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A cyclic catalysis enhanced electrochemiluminescence aptasensor based 3D graphene/photocatalysts Cu_2O-MWCNTs

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ABSTRACT

Here, we developed an electrochemiluminescence (ECL) aptasensor for ultrasensitive detection of thrombin (TB) based on a synergistic effect of nanoparticles sensitization and cyclic catalysis of Cu_2O. The aptasensor is constructed from three parts: firstly, three dimensional (3D) graphene was dropped onto the electrode to accelerate electron transfer. Then, the synthesized Cu_2O-multiwalled carbon nanotubes (MWCNTs) were loaded onto the surface of 3D graphene. Finally, after TB aptamers linked to MWCNTs, $Ru(bpy)_3^{2+}$- doped silica nanoparticles (RuSiNPs) were marked onto the surface. When detection of TB, the aptamers folded into G-quadruplex's that made the RuSiNPs get closer to the Cu_2O. As a photocatalysis, Cu_2O was excited by the ECL of RuSiNPs and generated holes and electrons which could catalyze the ECL reaction to emit much light, and the light could excite Cu_2O again. Hence, a cyclic catalytic aptasensor was built. Under the optimal conditions, this aptasensor for TB detection showed good sensitivity with wide linearity (5×10^{-15} M - 5×10^{-11} M) and low detection limit (1.3×10^{-15} M).

1. Introduction

Since 1990, aptamers have been found that can specifically bind to some kind of targets, such as RNA enzymes [1] and organic dyes [2]. Aptamers are single-stranded DNA or RNA oligonucleotides with unique three dimensional structure, that could be used as bio-recognition parts to fabricate selective and sensitive biosensors to detect cells [3], proteins [4,5], ions [6], drugs [7,8], and so on. Compared to antibodies and enzymes, aptamers not only present advantages of smaller size, better thermal and chemical stability, but also can be synthesized in vitro or modified with functional groups which make them as promising sensing parts for bioassay [9]. Up to now, various methods based on aptamer sensors have been widely developed for analysis and detection, such as fluorescence [10], electrochemistry [11], and electrochemiluminescence (ECL) [12,13]. Especially, ECL obtained more attention due to its high sensitivity and selectivity [14–19]. ECL is generated by electron-transfer during electrochemical reactions to form excited states that emit light at specific wavelengths. Tris (2,2′-bipyridyl)ruthenium(II) ($Ru(bpy)_3^{2+}$) is a stable and efficient chemiluminescent reagent that has been widely applied in ECL system. However, $Ru(bpy)_3^{2+}$ is so expensive that using $Ru(bpy)_3^{2+}$ in the solution-phase ECL is not a good deal, so construct solid-state $Ru(bpy)_3^{2+}$ ECL sensor is very important [20–23]. Doping silicon dioxide (SiO_2) nanoparticles has become a popular approach to obtain cost-saving, brilliant, stable biocompatible solid-state ECL sensor. $Ru(bpy)_3^{2+}$–dopped SiO_2 can immobilized $Ru(bpy)_3^{2+}$ firmly by the strong electrostatic interaction, it also can reserve the original electrochemical and luminescent properties of $Ru(bpy)_3^{2+}$ [22,24,25].

Thrombin (TB) is a specific serine protease responsible for blood clotting, and it is also a useful marker which can help diagnose the pulmonary metastasis. As thrombin combines with thrombin aptamer (TBA), the conformation of TBA changes and folds into a unique G-quadruplex structure [26,27]. Up to now, some ECL aptasensors have been built for thrombin detection, including designing affinity aptamers and ECL labels, introducing nanomaterials and designing novel ECL systems [28–35]. However, wider linear ranges or lower detection limits are still great challenges for ultrasensitive detection.

Cuprous oxide (Cu_2O) is one of the most promising semiconductor photocatalysts due to the properties of nontoxicity,

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stability and ease synthesized [36,37]. Cu_2O is a p-type semiconductor that can be excited by light at 400–800 nm wavelength, and then generate electrons (e^-) and holes (h^+) [38], both of which can catalyze chemical reactions. So $Ru(bpy)_3^{2+}$ generates light at 520–800 nm can excite the Cu_2O, following the produced e^- or h^+ will facilitate the redox reaction of $Ru(bpy)_3^{2+}$ to generate more light, thus the ECL signal will be greatly enhanced. Based on this principle, a cyclic catalysis enhanced system can be built. In addition, carbon materials like multiwalled carbon nanotubes (MWCNTs) can enhance the electron transfer to improve the catalytic capacity of Cu_2O [39]. Similarly, 3D graphene is also a carbon material that possesses a lot of advantages, such as high conductivity, great mechanical strength and large specific surface [40]. 3D graphene not only retains all good properties of 2D graphene, but also has more wrinkles and ripples [41,42]. These excellent characters make it an ideal material to modify electrode [19].

Here, we developed a cyclic catalysis enhanced electrochemiluminescence aptasensor based on 3D graphene/photocatalysts Cu_2O-MWCNTs for thrombin detection. For aptasensor fabrication, 3D graphene used as substrate material to accelerate electron transferred, then Cu_2O-MWCNTs was loaded onto the surface of 3D graphene, finally TBA was linked to MWCNTs and followed by $Ru(bpy)_3^{2+}$ doped SiO_2 nanoparticles (RuSiNPs) was fixed onto the electrode. While detection of TB, the structure change of TBA caused the distance between $Ru(bpy)_3^{2+}$ and Cu_2O became closer, so the cyclic photocatalytic reaction would improve the ECL intensity. The developed aptasensor could specifically and quickly detect thrombin with high sensitivity and wide linear range.

