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Self-enhanced PEI-Ru(II) complex with polyamino acid as booster to construct ultrasensitive electrochemiluminescence immunosensor for carcinoembryonic antigen detection

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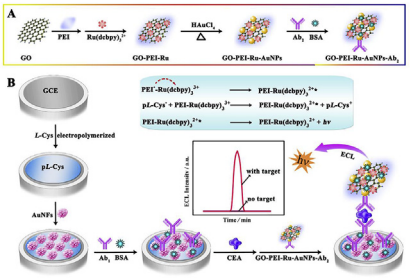
HIGHLIGHTS

- A sensitive ECL immunosensor was constructed with the amplification of p*L*-Cys toward self-enhanced PEI-Ru(II).
- The intramolecular electron transfer could efficiently amplify ECL signal owing to its benefit for electron transmission.
- P*L*-Cys as a strongly reducing radical could react with PEI-Ru(III) to generate PEI-Ru(II)* with further enhanced ECL signal.
- The prepared ECL immunosensor exhibited great advantages in simplification, rapidity, stability and selectivity.

GRAPHICAL ABSTRACT



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ABSTRACT

In this work, the luminophor tris (4,4′-dicarboxylicacid-2,2′-bipyridyl) ruthenium (II) dichloride ($Ru(dcbpy)_3^{2+}$) was linked to coreactant polyethylenimine (PEI) with effectively shorted distance, forming a novel self-enhanced PEI-Ru(II) complex to fabricate ultrasensitive electrochemiluminescence immunosensor for carcinoembryonic antigen (CEA) detection by using polyamino acid L-cysteine (p*L*-Cys) as booster for further signal amplification. The light-emitting species (PEI-Ru(II)*) based on intramolecular electron transfer between $PEI^{\bullet}$ and Ru(III) could efficiently amplify the ECL signal of $Ru(dcbpy)_3^{2+}$ owing to its benefit for electron transmission. Secondly, the p*L*-Cys film was fabricated on the glassy carbon electrode by cyclic voltammogram. After that, the p*L*-Cys acted as a strongly reducing radical could react with PEI-Ru(III) to generate the excited state PEI-Ru(II)*, which further amplify the ECL signal. With the signal amplification factors, the prepared ECL immunosensor exhibited great advantages in simplification, rapidity, stability and selectivity. Furthermore, the linear range for the determination of CEA was from 0.10 pg/mL to 80 ng/mL with a correlation coefficient of 0.9942 and a detection limit of 0.045 pg/mL ($S/N = 3$).

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1. Introduction

Carcinoembryonic antigen (CEA), as one of the common tumor markers for clinic diagnosis and treatment of cancer, has attracted broad interest of scientists to develop sensitive, precise and accurate measurement assays for the level of its in human serum [1,2]. Up to now, a series of analysis techniques have been used for the quantitative detection of CEA, such as enzyme-linked immunosorbent assay (ELISA) [3,4], photoelectrochemistry (PEC) [5], surface-enhanced resonance raman scattering (SERRS) [6], colorimetry [7], electrochemical immunoassay (EC) [8–10] andelectrochemiluminescence (ECL) immunoassay [11]. Among these methods, ECL immunoassay presents not only advantages of high sensitivity with wide dynamic concentration response range but also biospecific recognition interactions between antigens and antibodies [12–14]. Therefore, many different ECL immunosensors have been proposed and extensively applied for the detection of CEA [15,16].

Since the ECL of tris (2,2′-bipyridyl)ruthenium(II) ($Ru(bpy)_3^{2+}$) was first reported in the literature by Tokel and Bard [17], it has become one of the most widely studied and used ECL compounds due to its chemical stability and redox properties [18,19]. In the process of the Ru(II) complex-based ECL system research, it can be found that when $Ru(bpy)_3^{2+}$ exist alone, its ECL intensity is relatively low; but after introducing the corresponding co-reagents into this system, the luminous efficiency could be improved significantly owing to its benefit for promoting ECL reaction [20–22]. Besides, a variety of researches demonstrated that some amine compounds (such as polyethylenimine (PEI), polyamide, Histidine, Arginine, etc.) could serve as co-reactants of $Ru(bpy)_3^{2+}$ or its derivatives [23,24]. In the traditional method, co-reactants are often added into detecting solution [25] or immobilized on the electrode [26] *via* intermolecular electron transfer to enhance the ECL signal. However, some of them had disadvantages of toxic and unstable, increasing the operation difficulty and measurement error. What's more, the electron transfer between luminescent reagents and co-reactants can result in the increase of electron transfer distance and energy loss. To solve these problems, our team has developed a series of self-enhance ECL reagents, which can overcome these difficulties by using intramolecular electron transfer to significantly improve the luminous efficiency [27–29].

