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Photoelectrochemical platform for cancer cell glutathione detection based on polyaniline and nanoMoS$_2$ composites modified gold electrode

Xu Hun*, Yanxin Li, Shiyu Wang, Yao Li, Jikuan Zhao, Hui Zhang, Xiliang Luo

Key Laboratory of Sensor Analysis of Tumor Marker, Ministry of Education; State Key Laboratory Base of Eco-chemical Engineering; Key Laboratory of Rubber-Plastics of Ministry of Education/Shandong Provincial Key Laboratory of Rubber Plastics; Key Laboratory of Biochemical Analysis, Shandong Province; College of Chemistry and Molecular Engineering, Qingdao University of Science and Technology, Qingdao 266042, China

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ABSTRACT

Herein, a visible light photoelectrochemical (PEC) platform based on polyaniline (PANI) and nanoMoS$_2$ composites as optoelectronic material for glutathione detection without any auxiliary of biomolecules or labeled materials was developed. Firstly, the nanoMoS$_2$ was prepared via a simple ultrasound exfoliation method. The PANI was synthesized by chemical oxidative polymerization method. Then composite of PANI and nanoMoS$_2$ was used to modify gold electrode. It was found that the composite membrane showed excellent PEC properties. And glutathione enhanced the PEC signal greatly. Based on this finding a method for glutathione detection was fabricated. Under the optimum conditions, the linear response of glutathione concentrations ranged from 1.0×10^{-10} to 1.0×10^{-4} mol L^{-1} was obtained with a detection limit of 3.1×10^{-11} mol L^{-1}. The relative standard deviation was 2.9% at 2.0×10^{-9} M (n = 10). This method showed high sensitivity and simpleness which opened up a new promising signal-on PEC platform for future bioassay.

1. Introduction

Glutathione (GSH, L-γ-glutamyl-L-cysteinyl-glycine) is a critically important thiolated tripeptide and endogenous antioxidant, which exists broadly in the intracellular environment (Deng et al., 2011; Gutscher et al., 2008; Huang et al., 2013). It plays a pivotal role in human physiology such as xenobiotic metabolism, protection against oxidative stress, endogenous toxic metabolite detoxification, enzyme activity and sulphur or nitrogen metabolism. Therefore, an abnormal level of GSH is associated with many diseases directly, including cancer, Alzheimer's disease, and cardiovascular disease. So, detecting GSH has attracted a great deal of attention. Currently, a number of analytical techniques have been developed to analyze the GSH level, including luminescence analysis (Yang et al., 2013), fluorometry (Niu et al., 2012), colorimetry (Xie and Xie, 2014), electrochemistry (Wang et al., 2009), magnetic resonance spectroscopy (Mandal et al., 2012), and surface enhanced Raman scattering (SERS) (Huang et al., 2009a, 2009b). However, these methods showed several drawbacks, for instance, low sensitivity, low selectivity, long detection time, laborious and costly procedures.

PEC on semiconductor electrodes is a promising strategy, which has been wildly applied in bioanalytical chemistry owing to its low-cost, high sensitivity, rapid detection and low back-ground current (Okoth et al., 2016; Zhao et al., 2015). The mechanism of PEC is based on the oxidative capacity of photogenerated hole or reductive property of photoelectron and the PEC active materials' photogenerated electron transfer between the analyte and a semiconductor electrode (Gao et al., 2015; Goswami et al., 2013; Ou et al., 2014). The performance of PEC sensing is largely depended on the optoelectronic active materials. Recently, a series of PEC active materials have been investigated such as organic dyes (Niu et al., 2012) and inorganic semiconductor materials (Chen et al., 2014). Specially, as a 2D layered structure analogous of graphene, MoS$_2$ has aroused increasing academic interest due to its extraordinary properties such as unique optical properties, superior electrical performance and robust mechanical properties and a considerable effort have been developed to design a detection platform based on MoS$_2$. For instance, Wang et al. reported a method for a sensitive nucleic acid sequence detecting platform with MoS$_2$ nanosheet modified electrode which demonstrated that MoS$_2$ nanosheet is biocompatible and suitable for DNA analysis in electrochemical measurements (Wang et al., 2015). Zang et al. used MoS$_2$ to fabricate a series of sensors for NO, dopamine and glucose detection (Li et al., 2012; Zang et al., 2016).

