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Novel redox species polyaniline derivative-Au/Pt as sensing platform for label-free electrochemical immunoassay of carbohydrate antigen 199

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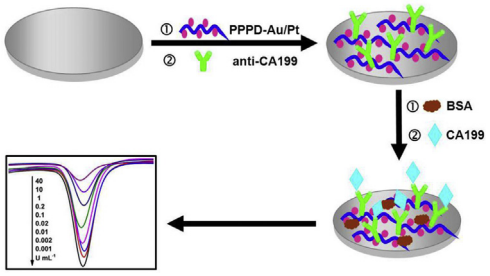
HIGHLIGHTS

- A novel electrochemical redox composite PPPD-Au/Pt was synthesized by one-pot method.
- PPPD-Au/Pt was used as sensing substrate for label-free electrochemical immunosensor.
- The immunosensor showed wide detection range and ultralow detection limit for the detection of CA199.

GRAPHICAL ABSTRACT



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ABSTRACT

A novel electrochemical redox-active nanocomposite was synthesized by a one-pot method using N,N′-diphenyl-p-phenylediamine as monomer, and $HAuCl_4$ and K_2PtCl_4 as co-oxidizing agents. The as-prepared poly(N,N′-diphenyl-p-phenylediamine)-Au/Pt exhibited admirable electrochemical redox activity at 0.15 V, excellent H_2O_2 electrocatalytic ability and favorable electron transfer ability. Based on these, the evaluation of the composite as sensing substrate for label-free electrochemical immunosensing to the sensitive detection of carbohydrate antigen 199 was described. This technique proved to be a prospective detection tool with a wide liner range from 0.001 U mL^{-1} to 40 U mL^{-1}, and a low detection limit of 2.3×10^{-4} U mL^{-1} (S/N = 3). In addition, this method was used for the analysis of human serum sample, and good agreement was obtained between the values and those of enzyme-linked immunosorbent assay, implying the potential application in clinical research. Importantly, the strategy of the present substrate could be extended to other polymer-based nanocomposites such as polypyrrole derivatives or polythiophene derivatives, and this could be of great significance for the electrochemical immunoassay.

1. Introduction

Carbohydrate antigen 199 (CA199) is a kind of carbohydrate antigen that shows great promise for malignant tumor detection such as pancreatic cancer, colorectal cancer, liver cancer, gastric cancer, and ovarian cancer [1–4]. Therefore, sensitive and reliable detection of CA199 tends to be an urgent need in early clinical

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diagnosis, disease screening, and cancer prognosis [5,6]. To date, great efforts have been made to the detection of CA199 such as enzyme-linked immunosorbent assay (ELISA) [7–9], fluorescent immunoassay [10], and chemiluminescence immunoassay [11–13]. In spite of the promising results reported, there are some inevitable drawbacks such as enzyme deactivation or time-consuming process. Thus, the attempts to develop the immunoassay which is high sensitive, efficient, low cost and user-friendly are desirable.

Taking advantages of exceptional attributes, such as simple operation, fast response, low-cost, high sensitivity and specificity, considerable attention has been devoted to develop the electrochemical immunoassay [14–18]. Among these electrochemical immunoassays, label-free electrochemical immunoassay is attractive owing to its easy fabrication, low cost and time-saving advantages. Commonly, the construction of label-free electrochemical immunosensor is based on sensing substrate nanomaterials with electrochemical redox activity or electrocatalytic ability [15,16,19–21]. Compared with the electrocatalytic substrate-based immunosensors, the redox substrate-based sensor holds enormous potential for its fast response and electrolyte reusability [15,19,21]. Additionally, for the purpose of increasing the sensitivity, three basic points are extraordinary important to the sensing substrate nanomaterials: (1) good conductivity and electron transfer ability; (2) large specific surface area for antibodies attachment; (3) signal amplification [22,23].

The polyaniline derivative-noble metal composites can be easily synthesized by oxidation polymerization using noble metal salt as chemical oxidizing agents and aniline derivatives as monomers [24]. Combining the advantages of polyaniline derivatives and noble metals, the composites are considered as ideal platform materials for electrochemical immunosensors. The polyaniline derivatives can produce single electrochemical redox signals because of the functional groups in their monomers and the noble metals can greatly improve the electron transfer ability of these composites [25–27]. Additionally, it has been reported that bimetallic noble metal possesses better electrocatalytic ability than the monometal, which can be used to enhance the performance of electrochemical immunoassay [21,28,29].

