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Sensitive detection of superoxide anion released from living cells using silver nanoparticles and functionalized multiwalled carbon nanotube composite

Xinhe Liu, Xiuhui Liu*, Hongwei Wei, Guangjie Song, Huixia Guo, Xiaoquan Lu**

Key Laboratory of Bioelectrochemistry & Environmental Analysis of Gansu Province, College of Chemistry & Chemical Engineering, Northwest Normal University, Lanzhou 730070, China

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ABSTRACT

Considering the crucial roles of superoxide anion ($O_2^{\bullet-}$) in pathological conditions, it is of great urgency to establish a reliable approach for real-time determination of $O_2^{\bullet-}$. Herein, a sensitive non-enzymatic sensor was constructed based on silver nanoparticles (AgNPs) and sodium dodecyl sulfate functionalized carbon nanotubes (SDS-MWCNTs) composites to measure the release of $O_2^{\bullet-}$ from living cells. As an analytical and sensing platform, the AgNPs/SDS-MWCNTs modified glassy carbon electrode exhibited excellent electrochemical performance toward $O_2^{\bullet-}$ with a determination limit as low as 0.0897 nM and wide linear range of 6 orders of magnitude, which was superior to other $O_2^{\bullet-}$ electrochemical sensors. The excellent performance was attributed to the SDS-MWCNTs composites being used as effective load matrix for the deposition of AgNPs. Importantly, this novel non-enzymatic sensor could be applied to determination of $O_2^{\bullet-}$ released from living cells, and the amount of flux of $O_2^{\bullet-}$ increased accordingly with the improving of the concentration of AA, which has the possibility of application in clinical diagnostics to assess oxidative stress of living cells.

1. Introduction

Recent clinical evidence indicates that the reactive oxygen and nitrogen species (ROS/RNS) have significant physiological functions and play a critical role in redox regulation and oxidative stress, which has been connected with the pathophysiology and development of many diseases (Alzheimer's disease, autoimmune diseases and inflammatory diseases) [1–3]. Therefore, quantification of ROS is indispensable for the study and understanding of oxidative stress. Superoxide anion radical ($O_2^{\bullet-}$), a common type of reactive oxygen species (ROS), is a short-lived reactive oxygen intermediate resulting from the process of monovalent reduction of molecular oxygen. Owing to its central role as a major contributor to oxidative stress, designing an analytical strategy to assess $O_2^{\bullet-}$ dynamic release process from living cells is necessary to adequately understand the roles of the reactive species in cellular physiology [4–6].

So far, various kinds of analytical techniques for the detection of $O_2^{\bullet-}$ have been developed such as fluorometry [7,8], electron spin resonance [9], spectrophotometry [10], chromatography [11], and electrochemical methods [12–14]. Among these techniques, the electrochemical methods have received widespread attention owing to they are nondestructive, facile operation, and sensitive in comparison, and they can provide real-time data in complex biological environments [15–17]. In particular, electrochemical methods based on the catalytic reduction of $O_2^{\bullet-}$ by the enzyme (cytochrome-c (cyt-c) or superoxide dismutase (SOD)) have witnessed an increasing interest because of their high efficiency, good selectivity and sensitivity toward $O_2^{\bullet-}$. Ganesana et al. recently functionalized a gold wire with cytochrome-c (cyt-c) and measured the accumulation of superoxides in mouse brain slices in micro-molar range [18]. Another example is the work of Zhu et al., who reported the use of a porous Pt-Pd construct, decorated with SOD as a platform for amperometric measurement of $O_2^{\bullet-}$ in living Hela cells [19]. Nevertheless, the preparation of enzyme is generally time-consuming and expensive. Simultaneously, enzymes are of easy denaturation and leakage during their immobilization and storage process [20,21]. Therefore, the poor stability of enzymatic sensor remains as a problem for application. So preference is given to nonenzymatic sensor. Nonenzymatic electrode would

* Corresponding author at: No. 967 Anning East Road, Lanzhou, Gansu, 730070, China.
** Corresponding author.
E-mail addresses: liuxh@nwnu.edu.cn, liuyr1008@sina.com (X. Liu), luxq@nwnu.edu.cn (X. Lu).

