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Synthesis of multiwall carbon nanotubes-graphene oxide-thionine-Au nanocomposites for electrochemiluminescence detection of cholesterol

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ABSTRACT

In this work, we developed a facile approach to synthesize multiwall carbon nanotubes-graphene oxide-thionine-Au (MWCNTs-GO-Thi-Au) nanocomposites with the synergistic effect of GO and Thi, resulting in reducing $AuCl_4^-$ to Au nanoparticles (AuNPs). Meanwhile, the AuNPs reduced by GO and Thi promoted the electrochemiluminescence (ECL) of luminol-H_2O_2 system and offered active sites for immobilization of numerous enzymes. Additionally, it was also found that the synergetic interactions of Thi with MWCNTs and GO was employed for enhancing ECL of luminol-H_2O_2, which was applied to develop an ECL biosensor for the first time. Using cholesterol oxidase as model enzyme, the proposed biosensor employed by MWCNTs-GO-Thi-Au nanocomposites showed a high sensitivity for cholesterol in a concentration range of 0.15-828 μM with a detection limit of 50 nM (signal-to-noise ratio of 3). In addition, the proposed cholesterol biosensor exhibited high sensitivity, good selectivity and excellent stability. Taking into account the integrated advantages of MWCNTs-GO-Thi-Au nanocomposites and ECL detection, we confidently expect that this biosensing approach would have potential applications in clinical diagnosis.

1. Introduction

Accurate and fast determination of cholesterol concentrations has attracted much attention because it is an important biomarker for the management of many diseases, such as hypertension, coronary heart disease, arteriosclerosis, cerebral thrombosis and lipid metabolism dysfunction [1,2]. Until now, several methods have been used for cholesterol measurements [3–6]. Among these analytical techniques, electrochemiluminescence (ECL) is a powerful tool that combines the simplicity of electrochemistry with the inherent sensitivity and owns a wide linear range of chemiluminescence (CL) method [7,8]. ECL is a process whereby species electrochemically generated at electrode surface undergo high-energy electron transfer (redox or enzymatic) reactions that emit light from the excited states [9]. A significant advantage of ECL over electrochemical techniques is the absence of electrical interferences. Another advantage of the ECL over conventional spectroscopic techniques is the spatial and temporal control over the reaction with concomitant improvement of the signal-to-noise ratio and the high selectivity [10]. Therefore, the high sensitivity, ease of control and simple equipment of ECL render it an attractive technique for analytical chemistry related applications.

Luminol, due to its low oxidation potential, inexpensive reagent consumption and the high emission yields, becomes one of the most popular ECL reagents. It has been widely used to fabricate luminol-based ECL biosensors to determine hydrogen peroxide (H_2O_2), especially in the field of bioanalytical application such as enzyme biosensors. In these biosensors, the enzymatic reaction between a substrate and an enzyme generates H_2O_2. As a co-reactant of luminol, the resulting H_2O_2 could enhance the ECL of luminol [11]. Hence, luminol-based ECL biosensors greatly extend the applications of enzyme biosensors. Luminol exhibits strong ECL emission in alkaline solution, but its ECL emission in neutral solution is extremely weak, hampering the application of luminol ECL in physiological conditions. Recently, much attention has been paid to extend the application of luminol-based ECL biosensors. As reported, luminol can be oxidized by H_2O_2, and the oxidized process can be efficiently catalyzed by metal nanoparticles, such as gold nanoparticles (AuNPs) and platinum nanoparticles (PtNPs) [12,13].

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With the rapid development of nanomaterials, application of nanomaterials modified electrode in ECL is becoming a commonly used method to enhance the ECL intensity and sensitize the biosensor [14–16]. For instance, carbon nanomaterials and metal nanoparticles are the most generally applied nanomaterials in ECL sensors and biosensors on account of their large specific surface area, good conductivity, promising catalytic properties [11,17]. These nanomaterials not only improve the electron transfer at the electrode interface, but also increase the surface area of the electrode to load large amounts of enzymes and achieve the high sensitivity of the ECL biosensor. For example, Wang and his coworkers constructed a sensitive ECL biosensor for choline and acetylcholine detection based on CdS-MWCNTs nanocomposites [18]. Qu's group obtained a label-free ultrasensitive ECL sensor using porphyrin-functionalized graphene [19]. An ECL immunosensor based on ABEI labeling and AuNPs amplification has been reported by Tian et al. [20]. Cao and colleagues successfully fabricated a novel ECL immunosensor for AFP detection with the merits of PtNPs [21]. However, contrast to the single component of nanomaterial, nanocomposites combine the virtues of each component and exhibit integrated properties such as excellent electrocatalytic activity, good electrical conductivity and well biocompatibility [22–24]. Recent reports have demonstrated that carbon nanoparticles and AuNPs play an important role on the ECL behavior of luminol [25–27]. Based on the mentioned above, MWCNTs-GO and AuNPs could potentially provide an excellent opportunity for ECL signal amplification.

Thionine (Thi), a small planar molecule, contains two amino groups symmetrically distributed on each side [28]. It has recently been reported that amino groups of Thi possess high binding affinity for MWCNTs and GO *via* π-π stacking, and in solution, Thi in protonated form (Thi^+) is adsorbed to GO *via* electrostatic interaction. The positively charged Thi molecules promote the ability of adsorbing $AuCl_4^-$, and $AuCl_4^-$ was reduced by some oxygen-functional groups of GO and amino groups of Thi to form AuNPs [29].

