



Study on glutathione's inhibition to dopamine polymerization and its application in dopamine determination in alkaline environment based on silver selenide/molybdenum selenide/glassy carbon electrode



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ABSTRACT

Cellular glutathione plays critical roles in protecting neuronal cells against dopamine induced oxidative stress and electrophilic cellular damage; inspired by above mentioned properties of glutathione, we used glutathione as a polymerization inhibitor to develop a dopamine biosensor which can be used in alkaline environment. The detection of dopamine in alkaline environment has been challenged by its polymerization for many years, glutathione as a kind of thiol can be used to prevent the polymerization of dopamine in alkaline environment by eliminating the electrophilic quinone molecules needed during the polymerization. In order to enhance the electrochemical performance of electrode, Ag₂Se/MoSe₂ composite with large exposed surface areas was synthesized, which improved the sensitivity and stability for dopamine detection. The low oxidation peak potential for dopamine, 0.04 V vs. SCE, indicates that glutathione can realize the electrooxidation of dopamine at lower potential in alkaline environment. In this study, by introducing glutathione to inhibit the dopamine polymerization, sensitive and stable detection of dopamine can be achieved at pH 8.5 based on the Ag₂Se/MoSe₂/GCE.

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1. Introduction

Parkinson's disease caused by selective loss of dopaminergic neurons in the substantia nigra results in the reduction of the dopamine (DA) levels in the striatum [1–3]; it is characterized by the motor deficits including the resting tremor, bradykinesia, rigidity, and impairment of postural reflexes [4–6]. Among various pathological signs of Parkinson's disease, oxidative stress is one of the major factors which could exacerbate the patient's condition [7,8]. Previous reports indicate that one of the reasons to produce oxidative stress is the imbalance of DA metabolism, which results in the proliferated production of reactive oxygen species and the consequent oxidative/electrophilic stress, as well as mitochondrial dysfunction, leading to the mitochondrial dysfunction [9–12]. To minimize the oxidative/electrophilic damage, cells have evolved lots of antioxidants. For example, the cellular glutathione (GSH) is demonstrated to play critical roles in protecting neuronal cells against DA induced oxidative stress and electrophilic cellular damage [13,14]. GSH can react with electrophilic quinone molecules and reactive oxygen species to minimize DA induced neurocy-

tototoxicity, GSH alteration precede loss of dopaminergic neurons as well as accumulation of "Lewy bodies" in Parkinson's disease patients, and it is directly related to severity of Parkinson's disease [15]. In fact, alterations of cellular GSH in substantia nigra have been described as biochemical characteristics of Parkinson's disease, the levels of total as well as reduction of GSH are significantly depleted in the substantia nigra pars compacta of Parkinson's disease patients [16,17]. The reactive oxygen species/free radicals which can lead to the mitochondrial dysfunction and exacerbate the patient's condition is resulting from DA oxidation or enzyme degradation metabolism, and which can be eliminated by GSH. Based on above medical research, an idea is sprouted; the special property of GSH mentioned here can be used to prevent the polymerization of DA in alkaline environment. If this favorable anticipation can be realized, the challenge, detection of DA in alkaline environment, which has puzzled researchers for years, will be solved perfectly.

To date, the DA determination has been carried out using conventional analytical techniques including surface-enhanced raman scattering [18,19], fluorescence [20], self-powered triboelectric nanosensor [21], NMR relaxation method [22], colorimetric sensor [23], capillary electrophoresis [24], and spectrophotometry [25]. Electrochemical methods have appeared to be suitable and more often employed in the clinical analysis to determine the concen-

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tration of DA owing to easy operation, cost effectiveness, and it provides enough sensitivity to real time monitoring of the analytes [26–28]. Nonetheless, electrochemical observation of DA under physiological conditions is a challenging issue because its presence in the biological fluids is extremely low compared to ascorbic acid (AA) and uric acid (UA) and their oxidation potentials are extremely close in physiological media (pH 7.4). And worse still, the oxidized form of DA can accelerate the oxidation of AA resulting in the poor selectivity of biosensors [29–33]. Hence, the elimination of the interference caused by these species is very crucial as the overlapped voltammetric signal can result in the fouling effects and poor selectivity of electrode. To overcome the influence of these factors, some researchers try to determine DA under alkaline environment; however, the problem is the polymerization of DA in alkaline environment [34–36]. The DA polymerization in alkaline environment can cause lower sensitivity and stability for DA detection. Therefore, there are some works focused on transition-metal dichalcogenides. Layered transition-metal dichalcogenides are very fascinating in electrocatalysis and biosensing in terms of good electronic and electrochemical properties [37]. Material with special morphology has shortened path lengths for ions diffusion and large exposed surface areas, which play important roles in enhancing the electrochemical performance [38]. Among the many transition-metal dichalcogenides, metal selenide might be a good choice for electrochemical application [39]. Reports show that MoSe₂ has higher intrinsic electrical conductivity than MoS₂ due to the more metallic nature of Se [40,41]. In recent years, the application of MoSe₂ in electrochemical sensing is very popular. For example, Huang et al. successfully synthesized aptamer/Au nanoparticles/cobalt sulfide nanosheets biosensor for 17 β -estradiol detection using a guanine-rich complementary DNA sequence for signal amplification [42].

