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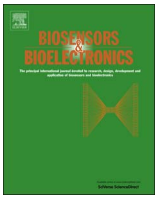

Sensitive electrochemiluminescent immunosensor for diabetic nephropathy analysis based on tris(bipyridine) ruthenium(II) derivative with binary intramolecular self-catalyzed property

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ABSTRACT

Electrochemiluminescence (ECL) co-reaction and ECL resonance energy transfer (ECL-RET) are two main methods for enhancing the ECL efficiency. In this work, a novel tris(bipyridine) ruthenium(II) derivative ($Ru(bpy)_2(mcbpy)^{2+}$-PEI-ABEI) with high ECL efficiency due to the binary intramolecular ECL self-catalyzed property including intramolecular co-reaction and intramolecular ECL-RET, was prepared for the construction of immunosensor. Firstly, through intramolecular co-reaction, polyethyleneimine (PEI) with large amount of amine co-reactive groups (primary, secondary and tertiary amine) not only increased the ECL intensity of $Ru(bpy)_2(mcbpy)^{2+}$, but also improved the ECL signal of N-(aminobutyl)-N-(ethylisoluminol) (ABEI). Meanwhile, ABEI, as an effective energy transfer donor, could further increase the ECL intensity of $Ru(bpy)_2(mcbpy)^{2+}$ (as energy transfer receptor) by intramolecular ECL-RET. It is worth noting that the strategy combined intramolecular co-reaction and intramolecular ECL-RET in the same ECL process was more effective for ECL efficiency enhancement compared with the traditional intermolecular interaction or individual intramolecular interaction, which would improve the sensitivity of immunosensor obviously. Thus, the proposed signal-on ECL immunosensor using convex hexoctahedral Pd@Au core-shell nanocrystals (Pd-Au HCDs) as immobilized platform exhibited a detected range for collagen type IV (Col IV), a typical biomarker of diabetic nephropathy (DN), from 0.5 pg mL^{-1} to 7.2 ng mL^{-1} with an estimated detection limit of 0.17 pg mL^{-1}. The developed strategy combined intramolecular co-reaction with intramolecular ECL-RET offered an effective mean for ECL methodology in ECL efficency enhancement and also provided possible road for early diagnosis and treatment monitoring of DN.

1. Introduction

As one of the most serious and common long-term complications of diabetic patients, diabetic nephropathy (DN) is the leading cause of death, especially for end stage renal disease (ESRD), which attracts increasing attention worldwide (Peng et al., 2015). As similar with other diseases, early diagnosis and treatment for DN prossess remarkable significance. Collagen type IV (Col IV) is reported as a typical biomarker for DN, and its sensitive and accurate detection would be significant for DN in early assessment and subsequent therapeutic evaluation (Jha et al., 2014). However, the analytical approaches for Col IV are relatively limited at present, and development of new techniques for accurate measurement of Col IV is essential. Electrochemiluminescence (ECL), a powerful analysis technique attracted increasing attention for its outstanding controllability, simplified optical setup and low background signal, could be employed for Col IV monitoring (L.L. Li et al., 2017; Rizzo et al., 2017; Muzyka et al., 2017). However, the sensitivity is the most important restraining factor for its better application, which is directly associated with the ECL efficiency and the signal amplification.

Numerous studies have shown that the addition of co-reactants to electrochemiluminescent systems can significantly improve the luminous efficiency of electrochemiluminescent reagents (Richter, 2004; Hesari et al., 2017). Therefore, various co-reactants are introduced into the construction of ECL biosensors by different ways, such as directly adding co-reactant in the detection solution, immobilizing co-reactant

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on the electrode, or in situ generating co-reactant around the electrode and so on (Xu et al., 2017; Wang et al., 2015; Poulpiquet et al., 2016). However, the long electron transfer path and massive energy loss in the intermolecular ECL reaction restrict the further application of ECL. Under this situation, a new ECL reaction pattern through intramolecular interaction has been developed based on luminous reagents simultaneously containing luminophore and its co-reactive groups in the molecular structure, by which the electronic transmission distance is shortened and energy loss is reduced, leading to improved luminous efficiency and stability (Carrara et al., 2017; Xiong et al., 2017; Fu et al., 2017).

