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An ECL biosensor for sensitive detection of concanavalin A based on the ECL quenching of Ru complex by MoS_2 nanoflower

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ABSTRACT

A novel signal-off electrochemiluminescence (ECL) biosensor was constructed for concanavalin A (Con A) detection based on the ECL quenching of Ru complex by MoS_2 nanoflower (MoS_2 NF). In brief, PTCA-Ru-Au NPs as matrix was dropped on the electrode to immobilize phenoxy dextran (DexP) through π-π interaction, where 3,4,9,10-perylenetetracarboxylic acid (PTCA) and Au nanoparticles (Au NPs) could greatly increase the luminous efficiency of Tris (2,2′-bipyridyl-4,4′-dicarboxylato) ruthenium(II) ($Ru(dcbpy)_3^{2+}$). Then, DexP as recognition element was bonded with Con A via specific carbohydrate-Con A interaction. Finally, the MoS_2 NF-multi-walled carbon nanotubes (MWCNT)-DexP as quenching probe of ECL signal was combined with Con A to successfully fabricate a sandwich type ECL biosensor. Importantly, MoS_2 NF as a new ECL quencher showed high ECL quenching efficiency toward Ru complex. Under the optimum detection conditions, a wide liner range of 1.0 pg/mL to 100 ng/mL was achieved with relatively low detection limit of 0.3 pg/mL.

1. Introduction

Concanavalin A (Con A) is a legume lectin from Jack beans including four binding sites to various carbohydrates such as mannose, glucose and dextran [1]. The interaction between Con A and carbohydrates has been used to drug development and clinical diagnostics such as the detection of pesticide, cancer cells and leukemia cell [2–4]. Phenoxy-dextran (DexP), as one of derivatives of dextran, not only can interact with Con A by means of specific carbohydrate-Con A interaction, but also can be immobilized onto surface of nanomaterials through π-π interaction [5]. Based on the Con A-dextran affinity, the assay methods for Con A containing surface plasmon resonance [6], electrochemical analysis [5] and electrochemiluminescence (ECL) [7] have been reported. Among various methods, ECL is a promising method in analytical chemistry because of high sensitivity, rapid response and simplified operation.

MoS_2, one of transition metal dichalcogenides, has the 2-dimensional (2D) layer structure analogous to graphene [8,9]. Single-layer MoS_2 is consisted of a positively charged molybdenum plane and two negatively charged sulfur planes to form an "S-Mo-S" sandwich structure [10]. Considering the morphology and size of electrode materials are relevant with their electrochemical properties, different morphology of MoS_2 such as nanosphere [11], nanosheet [12] and nanoflower [13] have been successfully synthesized. Hereinto, MoS_2 nanoflower (MoS_2 NF) with outstanding advantages of excellent conductivity, good catalytic activity, facile large-scale production and splendid repeatability of the products has been applied in many fields including photocatalysis [14], electrochemistry [15] and ECL [16]. The catalysis of MoS_2 NF has been used to fabricate ECL biosensor through the combination with polyaniline [17]. However, the quenching effect of MoS_2 NF on ECL response has not been investigated and applied in ECL sensing field.

It is critical to achieve the strong ECL background signal in the construction of signal off ECL biosensor [18]. Ruthenium(II) tris(2,2-bipyridyl) ($Ru(bpy)_3^{2+}$) and its derivatives, as familiar ECL reagents, has attracted increasing attention due to high luminescence, excellent stability, wide application range of pH and electrochemical reversibility [19,20]. However, $Ru(bpy)_3^{2+}$ leak out easily on a solid electrode surface because of its good water-solubility, inducing unstablity of ECL sensor [21]. 3,4,9,10-perylenetetracarboxylic acid (PTCA) as an organic perylene dye has good membrane-forming property to hinder the loss of $Ru(bpy)_3^{2+}$ [22]. Meanwhile, PTCA could combine with $Ru(bpy)_3^{2+}$ via π-π stacking to enhance the luminescence of $Ru(bpy)_3^{2+}$ [23]. In addition, It has been reported that citrate-capped Au nanoparticles (Au NPs) with neg-