In this work, GSH was used as the polymerization inhibitor which can effectively prevent the polymerization of DA in alkaline environment. In addition, the oxidation potential of DA linearly shifted in negative direction with increasing pH of the solution; the oxidation potential is closely related to the energy needed to oxidize the target analyte, lower oxidation potential means lower energy requirement. So increasing pH of the solution also can be considered as a way to reduce the energy requirement during the precise monitoring of DA in alkaline environment. Despite the performances of above mentioned polymerization inhibitor, improving the electrocatalytic properties of substrates for highly sensitive and target selective sensing is still considered as a challenge for bioanalytical research and medical diagnosis. In this study, hollow spherical Ag₂Se/MoSe₂ selected as the modifier because its excellent electrochemical selectivity and stability, the electrochemical property of different electrode toward DA was studied as Fig. S1. Based on the polymerization inhibitor GSH and electrode modifier Ag₂Se/MoSe₂, the highly sensitive and selective electrochemical detection for DA is achieved.

2. Experiment part

2.1. Chemicals

Hydrazine hydrate, potassium ferricyanide (K₃Fe(CN)₆), and potassium ferrocyanide (K₄Fe(CN)₆) were purchased from Shanghai Chemical Reagent Co. Ltd. Ammonium molybdate tetrahydrate ((NH₄)₆Mo₇O₂₄·4H₂O, analytically pure) and selenium powder (Se) were purchased from Aladdin. AgNO₃, DA, AA and UA were obtained from Alfa Aesar. GSH was purchased from Sigma-Aldrich, Ltd. The phosphate buffer solution (0.2 M) was prepared from Na₂HPO₄ and NaH₂PO₄, the pH was adjusted regularly with NaOH. Ultrapure water (resistivity of 18.25 MV cm) was used throughout the work.

2.2. Preparation of Ag₂Se/MoSe₂

The precursor MoO₃ and MoSe₂ were prepared under a conventional hydrothermal reaction as implemented in Supporting information. The synthesis process of Ag₂Se/MoSe₂ was described as follows: 5 mL of 0.05 M AgNO₃ solution was added to the prepared MoSe₂ aqueous dispersion (0.05 g/10 mL); then, 11 mL 0.05 M AA was gradually added with stirring. The products were separated from the solution by centrifugation, and the un-reacted species were removed by multiple washing steps with ultrapure water.

2.3. Characterization

The morphology of the material was investigated by scanning electron microscopy (SEM, J4800 Japan) and transmission electron microscopy (TEM, Tecnai G2 F30, FEI, USA). The phase structures of the samples were measured by X-ray diffraction (XRD, Rigaku D/max-2400, Cu K radiation). The chemical composition and valence state of these samples was determined by X-ray photoelectron spectroscopy (XPS, ESCALAB250xi X-Ray Monochromatization 200 W Spot/power: Mono 650 μ m). Raman was carried out using a HORIBA Jobin Yvon LabRAM HR 800. The electrochemical experiments were carried out in an electrochemical cell with a three-electrode configuration with a CHI660C electrochemical workstation (CHI, USA). Material modified glassy carbon electrode (GCE) as the working electrode, the Pt wire and saturated calomel electrode (SCE) served as the counter electrode and reference electrode, respectively.

2.4. Research method for polymerization inhibition of GSH

The inhibition performance of GSH on DA polymerization was researched using Ag₂Se/MoSe₂/GCE in alkaline environment. The electrochemical cyclic voltammetry (CV) and electrochemical impedance spectra (EIS) were employed to study the changes in the electrochemical properties of electrode surface before and after the presence of GSH. The SEM was employed to study the changes in the morphology of electrode surface before and after the presence of GSH. Raman measurements were carried out to confirm the generation of polydopamine and the inhibition property of GSH on DA polymerization in alkaline environment.

3. Results and discussion

3.1. Structure characterization of Ag₂Se/MoSe₂

As shown in Fig. 1a, the regular spherical structure of this prepared Ag₂Se/MoSe₂ can be observed, the radius of these spheres is about 250 nm. The TEM image shown in Fig. 1b displays that there are some particles with a radius of 15 nm attach onto the sphere surface. As shown in Fig. 1c, the high resolution transmission electron microscopy (HRTEM) image of the sphere surface displays two sets of crystal lattice fringes with spacing of 0.33 and 0.27 nm, corresponding to the in-plane lattice parameters of the MoSe₂ monolayer and the interplanar distances of (200) planes, respectively [43]. The HRTEM image of the nanoparticles attached on sphere surface shows a well-defined crystal structure with the lengths of the lattice spacing is about 0.31 and 0.26 nm, corresponding to the (121) planes of orthorhombic Ag₂Se [44]. Fig. 1d and e show the fast fourier transform (FFT) pattern of the selected areas marked by frame A and B, respectively. Fig. 1f displays the XRD pattern of Ag₂Se/MoSe₂. The diffraction peak marked with diamond corresponding to the (110) reflections of MoSe₂ [45], and the diffraction peak (120), (121) and (201) marked with asterisks originating from the orthorhombic Ag₂Se phase [46].

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