Electrochimica Acta 278 (2018) 352–362

Contents lists available at ScienceDirect

Electrochimica Acta

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

Multiple signal amplification electrochemiluminescent immunoassay for Sudan I using gold nanorods functionalized graphene oxide and palladium/aurum core-shell nanocrystallines as labels

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ARTICLE INFO

Article history:
Received 2 April 2018
Received in revised form
2 May 2018
Accepted 7 May 2018

Keywords:
Electrochemiluminescence
Competitive immunoassay
Sudan I
Pd/Au nanocrystallines
GNRs functionalized GO

ABSTRACT

Herein, a novel ultrasensitive competition-type electrochemiluminescent (ECL) immunosensor for detecting Sudan I was constructed using palladium/aurum core-shell nanocrystallines (Pd/Au CSNs) and CdSe@CdS QDs as signal bioprobe. The Pd/Au CSNs as outstanding nanocarrier equipped with superior performance like highly catalytic activity, good biocompatibility and large specific surface area, which enhanced ECL intensity significantly. The surface of electrode was coated with gold nanorods functionalized graphene oxide (GNRs/GO) nanocomposite film as the substrate, which provided an effective matrix to immobilize more coating antigen but also facilitated the electronic transmission to increase the ECL signal. The proposed immunoassay based on the synergistic effect of Pd/Au CSNs and GNRs/GO for determining Sudan I exhibited good stability, high sensitivity and wide linearity with a lower detection limit of 0.3 pg mL^{-1} (S/N = 3) and the wide range from 0.001 to 500 ng mL^{-1}. Furthermore, the developed immunosensor was successfully applied in determination of real samples with acceptable accuracy, and initiated a new route for detecting other small molecule in the future.

1. Introduction

Sudan I, a kind of azo dyes, was used as food colorants in several countries [1], but it has been recommended as unsafe, because of its possible carcinogen and mutagen for humankind [2–5]. Besides its carcinogenicity, Sudan I is a potent contact allergen and sensitizer, eliciting pigmented contact dermatitis in humans [6]. And it has been strictly banned for food usage by Food Standards Agency and the European Union [7]. Nevertheless, it is widely used as additives to chemical products like hydrocarbon solvents, oils, waxes and so forth [8,9]. Moreover, Sudan I is used in extremely great quantities and occurs everywhere in red and orange colored consumer products, foods, and printed matter owing to its low cost and stability in a series of dyes and pigments [10,11]. Such extensive use of these azo dyes might result in a considerable peril. Therefore, rapid and effective methods for the sensitive detection of Sudan I in foods are of great significance and urgency.

In the past decades, great efforts have been made by the researchers to develop various methods for determination of Sudan I in foodstuffs and water samples, including liquid chromatography [12,13], surface enhanced Raman scattering [14], flow injection chemiluminescence [15] and enzyme linked immunosorbent assay [16]. However, the majority of these approaches require expensive equipment, complex pretreatment steps, long time consuming and general pertinence, which restrict their practical applications [17–19]. Electrochemiluminescence immunoassay (ECLIA) techniques have witnessed significant progress in the electrochemical sensor field, which integrates the excellent sensitivity of electrochemiluminescence (ECL) and the high specificity of immunoreaction [20–22].

Noble-metal nano-compounds have been intensively studied for many decades attributed to their unique properties [23–25]. Among various noble metal nanomaterial, the bimetallic core-shell nanostructures have attracted growing attention, and multifarious invaluable applications in catalysis and sensing have been reported [26–28]. Herein, palladium/aurum nanocrystallines with core-shell structure (Pd/Au CSNs) were synthesized, which acted as an outstanding nanocarrier with good biocompatibility and high specific surface area. Moreover, owing to the highly catalytic

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https://doi.org/10.1016/j.electacta.2018.05.061
0013-4686/

activity of Pd/Au CSNs which accelerated electron transfer of the reduction of $S_2O_8^{2-}$, ECL reaction between co-reactant ($K_2S_2O_8$) and CdSe@CdS QDs was promoted and intensity of the luminescence was significantly enhanced [29]. On the other hand, polyamidoamine dendrimers (PAMAM, G2), a three dimensional macromolecule with multi-amidogen at the globular periphery [30], provided many amidogen active sites for Pd/Au CSNs as well as QDs. Herein, the QDs-PAMAM- Pd/Au CSNs composite working as ECL immunosensor probe was fabricated for bioconjugate with Sudan I antibody.

Gold nanoparticles (GNPs) have received widespread interest due to their unique optical properties, ease of bioconjugation and potential non-biotoxicity [31]. Nevertheless, electrochemical sensing applications of other gold nanostructures such as gold nanorods (GNRs) have been less deep-explored in spite of the fact that they have more advanced capabilities over the spherical gold nanoparticles like superior biocompatibility, good stability and catalytic activity, and so forth [32]. Moreover, the size and structure of GNRs would be controlled through many method such as reaction time, temperature, organic stabilizer and so on, which are of great significance to the property of nanomaterials [33–35]. Herein, GNRs were proposed as the signal amplification nanocarriers for immobilizing Sudan I coating antigen. Furthermore, for making further efforts to amplify the ECL signal of immunosenor, it is an important measure to find an efficient supporter which has sufficient capability to effectively load GNRs. Graphene oxide (GO), a single layer of two-dimensional carbon nanomaterial, has recently attracted great interest with its remarkable properties, such as larger surface area, high aqueous solubility and thermal stability [36–38]. But poverty of electrical conductivity limits its practical application [39]. To solve this trouble, much measure has been reported by decorating GO with various metal nanoparticles [40–42]. Therefore, we embellished GNRs on GO which solved the dilemma of conductivity but also provided a larger platform to immobilize Sudan I coating antigen with good biocompatibility and stability.