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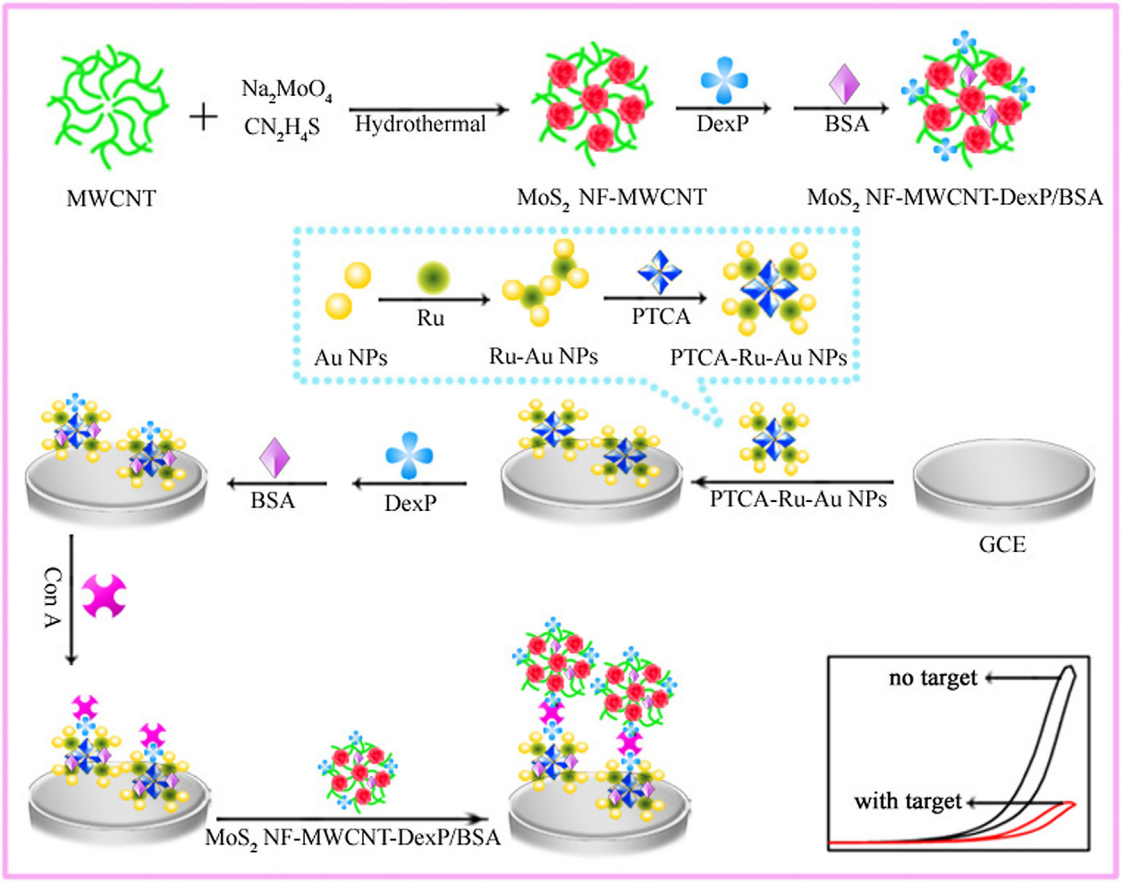

Scheme 1. The illustration of the preparation process of the proposed ECL biosensor.

ative charged could immobilize $Ru(bpy)_3^{2+}$ through electrostatic interactions to improve the stability and amplify ECL signal of $Ru(bpy)_3^{2+}$ [24].

Herein, based on the quenching effect of MoS_2 NF to Ru complex, a sandwich-configuration ECL biosensor was developed for ultrasensitive detection of Con A. Such a sandwich construction strategy mainly exhibited following attractive advantages. Firstly, PTCA and Au NPs functionalized tris (2,2′-bipyridyl-4,4′-dicarboxylato) ruthenium(II) ($Ru(dcbpy)_3^{2+}$) as matrix exhibited greatly enhanced ECL signal. Secondly, phenoxy dextran (DexP) as linker was bound to PTCA-Ru-Au NPs via π-π stacking and recognized the target Con A through specific carbohydrate-Con A interaction. Thirdly, the quenching probe of MoS_2 NF- multi-walled carbon nanotubes (MWCNT)-DexP nanocomposites was bonded with Con A to achieve the fabrication of sandwich biosensor. Here, MoS_2 NF as ECL quencher could efficiently and stably quench ECL signal of Ru complex. MWCNT was excellent supporter for MoS_2 NF and DexP because of its large surface area and flat π system. Owing to these favorable conditions, the proposed biosensor achieved sensitive detection for Con A. Furthermore, the proposed biosensor showed acceptable selectivity, stability, and reproducibility, showing potential application in bioanalysis.

2. Experiment

2.1. Apparatus

The ECL measurements were monitored using a MPI-A multifunctional chemiluminescent analytical system (Xi'an Remax Electronic Science&Technology Co. Ltd., Xi'an, China). The voltage of the photomultiplier tube (PMT) was set at 800 V and the potential scan was from 0.2 to 1.2 V with a scan rate of 100 mV/s during measurements. The electrochemical experiments were detected with a CHI660D electrochemical workstation (Shanghai CH Instruments Co., Shanghai, China). The three electrode system was used for the experiment containing a platinum wire as auxiliary electrode, an Ag/AgCl for ECL experiments or a saturated calomel electrode (SCE) for electrochemical experiments as the reference electrode and bare or modified glassy carbon electrode (GCE, $\Phi = 4.0$ mm) as working electrode. Scanning electron micrographs (SEM) were obtained by a scanning electron microscope (SEM, S-4800, Hitachi, Japan). The X-ray diffraction pattern (XRD) was performed by a XRD-7000 (Shimadzu, Japan) with Cu-Kα radiation. X-ray photoelectron spectroscopy (XPS) analysis was conducted with the Thermo Scientific Escalab 250Xi spectrometer (Thermoelectricity Instruments, USA). UV–visable (UV–vis) absorption spectra were obtained with an UV-2450 UV–vis spectrophotometer (Shimadzu, Japan).

2.2. Reagent and materials

Sodium molybdate, thiourea, oxalic acid, sodium citrate and multi-walled carbon nanotubes (MWCNT, 95% purity) were purchased from Ke Long Chemical Reagent Co. Ltd. (Chengdu, China). Gold chloride tetrahydrate ($HAuCl_4{\cdot}4H_2O$) was obtained from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Tris (2,2′-bipyridyl-4,4′-dicarboxylato) ruthenium(II) ($Ru(dcbpy)_3^{2+}$), tripropylamine (TPrA), concanavalin A (Con A, from canavalia ensiformis (jack bean)), bovine serum albumin (BSA, 96–99%), dextran (MW ≈ 70,000) and 1,2-epoxy-3-phenoxypropane (Epoxy) purchased from Sigma (St. Louis, MO, USA). 3,4,9,10-perylenetetracarboxylic dianhydride ($C_{24}H_8O_6$,