ECL resonance energy transfer (ECL-RET) is another effective way for enhancing the luminous efficiency of ECL (Dong et al., 2017; Chen et al., 2017; Liu et al., 2017). By introducing into the appropriate energy transfer donor, the energy could transfer to the energy receptor, which makes the ECL efficiency significantly improve. It is gradually intensively explored in recent years for the reason that it has low background noise, avoids the scattered light, and does not need activation of the light source. Recently, researchers have fabricated multiple sensitive ECL biosensors by using ruthenium complexes as energy receptors and selecting different energy donors (such as gold nanoclusters and C_3N_4, etc.) (Feng et al., 2016; Swanick et al., 2015; Yu et al., 2015). However, as similar with the intermolecular co-reaction, these present studies are also constructed by intermolecular interactions between the energy donors and energy receptors, existing shortcomings of large energy loss and poor stability. Accordingly, the construction of intramolecular ECL-RET system has remarkable significance for the enhancement of ECL efficiency and stability, which has been rarely reported until now.

Nevertheless, the ECL efficiency of individual intramolecular co-reaction or individual intramolecular ECL-RET is still limited. The ECL efficiency would be further greatly improved if the two intramolecular interactions could be combined in the same ECL process. Furthermore, in the preparation of new intramolecular self-catalyzed ECL reagents that simultaneously include intramolecular co-reaction and the intramolecular ECL-RET, how to improve the content of luminophore and co-reactive group, or the energy transfer donor and the energy transfer receptor, is another key limiting factor for the ECL efficiency. Polyethyleneimine (PEI) is one of the most widely used organic high-molecular polymers (Jäger et al., 2012). Its polymeric structure contains a large amount of amine co-reactive groups (primary, secondary and tertiary amine), making it an outstanding co-reagent for the ECL of ruthenium complex, and its catalytic effect is better than that of some co-reactive small molecules (X.J. Li et al., 2017). Especially, in this research, it has been found that PEI could also obviously enhance the ECL intensity of N-(aminobutyl)-N-(ethylisoluminol) (ABEI) that acts as an energy donor for the ECL of ruthenium complex (receptor). Moreover, PEI possesses abundant -NH_2 active groups, making it can be easily modified or immobilized. Based on these considerations, this study intends to prepare a novel tris(bipyridine) ruthenium(II) derivative, with binary intramolecular ECL self-catalyzed property including intramolecular co-reaction and intramolecular ECL-RET, by using PEI to simultaneously couple with ruthenium complex and ABEI. It can be confirmed that the novel binary intramolecular self-catalyzed ECL reagent would possess high ECL efficiency and stability due to the intramolecular co-reaction and the intramolecular ECL-RET.

Herein, a novel tris(bipyridine) ruthenium(II) derivative ($Ru(bpy)_2(mcbpy)^{2+}$-PEI-ABEI) was prepared to construct a sensitive immunosensor, which possessed extremely high ECL efficiency due to the binary intramolecular ECL self-catalyzed property including intramolecular co-reaction and intramolecular ECL-RET. Concretely, PEI, acted as a classic co-reactant with large amount of amine co-reactive groups, could obviously increase the ECL intensity of $Ru(bpy)_2(mcbpy)^{2+}$, especially through the intramolecular co-reaction. Meanwhile, the ECL of $Ru(bpy)_2(mcbpy)^{2+}$ could be further enhanced through the intramolecular ECL-RET from ABEI whose ECL intensity was also improved by intramolecular co-reaction with PEI. Then, owing to their large specific surface area and excellent electrocatalytic activity, convex hexoctahedral Pd@Au core-shell nanocrystals (Pd-Au HCDs) were synthesized to load antibody. On the Au nanoparticles (AuNPs) modified electrode, a sensitive ECL immunosensor was fabricated for detection of Col IV, a typical biomarker of diabetic nephropathy (DN), through the sandwiched immunoreactions. The strategy combined intramolecular co-reaction with intramolecular ECL-RET developed in this work provides a novel and effective thread to improve ECL efficency, and the sensitive detection of Col IV also offers possible road for early diagnosis and treatment monitoring of DN.