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show conveniences and advantages to circumvent the defects of enzymatic sensors. Up to now, various electrode materials such as metal nanoparticles (NPs) and conducting polymers have been used for this purpose [13,22].

It is reported that metal nanoparticles (NPs), especially noble metals (AgNPs, PdNPs, PtNPs, AuNPs etc.), have large surface areas, special binding sites [23–25], and accelerate electron-transfer which could substitute for enzymes to catalyze the $O_2^{\bullet-}$. Particularly, metallic silver nanoparticles (AgNPs) exhibit superior catalytic activity in all kinds of applications [23,26]. Recent studies found that the catalytic property of the sensor relies principally on the size, structure and dispersion degree of AgNPs on the electrode [27]. Hence, the matrix for the preparation of highly dispersed AgNPs is extremely significant.

Since being discovered in 1991, carbon nanotubes (CNTs) have been largely used for electrochemical sensing because of their outstanding electrical, mechanical and chemical properties [28]. Increasingly researchers focused on employing CNTs as the templates for supporting metal nanoparticle catalysts. However, a major obstacle to such efforts is that CNTs aggregate into bundles due to highly attractive van der Waals forces, which result in a poor dispersion in aqueous solutions [29]. Therefore, several alternatives have been proposed to get homogenous dispersion of CNTs, and they have been used in CNTs-modified electrodes. Among them, the non-covalent functionalization is particularly attractive because of the possibility of adsorbing various groups onto the CNTs surface without disturbing the π system of the tube lattice [30].

In this study, we utilize sodium dodecyl sulfate (SDS) functionalized MWCNTs to disperse MWCNTs effectively. SDS disperse CNTs in aqueous solutions mainly through hydrophobic/hydrophilic interactions, in which the hydrophobic tail of a SDS surfactant molecule adsorbs on the surface of CNT bundles while its hydrophilic head associates with water for dissolution. In addition, the adsorbed SDS molecules on the surface of CNTs could act as anchoring sites for metal nanoparticles [31]. Thus, these results indicated that SDS-functionalized CNTs are expected to create more opportunity for applications in sensors. As expected, AgNPs/SDS-MWCNTs/GCE sensor exhibited remarkable analytical performance towards $O_2^{\bullet-}$ with good reproducibility, wide linear range and low detection limit. Importantly, the constructed sensor could be applied to determination of $O_2^{\bullet-}$ released from living cells, which has the possibility of application in clinical diagnostics to assess oxidative stress of living cells.

2. Experimental section

2.1. Apparatus

The surface morphology of AgNPs/SDS-MWCNTs composite was characterized by Zeiss scanning electron microscopy (SEM, Zeiss, Oberkochen, Germany). All electrochemical measurements and characterization were performed on a CHI660C electrochemical workstation (Austin, TX, USA) using a three-electrode system with a glassy carbon electrode (GCE, 3 mm in diameter) or modified GCE as the working electrode, a saturated calomel electrode (SCE) and a platinum electrode as the reference and counter electrode, respectively. All potentials given in this paper were referred to the SCE. Electrochemical impedance spectroscopy (EIS) experiments were performed on Multi-potentiostat (VMP2, Princeton Applied Research, USA). Ultraviolet-Visible spectroscope (UV–vis, EVOLUTION 220, Thermo Scientific) was used to detect the concentration of $O_2^{\bullet-}$ obtained from the KO_2 stock solution. Before each electrochemical measurement, solutions were thoroughly deoxygenated by bubbling nitrogen through the solution for at least 20 min to remove dissolved oxygen.