In this work, a sensitive ECL immunosensor was constructed for CEA detection by using the signal amplification effect of polymer L-cysteine (p*L*-Cys) toward the self-enhanced PEI-Ru(II) complex. First of all, PEI was linked with $Ru(dcbpy)_3^{2+}$ *via* amide bond to form a self-enhanced luminescent reagent (PEI-Ru), in which PEI acted as an intramolecular co-reactant of $Ru(dcbpy)_3^{2+}$. Secondly, p*L*-Cys was immobilized on the glassy carbon electrode (GCE) by cyclic voltammogram (CV) and act as a co-reactant of the self-enhanced PEI-Ru(II) complex to further amplify the ECL signal of $Ru(dcbpy)_3^{2+}$. Based on this strategy of signal amplification, a strong ECL signal was achieved. Furthermore, graphene and gold nanomaterials were introduced into this system to increase the immobilization amount of antibodies and luminescent reagents, further improving the sensitivity of the immunosensor. As a result, the proposed ECL immunosensor had a potential value and development prospects in the fields of clinical diagnosis to detect CEA.

2. Experimental

2.1. Materials

Poly(ethylenimine) (PEI, ~50 w/v in aqueous solution, the molecular weight 600–1000 kDa, the amino group concentration 56.4–93.9 μM) was supplied from Fluka (Switzerland). Tris (4,4′-dicarboxylicacid-2,2′-bipyridyl) ruthenium(II) dicbloride ($Ru(dcbpy)_3^{2+}$) was purchased from Suna Tech Inc (Suzhou, China). Graphene oxide (GO, 95%) was obtained from Shenzhen Nanomaterials Company (Shenzhen, China). *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide-hydrochloride (EDC) and *N*-hydroxy succinimide (NHS) were acquired from Shanghai Medpep Co. (Shanghai, China). Gold chloride ($HAuCl_4$) and bovine serum albumin (BSA, 96–99 *wt%*) were supplied from Sigma-Aldrich Co. (St. Louis, MO, USA). L-cysteine (*L*-Cys) and ascorbic acid (AA) were purchased from Kangda Amino Acid (Shanghai, China). Cetyltrimethylammonium chloride (CTAC) was acquired from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Carcinoembryonic antigen (CEA), carcinoembryonic antibody (anti-CEA), prostate specific antigen (PSA) and collagen IV (Col IV) were obtained from Biocell Company (Zhengzhou, China). Phosphate buffered solution (PBS, 0.1 M) was prepared by using 0.1 M Na_2HPO_4, 0.1 M KH_2PO_4, and 0.1 M KCl. Ferricyanide solution ($Fe(CN)_6^{3-/4-}$) was obtained by dissolving $K_3Fe(CN)_6$ and $K_4Fe(CN)_6$ in 0.1 M PBS (pH = 7.0).

2.2. Preparation of GO-PEI-Ru-AuNPs-Ab_2 bioconjugate

First of all, 20 mg $Ru(dcbpy)_3^{2+}$ was dispersed into 4 mL PBS (0.1 M, pH = 7.4) with slowly stirring, and then coupling reagents EDC/NHS (4:1) was added to activate the -COOH of $Ru(dcbpy)_3^{2+}$. Next, 1.5 mL GO-PEI mixed solution (prepared by dispersing 4 mg GO into 4 mL PEI (1%, *w*/*v*)) was added for combining the -COOH of $Ru(dcbpy)_3^{2+}$ with the $-NH_2$ of PEI to obtain the self-enhanced complex (GO-PEI-Ru). Subsequently, 0.5 mL $HAuCl_4$ (1%, *w*/*v*) aqueous solution was dropped into the obtained solution and heated to 80 °C for the incubation of 2 h. After naturally cooled to room temperature, the above solution was mixed with 0.5 mL anti-CEA under softly stirring at 4 °C and incubated for 12 h. Then, the obtained GO-PEI-Ru-AuNPs-Ab_2 bioconjugate were collected by centrifugation (6000 rpm, 5 min) at 4 °C. Finally, the resulting bioconjugate was dispersed in 5 mL PBS (0.1 M, pH = 7.4) containing 0.3 mL BSA (1 *wt%*) and stored at 4 °C for further use.

2.3. Fabrication of the ECL immunosensor

Prior to modification, the GCE was polished with 0.3 and 0.05 μm alumina powder, which followed by ultrasonically cleaning with deionized water, anhydrous ethanol and deionized water, respectively. Thereafter, the cleaned GCE was scanned in 20 mM *L*-Cys between −0.5 V and 1 V by CV for 3 min to modify a layer of the p*L*-Cys film. After rinsing with ultrapure water, the modified electrode was dipped in prepared flower-like gold nanoparticles (AuNFs) solution (See Supporting information) at 4 °C for 8 h, and then incubated with 15 μL anti-CEA at 4 °C for 12 h. Subsequently, 15 μL BSA was dropped on the modified electrode surface at 37 °C for incubation of 1 h to eliminate nonspecific binding sites. Fallowing that, 15 μL CEA with different concentration was incubated with the modified electrode at 37 °C for another 50 min. Finally, the modified electrode was further incubated with 15 μL GO-PEI-Ru-AuNPs-Ab_2 bioconjugate at 37 °C for 1 h. Scheme 1 outlined the construction process of proposed ECL immunosensor, which includes the preparation procedure of GO-PEI-Ru-AuNPs-Ab_2 bioconjugate.

3. Results and discussion

3.1. The SEM characterization of different nanomaterials

The surface morphology of GO, GO-PEI-Ru-AuNPs complex and AuNFs were characterized by scanning electron microscope (SEM),

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