2. Experimental

2.1. Reagents and apparatus

Collagen type IV (Col IV) standards and its antibody, N-acetyl-β-D-glucosaminidase (NAG) were purchased from Shanghai HuaYi Biotechnology Co. Ltd. (Shanghai, China). Carcinoembryonic antigen (CEA) was got from Biocell Company (Zhengzhou, China). Bis(2,2′-bipyridyl)(4′-Methyl-[2,2′]bipyridinyl-4-carboxylicacid) ruthenium (II) dichloride ($Ru(bpy)_2(mcbpy)^{2+}$) was obtained from Suna Tech Inc. (Suzhou, China). N-(aminobutyl)-N-(ethylisoluminol) (ABEI) was purchased from TCI Development Co., Ltd. (Shanghai, China). Poly(ethylenimine) (PEI, 50%) was purchased from Fluka (Switzerland). Potassium tetra-chloropalladate (K_2PdCl_4), gold chloride tetrahydrate ($HAuCl_4 \cdot 4H_2O$), bovine serum albumin (BSA, 96–99%), glutaric dialdehyde (GA, 50%), N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC), N-Hydroxy succinimide (NHS) were obtained from Sigma-Aldrich Chem. Co. (St. Louis, MO, USA). Cetyltrimethylammonium bromide (CTAB) and cetylpyridinium chloride monohydrate (CPC) were got from Kelong Chemical Company (Chengdu, China). Ascorbic acid (AA) was obtained from Shanghai Chemical Reagent Company (Shanghai, China). The serum speciments were got from Southwest Hospital in Chongqing of China. Phosphate-buffered solution (PBS) (pH 8.0, 0.1 M) was prepared with KCl (0.1 M), KH_2PO_4 (0.1 M) and Na_2HPO_4 (0.1 M). Deionized water was used throughout this work.

The ECL detection was performed by MPI-E ECL analyzer (Xi'an Remax Electronic science & Technology Co. Ltd., Xi'an, China). The potential scanning range was 0.2–1.25 V with the voltage of the photomultiplier tube (PTM) being set at 800 V. CHI 660E electrochemical workstation (Shanghai Chenhua Instrument, China) was used for electrochemical experiments. Three-electrode system was used in the detection, which used the modified glassy carbon electrode (GCE) as working electrode, platinum wire as counter electrode, and Ag/AgCl (sat. KCl) as reference electrode. Scanning electron microscopy (SEM, S-4800, Hitachi, Japan), transmission electron microscope (TEM, H600, Hitachi Instrument, Japan) and X-ray photoelectron spectroscopy (XPS) were used for characterizing the successful preparation of nanomaterials.

2.2. Preparation of Pd-Au HCDs/$Ru(bpy)_2(mcbpy)^{2+}$-PEI-ABEI@Ab/BSA bioconjugate

The preparation of Pd-Au HCDs/ $Ru(bpy)_2(mcbpy)^{2+}$-PEI-ABEI@Ab/BSA bioconjugate was illustrated in Scheme 1A. Primitively, convex Pd-Au HCDs were synthesized on the basis of literature with some modifications (Zhang et al., 2014). Firstly, nanocube-like palladium (PdNCs) seeds were prepared. CTAB (12.5 mM, 9.42 mL) and K_2PdCl_4 solution (10 mM, 0.5 mL) were mixed with stirring under 95 °C for 5 min. Then, AA (100 mM, 80 μL) prepared freshly was added into the mixture under stirring for 20 min. Through reduction reaction, the PdNCs seeds were obtained by centrifugation and washing with CPC aqueous, which were re-dispersed into CPC (5 mM, 10 mL) aqueous for