In this work, we designed the multiple signal amplification strategies by using GNRs/GO and QDs-PAMAM-Pd/Au CSNs nanomaterials to construct competitive-type immunosensor for sensitive detection of Sudan I. The GNRs/GO, a nanocarrier with good biocompatibility and conductivity, could provide a substrate to increasing the load capacity of Sudan I coating antigen. PAMAM-Pd/Au CSNs nanocomposite coupled with the CdSe@CdS QDs emerged a significantly promoted signal owing to its catalysis and large load bioconjugating with Sudan I antibody. As a consequence, multiple-assisted signal amplification electrochemiluminescent immunoassay for detecting Sudan I had been obtained via amplification of GNRs/GO as substrate and QDs-PAMAM- Pd/Au CSNs nanomaterials as bioprobe for the first time. Moreover, the proposed immunosensor revealed a wide detection and low detection limit. This strategy had been used to real samples with acceptable accuracy, which will also open a new way for determining other small molecule in the future.

2. Experimental

2.1. Chemicals and apparatus

Chloroauric acid ($HAuCl_4 \cdot 4H_2O$, 47.8%) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Polyamidoamine dendrimers (PAMAM, G2) was purchased from Weihai CY Dendrimer Technology Co., Ltd. (Weihai, China). Potassium tetrachloropalladate (K_2PdCl_4), sodium borohydride ($NaBH_4$, 96%) and L-ascorbic acid (AA) were bought from Adamas Reagent Co., Ltd. (Shanghai, China). Graphene Oxide (GO) was purchased from XF NANO, INC. (Nanjing, China). Cetyltrimethylammonium bromide (CTAB) and hexadecylpyridinium chloride monohydrate (CPC) were acquired from Aladdin Industry Corporation. (Shanghai, China). Ethyl-3-(dimethyl aminopropyl) carbodiimide (EDC, 98%), N-hydroxysuccinimide (NHS, 97%) were supplied by J&K Scientific (Beijing, China). Bovine serum albumin (BSA, 98%) and ovalbumin (OVA) were acquired from Sigma-Aldrich Co., Ltd. (St. Louis, MO, USA). Aluminum oxide polishing powder (Al_2O_3, 0.3 and 0.05 μm) was obtained from Tianjin Aidahengsheng Technology Co., Ltd. (Tianjin, China). All other reagents and materials were commercially available and of analytical reagent grade.

The ECL measurements were performed by a MPI-A multifunctional electrochemical and chemiluminescent analytical system (Xi'An Remax Elcetronic Science & Technology Co., Ltd., Xi'An, China) with the voltage of the photomultiplier tube (PMT) being biased at −650 V and the potential scanning from 0 to −1.7 V in the course of detection. Three electrode system was used in the experiment, which contained the modified glassy carbon electrode (GCE, $\Phi = 3$ mm) as working electrode, platinum as the counter electrode and Ag/AgCl (saturated KCl solution) electrode as reference electrode. Electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) were carried out with a RST electrochemical working station (Suzhou Risetest Instrument Co., Ltd., Suzhou, China). UV–vis absorption spectra was performed with an Agilent 8453 UV–vis spectrophotometer (Agilent Co., America). X-ray photoelectron spectroscopy (XPS) was performed on an ESCALab 220i-XL electron spectroscopy from VG Scientific (Thermo VG Scientific, USA). Scanning Electronic Microscopy (SEM) was carried out using a Hitachi SU8010 SEM (Hitachi Co., Ltd. Japan). Transmission Electron Microscope (TEM) images were obtained from Tecnai G2 F20 S-TWIN 200 KV (FEG, FEI Co., USA).

2.2. Buffers and solutions

Phosphate buffered saline (PBS, pH = 7.4, 0.1 M) was prepared using KCl (0.1 M), NaCl (0.1 M), Na_2HPO_4 (6.4 mM) and KH_2PO_4 (1.0 mM) throughout the entire work. ECL detection buffer was prepared by PBS containing 0.1 M $K_2S_2O_8$. Sudan I stock solution (1 mg mL^{-1}) was obtained by dissolving Sudan I red powder in DMF. Sudan I standard solutions at the concentrations of 0.001, 0.01, 0.1, 1, 10, 100, 500 and 1000 ng mL^{-1} were prepared by diluting the stock solution with methanol: water (v v^{-1} = 5: 95). All aqueous solutions were prepared with sub-boiling doubly distilled water.

2.3. Preparation of the Sudan I coating antigen and Sudan I antibody

The preparation of the Sudan I coating antigen and antibody were followed by an anhydride ester method [16]. First of all, the carboxylic acid-derivative Sudan I, named Sudan I- 3- propanoic acid (Sudan I-C3), was synthesized according to previous work [43]. Subsequently, equimolar amounts (0.15 mmol) of Sudan I-C3, DCC and NHS were dissolved in 300 μL DMF under slow stirring for overnight at 25 °C to obtain activated derivatives. After the centrifugation (12,000 rpm, 10 min), the supernatant was added slowly to 100 mg protein (BSA or OVA) which was dissolved in 5 mL $NaHCO_3$ (0.13 M). Then, the solution was kept stirring for 4 h. Afterwards, the solution was centrifuged and the supernatant was dialyzed in $(NH_4)_2CO_3$ (0.01 M) for 4 days. Finally, the Sudan I–protein conjugates were lyophilized and stored at −40 °C until use. Sudan I–C3–BSA was served as immunogen for antibody preparation, while the Sudan I–C3–OVA was selected as coating antigens for ECLIA establishment.

Polyclonal antibodies (pAb) against Sudan I were extracted from the antisera of two adult New Zealand rabbits immunized with