PANI is an important polymer due to its facile synthesis, low cost, excellent high electrical conductivity, which has potential applications in light weight batteries, microelectronics, electrochemical sensors, and

* Corresponding author.
E-mail address: hunxu@sohu.com (X. Hun).

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optical display (Liu et al., 1998; Reuven et al., 2012; Varela-Alvarez et al., 2005; Ansari et al., 2016). Recently, some researches about self-assembly of biofunctional polymer on graphene nanoribbons have developed (Reuven et al., 2012). As a graphene analogue constructed material, one expects that MoS_2 was allowed and properly synthesized, composites with PANI could make those of each component materials possess superior properties. Some researches focusing on the composites of MoS_2 and PANI have been done recently, such as supercapacitor electrode material (Ansari et al., 2017), and the composites of MoS_2 and PANI showed good photoelectric properties but the composites applied to PEC sensors were rarely reported. Herein, we developed a PEC method for analysis of glutathione based on MoS_2 nanosheet and PANI composites with high sensitivity. To the best of our knowledge, there is the first research based on nanoMoS_2 and PANI composites for intracellular glutathione detection with PEC detection system.

2. Experimental

2.1. Apparatus and reagents

The PEC measurements were performed with a homemade detection system (Wang et al., 2018). A 10 W LED lamp was used as irradiation source. All experiments were carried out on a CHI 830D electrochemical working station using a conventional three-electrode system at room temperature. A gold electrode or modified gold electrode was used as the working electrode, calomel electrode as reference electrode and a platinum wire as auxiliary electrode. The used bulk MoS_2 or PANI/nanoMoS_2 was characterized by scanning electron microscopy (SEM, JSM-6700Fmachine, JEOL, Tokyo, Japan). Raman spectra were obtained with a Raman spectrometer (In-Via-Reflex, Renishaw, England).

The bulk MoS_2 was purchased from Sigma-Aldrich Co., LLC (USA). N,N-Dimethylformamide (DMF) was provided by Sangon Biotech Co., Ltd. (Shanghai, China). Glutathione (GSH), ammonium peroxydisulfate and aniline were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Metallothionein (MT) was purchased from Hunan Lugu Biotechnology Co. Ltd. (China). Glutathione reductase (GTR) was purchased from Shanghai Sangon Biological Engineering Technology & Services Co. Ltd. (China). All chemicals were of analytical reagent grade. All aqueous solutions were prepared with ultrapure water from an Aquapro ultrapure water system (Ever Young Enterprises Development. Co. Ltd., Chongqing, China). Phosphate buffer solution (PBS) was prepared by mixing the stock solutions of 0.2 mol L^{-1} NaH_2PO_4 and 0.2 mol L^{-1} Na_2HPO_4 containing 0.1 mol L^{-1} KCl.

2.2. Pretreatment of nanoMoS_2

The nanoMoS_2 was fabricated via a simple ultrasound exfoliation method. Detailed procedure was as follows: 10 mg of bulk MoS_2 was transferred into 20 mL DMF and disposed with constant stirring for 5 min. Followed by ultra-sonication for 4 h, the homogenous suspension of nanoMoS_2 was formed. The suspension was centrifuged at 3000 r min^{-1} for 10 min. The supernatant was then centrifuged at 12,000 r min^{-1} for 10 min. At last the precipitation was collected and dispersed in a certain amount of DMF. And the obtained nanoMoS_2 solution was kept at 4 °C for further using.