2.2. Chemicals and reagents

The multi-walled carbon nanotubes (MWCNTs) used (diameter: 20–40 nm, length: 1–2 μm, purity: ≥95%) came from Shenzhen Nanotech Port Co. Ltd. (Shenzhen, China). Before use, MWCNTs were purified according to the reported literature with slight modification [32]. KO_2 was obtained from Aladdin Industrial Inc. DMSO was purchased from Beijing Chemical Works (Beijing, China). 18-crown-6 was bought from Energy Chemical (Shanghai Chemical Industries, Ltd.). 4 Å molecular sieve was obtained from Tianjin Kermel Chemical Industries, Ltd (Tianjin, China). Superoxide dismutase (SOD) was bought from Sigma (USA). Sodium dodecyl sulfate (SDS, $C_{12}H_{25}SO_4Na$) from Tianjin Chemical Reagent (Tianjin, China). $AgNO_3$ and KNO_3 were purchased from Xi'an Chemical Reagent (Xi'an, China). Phosphate buffer solution (PBS, pH 7.0) was prepared by mixing suitable amounts of 0.2 M NaH_2PO_4/Na_2HPO_4. A physiological PBS solution containing KH_2PO_4 (1.76 mM), Na_2HPO_4 (10.14 mM), NaCl (136.75 mM), and KCl (2.28 mM), was mainly used for washing of PC12 cells and observing the release of $O_2^{\bullet-}$ from the cells. Other chemicals were all of analytical grade, and ultrapure water (18.25 MΩ cm) was used during all experiments.

2.3. Fabrication of SDS-MWCNTs nanocomposite

The synthesis of SDS-MWCNTs was performed by previously reported method [33]. In brief, one hundred milligram of MWCNTs were suspended in 400 mL of 0.25 M sodium dodecyl sulfate (SDS) aqueous solution, and then was ultrasonicated for 1 h and stirred for 24 h. After that, SDS-MWCNTs was filtered by nylon membrane with 0.22 μm pores, thoroughly washed with water to remove the free SDS in the solution, followed by drying in a vacuum oven at 60 °C for 24 h. So, SDS functionalized MWCNTs were obtained. The structure and composition of the SDS-MWCNTs have been verified by FT-IR as shown in Fig. S1.

2.4. Preparation of the $O_2^{\bullet-}$ sensor

Prior to the modification, the glassy carbon electrode (GCE) was polished with 1.0, 0.3 and 0.05 μm alumina slurry to a mirror-like, respectively, followed by rinsing thoroughly with ultrapure water. Then 8 μL of SDS-MWCNTs aqueous solution (0.3 mg mL^{-1}) was dropped on the surface of a GCE and dried in air, and then the SDS-MWCNTs/GCE was obtained. Next, the electrode was electrodeposited in the solution containing 1.0 mM $AgNO_3$ and 0.1 M KNO_3 for 240 s at 0 V to obtain AgNPs/SDS-MWCNTs modified electrode.

2.5. Generation of superoxide anion

The chemical generation of $O_2^{\bullet-}$ was performed by dissolving KO_2 in dimethyl sulphoxide (DMSO) solution (containing 18-crown-6), and stored together with 4 Å molecular sieve. The chemical of 18-crown-6 can bind K^+ of KO_2 and increase the solubility of KO_2 in DMSO [34]. After sonicating the solution for 5 min, additional 4 Å molecular sieve was added to remove traces of H_2O. According to the molar absorptivity of $O_2^{\bullet-}$ in DMSO (2006 M^{-1} cm^{-1} at 271 nm), the concentration of $O_2^{\bullet-}$ could be estimated by a UV–vis spectroscope [35].

2.6. Cell culture

PC12 (rat adrenal medulla pheochromocytoma) cells were obtained from Institute of Hematology, Chinese Academy of Medical Sciences. They were maintained in DMEM (high glucose, Gibco) medium supplemented with 10% heat-inactivated fetal calf serum (Evergreen, China), 100U/mL penicillin (Sigma, USA), and