Herein, a facile approach to synthesize MWCNTs-GO-Thi-Au nanocomposites has been developed in a mild condition by reducing the $AuCl_4^-$ to AuNPs with the synergistic effect of GO and Thi, which was employed to amplify the ECL of luminol to construct a cholesterol biosensor. To the best of our knowledge, this is the first report that the synergetic interactions of Thi with MWCNTs and GO was employed for enhancing ECL of luminol, which was applied to develop an ECL cholesterol biosensor. The proposed biosensor exhibited good selectivity, high sensitivity and excellent stability. Details of the preparation, characterization, optimal conditions and possible application of biosensor electrodes were described as follows.

2. Experimental

2.1. Reagents and chemicals

Cholesterol ($C_{27}H_{46}O$, *Mr* = 386.67, ≥99% purity, from lanolin), cholesterol oxidase (ChOx, EC 1.1.3.6, ≥50 U/mg, from *Brevibacterium* sp.), gold chloride ($HAuCl_4$), Thionine (Thi), Triton X-100, uric acid (UA), glucose and luminol (5-amino-2,3-dihydro-1,4-phthalazinedione) (98%) were obtained from Sigma Chemical Co. (St. Louis, Mo, USA). Cholesterol acetate ($C_{29}H_{48}O_2$, *Mr* = 428.70, >95.0% purity) was purchased from Tokyo Chemical Industry Co. (Tokyo, Japan). Graphene oxide (GO) was purchased from Nanjing Xianfeng Nanotechology Co. (Nanjing, China). Multiwall carbon nanotubes (MWCNTs) (>95% purity) were obtained from Chengdu Organic Chemicals Co. Ltd. of the Chinese Academy of Science. Tryptophan, ascorbic acid (AA), dopamine (DA), urea, sodium nitrite ($NaNO_2$), cupric sulfate ($CuSO_4{\cdot}5H_2O$), ferric chloride ($FeCl_3{\cdot}6H_2O$), potassium chloride (KCl) and phosphoric acid (H_3PO_4) were purchased from Chemical Reagent Co. (Chongqing, China). Human serum was obtained from Ninth People's Hospital (Chongqing, China). The phosphate buffer solution (0.05 M, containing 10% Triton X-100, pH 7.4), chosen as the supporting electrolyte, was prepared with 0.05 M NaH_2PO_4, 0.05 M Na_2HPO_4 and 0.1 M KCl. Ferricyanide solutions ($[Fe(CN)_6]^{4-/3-}$, 5 mM, 0.1 M KCl) were obtained by dissolving potassium ferricyanide, potassium ferrocyanide and potassium chloride with double distilled water. The stock solution was prepared by dissolving cholesterol in Triton X-100, and diluted the cholesterol solution with the as-prepared phosphate buffer solution. All the other chemicals and solvents employed were of analytical grade and used as received from commercial sources. Double distilled water was employed throughout the experiments.

2.2. Apparatus

The ECL emission was monitored with a model MPI-E electrochemiluminescence analyzer (Xi'an Remax Electronic Science & Technology Co. Ltd., Xi'an, China). Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were carried out with a CHI 600D electrochemical workstation (Shanghai CH Instruments, China). All measurements were performed with a conventional three electrode system: the modified glassy carbon electrode (GCE, Φ = 4.0 mm) as working electrode, a platinum wire and an Ag/AgCl electrode (sat. KCl), respectively, served as the auxiliary and reference electrode. The morphological characterization of the MWCNTs-GO-Thi-Au nanocomposites and the images of the stepwise assembly processes of the biosensor were characterized by scanning electron microscopy (SEM, S-4800, Hitachi, Japan). The ultraviolet-vis (UV-vis) absorption spectra of the MWCNTs-GO-Thi-Au nanocomposites were collected on a UV-vis spectrophotometer (Shimadzu, UV-2450, Japan). All the experiments were carried out at room temperature.

2.3. Preparation of MWCNTs-GO-Thi-Au nanocomposites

The MWCNTs-GO-Thi-Au nanocomposites were synthesized according to literature [29] with slight modification. Firstly, 2 mg MWCNTs and 2 mg GO were dispersed in 2 mL double distilled water by ultrasonic agitation for 1 h. Then 5 mL Thi (0.5 mM) and 2 mL $HAuCl_4$ (1%) solutions were added to the MWCNTs-GO suspension with stirring vigorously under dark conditions for 12 h at room temperature. Then the resulting nanocomposites were collected by centrifugation at 9000 rpm for 15 min and washed with double distilled water for three times. Finally, the precipitation was re-dispersed in 5 mL water and stored in 4 t for further use. The typical synthetic process was given in Scheme 1A.

2.4. Fabrication process of the cholesterol biosensor

The schematic illustration of the fabrication process of modified electrode was described in Scheme 1B. The GCE was polished with 0.3 and 0.05 μm alumina slurry repeatedly and ultrasonically cleaned in ethanol and water thoroughly, and it was allowed to dry at room temperature. Subsequently, 8 μL MWCNTs-GO-Thi-Au nanocomposites was dropped onto the pretreated GCE and dried in air. Finally, 3 μL ChOx (1 mg/mL in 0.05 M phosphate buffer solution, pH 7.0) was cast onto the surface of the electrode for 8 h to construct a cholesterol biosensor (denoted as ChOx/MWCNTs-GO-Thi-Au/GCE). For comparison, ChOx/MWCNTs/GCE, ChOx/GO/GCE, ChOx/MWCNTs-GO/GCE and ChOx/MWCNTs-GO-Thi/GCE were prepared similarly. The modified electrodes were stored at 4 t for further use.