2.3. Polyaniline synthesis and dispersion preparation

The synthesis is based on a previously reported method (Deshpande et al., 2009). Twenty five mmol ammonium peroxydisulfate was dissolved in 50 mL of 18.2 MΩ deionized water. Twenty mmol aniline was dissolved in 50 mL of 1 M HCl aqueous solution. Both solutions were stirred for 1 h at room temperature. Then, the ammonium peroxydisulfate solution was added slowly to the aniline solution. The mixture was stirred for 1 h at 5 °C. The precipitate, green polyaniline, was collected by filtration and washed repeatedly using 1 M HCl until the filtrate became colorless. The washed precipitate was dried under vacuum for 48 h. The prepared polyaniline was kept at 4 °C for further using.

2.4. Cell culture

MCF-7 and MDAMB- 231 as cancer cell, TPH-1 and HBE as normal cell were separately cultured in cell flasks according to the instructions from the American Type Culture Collection. MDAMB-231 and HBE were cultured in Dulbecco's Modified Eagle Medium. MCF-7 and TPH-1 were cultured in RPMI 1640 medium. Both the mediums were supplemented with 10% fatal bovine serum and 1% penicillin and streptomycin. To obtain the cell lysates, cells were detached from the dish using 0.25% trypsin. Then the cells were washed once with PBS. To obtain the cellular content and to remove the proteins, cell sedimentation volume 3 times of protein-removing reagent was added to the cell precipitate, followed by repeated freezing (liquid nitrogen) and thawing (37 °C water bath) twice. The solution was placed in ice bath for 5 min, followed by a centrifugation (10,000*g*, 10 min) to remove the cell debris. The supernatant was collected and transferred to a new tube for the GSH assay.

2.5. Detection of GSH in human serum samples

The human serum samples were collected from Qingdao Center Medical Hospital (Qingdao, China). The serum samples were pretreated according to the method reported before (Jia et al., 2015). The collected samples were diluted with PBS and then spiked with different concentrations of GSH. The concentrations of GSH in human serum samples were determined by the assembled PEC platform.

2.6. Preparation of the modified electrodes and PEC measurements

Before modification, the gold electrode was polished with 1, 0.3, 0.05 μm alumina slurries sequentially and washed ultrasonically with deionized and doubly distilled water. Then it was electrochemically cleaned in 0.5 M H_2SO_4 solution by cyclic potential scanning between 0.3 and 1.5 V until a standard CV was obtained. Subsequently, the gold electrode was rinsed with deionized and doubly distilled water and absolute ethanol in turn. After dried with nitrogen gas, 10.0 μL of composite of nanoMoS_2/polyaniline solution was dripped onto the fresh gold electrode surface and dried naturally in the air. The obtained electrode was denoted as PANI/nanoMoS_2/GE. The PEC activity of the PANI/nanoMoS_2/GE was investigated by i-t curves in 0.1 M PBS containing glutathione which was served as a sacrificial electron donor during the photocurrent measurement irradiated with 10 W white LED light. The light was switched on and off every 10 s, and the applied potential was − 0.1 to 0.5 V (vs SCE).

3. Result and discussion

3.1. Fabrication of glutathione biosensor and detection process

A PEC platform for GSH determination with high sensitivity was designed based on PANI/nanoMoS_2 composites modified gold electrode, as illustrates in Scheme 1. In this paper, the MoS_2 nanosheets were fabricated via a simple ultrasound exfoliation method. After ultrasonication, the bulk MoS_2 was reduced to nanoMoS_2, and the change of band gap of MoS_2 occurs as one of the most interesting distinguished features (Wang et al., 2013). The transformation from indirect to direct and the increase of the band gap of MoS_2 nanosheets greatly promote their applications in PEC platform (Cao et al., 2012; Karunadasa et al., 2012). Polyaniline is one of a crucial polymer. One expected that MoS_2 was composited with PANI which could possess superior properties to those of each component material. In order to analyze the potential