSE polyclonal antibody (1 mg mL^{-1}) and AP-labled goat anti-rabbit antibody (AP-anti-IgG, 1 mg mL^{-1}) were purchased from Beijing Biosynthesis Biotechnology Ltd. Co. (Beijing, China). $HAuCl_4{\cdot}4H_2O$ was purchased from Sinopharm Chem Ltd. Co. (Shanghai, China). Carboxylic group-functionalized SWNTs (<5 nm diameter) were purchased from Shenzhen Nanotech Port Ltd. Co. (Shenzhen, China). Bovine serum albumin (BSA), N-hydroxysuccinimide (NHS), 1-ethyl-3-(3-dimethyllaminopropyl) carbodiimide hydrochloride (EDC) and α-naphthyl phosphate (α-NP) were obtained from Sigma Aldrich (USA). Other reagents were of analytical grade. All aqueous solutions were prepared using 18 MΩ ultrapure water.

The electrochemical measurements were performed on a CHI 660D electrochemical analyzer (Shanghai Chenhua Instrument, China). The electrochemical system consisted of a three-electrode system where a SWNTs-modified GCE (3 mm in diameter) was used as a working electrode, platinum wire was used as an auxiliary electrode, and an Ag/AgCl was used as a reference electrode. UV–visible spectra were carried out on a UV (3150)-Vis spectrophotometer (Jiangsu Jialing Instrument, China). Transmission electron microscopic (TEM) image was taken with an H-7500 transmission electron microscope (Hitachi, Japan).

2.2. Preparation of gold nanoparticle

AuNPs were prepared according to the method reported previously by adding 1 mL of 1% trisodium citrate aqueous solution to 100 mL of boiling 0.01% $HAuCl_4$ deionized water solution [26]. The mixture was maintained at boiling point for 15 min and stirred to cool completely after removing the heating source to produce 24 nm-diameter AuNPs, which were stored at 4 °C. All glassware used in this procedure was cleaned in freshly prepared 1:3 HNO_3–HCl and then rinsed thoroughly in deionized water.

2.3. Preparation of gold nanoprobe

The bioconjugation of the synthesized AuNPs and AP-anti-IgG was prepared according to the literature [27]. 2 mL colloidal AuNP solution was initially adjusted to pH 9.0–9.5 using Na_2CO_3, and then 0.1 mL of the original AP-anti-IgG was added, after incubated for 12 h at 4 °C with slight stirring, the mixture was centrifugated (13,000 rpm) at 4 °C for 30 min, then the obtained AP-anti-IgG/AuNPs conjugates were resuspended into 3.0 mL pH 7.4 PBS. The synthesized gold nanoprobe was stored at 4 °C.

2.4. Preparation of immunosensor

The GCE was polished to a mirror using 0.3 and 0.05 μm alumina slurry followed by rinsing thoroughly with deionized water. After sonicated in 1:1 nitric acid and acetone, the electrode was sonicated again in deionized water, rinsed with deionized water and allowed to dry at room temperature. Then 2 μL of 1 mg mL^{-1} carboxylic group functionalized SWNTs solution was dropped on the pretreated GCE and dried in a desiccator. 10 μL of 4 mM EDC and 10 mM NHS mixed liquor was dropped onto the SWNTs-coated GCE surface and incubated for 1 h to activate the carboxylic group functionalized SWNTs. After the activated SWNTs/GCE was thoroughly rinsed with deionized water, 10 μL of 5 μg mL^{-1} NSE was immediately dropped on its surface and then incubated for 2 h. After rinsed thoroughly with 0.01 M pH 7.4 PBS, the obtained immunosensor was stored at 4 °C prior to electrochemical assays.

2.5. Electrochemical measurements

Firstly, the NSE-SWNTs/GCE was immersed in 1% BSA solution for 30 min to block the nonspecific binding sites on the surface. Then, the immunosensor was immersed in 50 μL incubation solution, which was prepared by mixing 40 μL NSE standard solution or serum sample with 10 μL of 5 μg mL^{-1} anti-NSE primary antibody, for 60 min at 37 °C for competitive immunoreaction (Incubation step I). After the immunosensor was washed carefully with 0.01 M pH 7.4 PBS, 5 μL of AP-anti-IgG/AuNPs was dropped on its surface and incubated at 37 °C for 60 min (Incubation step II). Following a rinse with 0.01 M pH 7.4 PBS, the electrochemical measurement was performed in pH 9.5 DEA solution (0.1 mol L^{-1} diethanolamine, 1 mmol L^{-1} $MgCl_2$, 100 mmol L^{-1} KCl) containing 1.2 mg mL^{-1} α-NP. The differential pulse voltammetric (DPV)