Herein, a novel electrochemical redox-active composite poly(-N,N′-diphenyl-p-phenylediamine)-Au/Pt (PPPD-Au/Pt) was synthesized by using $HAuCl_4$ and K_2PtCl_4 as co-oxidizing agents and N,N′-diphenyl-p-phenylediamine as monomer. The as-prepared nanocomposite with favorable conductivity exhibited single electrochemical redox signal at 0.15 V. Besides, it showed excellent H_2O_2 electrocatalytic ability because of the presence of Au/Pt, which exhibited higher catalytic ability than monometallic Pt or Au. Taking these outstanding properties into account, the PPPD-Au/Pt was used as sensing substrate in an electrochemical immunosensor for label-free detection of CA199.

2. Experimental

2.1. Materials

CA199, anti-CA199, cancer embryo antigen (CEA), alpha fetal protein (AFP), and prostate-specific antigen (PSA) were purchased from Shanghai Linc-Bio Science Co. Ltd (Shanghai, China). N,N′-diphenyl-p-phenylediamine were obtained from Aladdin (Tianjin, China). H_2O_2 (30%), $HAuCl_4$ xH_2O and K_2PtCl_4, uric acid (UA), ascorbic acid (AA), dopamine (DA) were obtained from Alfa Aesar (Tianjin, China). Phosphate buffer solutions (PBS) with different pH value were prepared by mixing NaH_2PO_4, Na_2HPO_4 and KCl. Clinical human serum samples were provided by the Capital Normal University Hospital (Beijing, China). NaH_2PO_4, Na_2HPO_4, KCl, $K_3Fe(CN)_6$, $K_4Fe(CN)_6$, ethanol and bovine serum protein (BSA) were achieved from Beijing Chemical Reagents Company (Beijing, China). All other reagents were of analytical grade and used without any further purification. All aqueous solutions were prepared with ultrapure water (resistivity > 18 MΩ).

2.2. Apparatus

In all the procedures, the water used was purified by an Olst ultrapure K8 apparatus (Olst, Ltd., resistivity = 18.2 MΩ cm^{-1}). Transmission electron microscope (TEM) image was obtained from a JEOL-100CX electron microscope. X-ray photoelectron spectroscopy (XPS) was conducted using an Escalab 250 X-ray Photoelectron Spectroscope (Thermofisher, American) employing a monochromatic Al Kα radiation. High angle annular dark field scanning transmission electron microscopy (HAADF-STEM), and EDX mapping characterizations were obtained through a JEOL-2011 electron microscope. Fourier transform infrared spectroscopy (FTIR) spectra were obtained from FTIR spectroscopy (Tensor37, Bruker, Germany). Electrochemical measurements were conducted on a CHI-832 electrochemical workstation (Chenhua Instruments Co., Shanghai, China). A three-electrode system was used in the experiment with a glassy carbon electrode (GCE) (4 mm in diameter) as the working electrode, a Ag/AgCl electrode (saturated KCl) as reference electrode and a Pt wire as counter-electrode, respectively.

2.3. Synthesis of PPPD-Au/Pt

The PPPD-Au/Pt composites were synthesized by adding 300 μL N,N′-diphenyl-p-phenylediamine ethanol solution (5.5 mg mL^{-1}) into 2.0 mL ultrapure water. And then 150 μL $HAuCl_4$ (101.4 mM) and 150 μL K_2PtCl_4 (101.4 mM) was quickly mixed, followed by injecting into the above solution under vigorous stirring for 4 h. The composite was centrifuged at 12000 rpm for 8 min and was washed with ultrapure water. The obtained purified PPPD-Au/Pt samples were redispersed into 2 mL ultrapure water for further use.

2.4. Fabrication of immunosensor

Prior to the fabrication procedure, the GCE was polished with 1.0, 0.3 and 0.05 μm alumina slurry, respectively, ultrasonically treated with ultrapure water and dried at 37 °C to get a mirror-like surface. After pretreatment, 20 μL PPPD-Au/Pt samples were dropped on the GCE and dried at 37 °C for about 10 min. Next, 80 μL anti-CA199 (200 μg mL^{-1}) was incubated on the modified electrode at 4 °C overnight. Following that, the anti-CA199/PPPD-Au/Pt/GCE was washed with ultrapure water to remove the physically absorbed antibodies and blocked with BSA to avoid non-specific adsorption. Then, 80 μL CA199 with various concentrations was incubated onto the electrode at 37 °C for 45 min.

2.5. Electrochemical measurement

Square wave voltammetry (SWV) and amperometric i-t were carried out in phosphate buffer saline (PBS) (pH 7.0). For amperometric i-t measurement, −0.4 V was selected as the detection potential, which is in favor of decreasing the background current and minimizing the responses of common interference species. After the background current was stabilized, 15 μL H_2O_2 was injected into 30 mL PBS (pH 7.0) under proper stirring to form a uniform solution, and the current changes were recorded. For SWV measurements, 7.5 μL H_2O_2 was mixed with 15 mL PBS (pH 7.0) before measurement. Then, the immunosensor acted as working electrode and SWV was conducted from −0.1 V to 0.4 V with pulse amplitude