2. Experimental section

2.1. Chemicals and materials

Tris (2,2′-bipyridyl) dichlororuthenium(II) hexahydrate ($Ru(bpy)_3Cl_2 \cdot 6H_2O$), nafion (5 wt%), bovine serum albumin (BSA) and thrombin (TB) were purchased from Sigma-Ardrich (Madrid, Spain). Tetraethyl orthosilicate (TEOS), hexylalchol, N-succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC), N-(3-dimethyl aminopropyl)-N-ethylcarbodiimidehydrochloride (EDC) and N-hydroxy succinimide (NHS) were purchased from Damas-beta (Shanghai, China). Sodium citrate, L-Asocbric acid (AA) and Copper sulfate pentahydrate were purchased from China national pharmaceutical group corporation (Beijing, China). Triton X-100(TX-100) was supplied by Biosharp (Hefei, China). Graphite powder was offered by Beilian chemical company (Tianjin, China). Hemoglobin (Hb) and Immunoglobulin G (IgG) were purchased from Solabio science and technology Ltd. (Beijing, China). Multiwalled carbon nanotubes (MWCNTs, purity>95%, diameter 20 nm, length 30 μm) were purchased from Chengdu organic chemicals Co. Ltd. (Chengdu, China). Thrombin aptamer (TBA) were acquired from Suzhou hongxun biotechnology Co. Ltd. (Suzhou, China) and has the following sequences:

5′-SH- TCTCTCAGTCCGTGGTAGGGCAGGGTTGGGGTGACT-3′.

Tris–HCl buffer (pH 7.4) containing 140 mM NaCl, 5.0 mM KCl and 1.0 mM $MgCl_2$ was used to dissolve the TBA. Double distilled water (DDW, 18.2 MΩ cm^{-1}) used all throughout the experiments was further purified by the Millipore system.

2.2. Apparatus

The ECL signals were obtained by a MPI-B model electrochemiluminescence analyzer (Xi'an Remax Electronic Science & Technology Co., Ltd., China) with voltage of 800 V provided by photomultiplier tube (PMT). The PGSTAT 128N electrochemical workstation (Metrohm China Ltd.) was employed for electrochemical studies. All the experiments were carried out with a three-electrode system including a glassy carbon electrode (GCE, 3 mm diameter) as a working electrode, a platinum pole electrode as an auxiliary and an Ag/AgCl electrode as a reference electrode. UV–visible spectrum was obtained by an UV–visible spectrophotometer (Ls55, Perkin Elmer Instruments, America). Powder X-ray diffraction (XRD) was carried out with a X-ray diffractometer (Ultima lti Rigaku Corporation, Japan). Field emission scanning electron microscopes (SEM) (SUPPRA 55 sapphire, German Carl ZESS, German) and transmission electron microscope (TEM) (Tecnai G20, FEI, America) were applied for observing the exterior and interior morphologies of synthetic materials.

2.3. Synthesis of 3D graphene

Graphene oxide (GO) was synthesized according to the modified Hummers' method [43]. Firstly, graphite powder (3.0 g), concentrated sulfuric acid (70 mL) and sodium nitrate (1.5 g) were mixed under an ice bath under stirring. Then $KMnO_4$ (9.0 g) was slowly added and the temperature was maintained below 20 °C. Next, the reaction was transferred to water bath at 35–40 °C and maintained for 0.5 h to form a thick paste. After that, water (140 mL) was added and the temperature was increased to 98 °C, and stirred for 40 min. Successively, 70 mL water was added to terminate reaction. Then, H_2O_2 was slowly added into the mixture until the color of the solution changed from brown to yellow. The yellow GO dispersion was filtered and washed with 1 M HCl aqueous solution and water repeatedly to remove the residuals. Secondly, 3D graphene was prepared by heating homogeneous GO aqueous dispersion (2 mg mL^{-1}) in a Teflon-lined autoclave at 180 °C for 12 h [44]. In order to improve the dispersion stability of 3D graphene, Nafion solution (5 wt%) was added in the 3D graphene aqueous solution (2 mg mL^{-1}).

2.4. Synthesis of Cu_2O -MWCNTs

In a typical synthesis [45], 40 mL H_2O and 1 mL sodium citrate (0.36 M) was mixed at 32 °C under vigorous stirring. After 20 min, 1 mL $CuSO_4 \cdot 5H_2O$ (1.2 M) solution was quickly added, followed by 1 mL NaOH (4.8 M) solution and held for 5 min. Then, 1 mL ascorbic acid (1.2 M) was injected and the reaction was maintained for 30 min. The product was washed with water and alcohol three times and dried.

Appropriate amount of MWCNTs was added into the mix acids H_2SO_4/HNO_3 (3:1) and ultrasonicated for 24 h to get –COOH functional groups. Then the mixture was thoroughly rinsed with water to remove residual acids. The carboxylated MWCNTs was dried and dispersed in ethanol (1 mg mL^{-1}). Next, the Cu_2O was mixed with the MWCNTs alcoholic solution, the mixture was sonicated to a well-distributed solution. The final product was obtained after washed with deionized water and ethanol several times and centrifuged.

2.5. Synthesis of RuSi NPs

$Ru(bpy)_3^{2+}$-doped silica nanoparticles were synthesized according to the previous report [46]. In brief, TX-100 (1.77 mL), cyclohexane (7.5 mL), 1-hexanol (1.8 mL), water (340 μL) and $Ru(bpy)_3^{2+}$ aqueous solution (0.1 M, 80 μL) were mixed. In the presence of TEOS (100 μL), the reaction was initiated by adding $NH_3 \cdot H_2O$ and completed after stirring for 24 h. Acetone was used to isolate the precipitate, followed by centrifuging and washing with ethanol and water for several times. The obtained RuSi NPs were