Short Communication

A Novel Hydrogen Peroxide Amperometric Sensor based on Thionin Incorporated onto a Mo$_6$S$_9$-xI$_x$ Nanowire Modified Glassy Carbon Electrode

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Abstract

A simple and enzymeless amperometric sensor for the detection of H$_2$O$_2$ based on a new kind of nanomaterial Mo$_6$S$_9$-xI$_x$ (MoSI) nanowires (NWs) was developed. The construction of the sensing platform was based on strong electrostatic interactions between negatively charged MoSI NWs and positively charged thionin molecules. MoSI NWs act as not only a good substrate for the immobilization of redox mediator thionin, but also a promoter for electrocatalysis of H$_2$O$_2$. The fabricated sensor showed a wide linear range over the concentration of H$_2$O$_2$ from 5 $\mu$M to 2.8 mM with a measurable lowest detection of 0.8 $\mu$M; furthermore, it exhibited good stability and reproducibility.

Keywords: Hydrogen peroxide, MoSI nanowires, Thionin, Electrocatalysis, Amperometric sensors, Sensors

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The immobilization of different electron transfer mediators on the surface of various electrode materials has been attracting increasing interest for applications of chemically modified electrodes in the fields of electrocatalysis and electroanalysis. Appearance of nanomaterials may provide a good chance for developing simple and efficient methods to fabricate modified electrodes due to their peculiar properties [1], and various nanomaterials have been used to incorporate electron transfer mediators for constructing electrochemical biosensors, such as carbon nanotubes (CNTs) [2 – 3], carbon nanofiber [4], nanogold [5 – 7] and nano-TiO$_2$ [8] etc.

Electroactive thionin (Th) has been widely utilized as an electron transfer mediator in fabrication of electrochemical biosensors, especially acting as a good mediator for sensing detection of H$_2$O$_2$ [8 – 18] because of the importance of H$_2$O$_2$ in various fields such as food, clinic, biochemistry, environment and industry [19 – 23]. Various approaches and strategies such as adsorption [9 – 11], covalent bonding [12, 14, 17], sol–gel technique [15 – 16] and electropolymerization [24] etc. have been used for the immobilization of Th on various electrode materials. These systems were usually divided into two types. One was based on the co-immobilization of horseradish peroxidase and Th on various electrode materials by covalent linking methods [11, 13 – 14, 17 – 18]. Most of these systems were complicated and time-consuming, involving multistep synthetic procedures; furthermore, the activity of enzymes was easily affected by temperature, pH, humidity and toxic chemicals. The other was based on enzymeless immobilization, and most of them were involved in CNTs, which were used as an immobilization substance due to their high electrical conductivity, high chemical stability, extremely high mechanical strength and large surface area [8 – 10, 12]. Unfortunately, these systems usually could not avoid complex organic synthesis work in order to make Th be immobilized stably on the electrodes. Consequently, to explore and develop a simple, time-saving and reliable strategy using new nanomaterials for the enzymeless immobilization of Th arouses our research interest.

Molybdenum-chalcogenide-halide nanowires (NWs) which are composed of molybdenum (Mo), sulfur (S) and iodine (I) in the form of Mo$_6$S$_9$-xI$_x$ (MoSI) are a new class of quasi-one-dimensional objects, having two different stochiometries Mo$_6$S$_9$I$_6$ and Mo$_6$S$_{10}$I$_6$ [25 – 26]. An identical skeletal structure is composed of indistinguishable one-dimensional polymer chains of molybdenum-sulfur-iodine clusters, strongly joined together by anions (either S or I). The individual nanowire is joined together into bundles by weak Van der Waals forces. The materials have strong anisotropy, large Young moduli along the wires, very small shear moduli, and controllable electronic properties. Compared with CNTs, MoSI NWs have some significant advantages such as straightforward synthesis, monodisperse...
diameters and metallic properties [27–30]. They have demonstrated their potential to become unique building elements in biosensors and electrocatalysis. Mihailovic et al. reported that the S atoms at the ends of MoSI NWs are capable of forming covalent bonds with a gold surface or with thiolated proteins [30]. We showed that bioassembled nanocircuits of MoSI NWs and anti-estrone antibody on glassy carbon electrodes provide an amplification and conductive pathway for the specific electrochemical sensing of estrone hapten [31].

In order to further explore the potential application of MoSI NWs as an immobilization substance for compositing redox mediators on the electrodes, herein, a simple, time-saving and effective strategy was used to immobilize redox mediator Th on a MoSI NWs modified GCE (Th-NWs/GCE) based on strong electrostatic interactions between negatively charged MoSI NWs and positively charged Th molecules, and electrocatalysis of H₂O₂ on the Th-NWs/GCE was also investigated. The resulting Th-NWs/GCE exhibited excellent electrocatalytic activity towards the reduction of H₂O₂ and showed excellent analytical performance for amperometric determination of H₂O₂.

Figure 1 shows the cyclic voltammograms of different electrodes in a 0.1 M pH 6.70 phosphate buffer solution at a scan rate of 0.1 V/s. None redox peak could be observed on a bare GCE (curve A) and NWs/GCE (curve B) in the potential range from 0.20 to −0.60 V, whereas, a pair of well-defined redox peaks was observed on a Th-NWs/GCE. The anodic and cathodic peak potentials were −0.181 V and −0.251 V respectively (curve C). Obviously, the response of the Th-NWs/GCE was attributed to two electron transfer reduction of the immobilized thionin on the basis of reported literatures [8, 10, 14]. The effect of pH value on the response of the Th-NWs/GCE electrode was studied between 4.76 and 8.05 in 0.1 M PBS. The results showed that the voltammetric responses increased from pH 4.76 to 6.70 and decreased from pH 6.70 to 8.05, and the best peak shape and the maximum current response can be obtained at pH 6.70. Thus, pH 6.70 was selected for the subsequent experiments. The peak potential shifted negatively as pH increased. The relationship between the cathodic peak potential and pH was also investigated, and a linear regression equation – \( E_{pc} = 0.213 - 0.0691 \times \text{pH} \) \( n = 5, R^2 = 0.994 \) was obtained, which indicates that the uptake of electrons is accompanied by an equal number of protons. This is in accordance with previous literatures [8–10]. The electrode reaction mechanism is as follows:

Furthermore, both the anodic and cathodic peak currents increased linearly with increasing scan rate in the range from 0.1 to 1.3 V/s (Fig. 1), displaying characteristics of surface-confined waves. These suggest that Th has been successfully incorporated on the MoSI NWs modified GCE. Raman spectra also confirm it.

Fig. 2 shows Raman spectra of a MoSI NWs modified ITO substrate, an ITO substrate coated with MoSI NWs dipped in the 0.5 mM Th solution of ethanol overnight as well as the bulk Th sample. It can be seen that the ITO substrate modified with MoSI NWs dipped in the Th solution and the Th powder sample have almost identical Raman signatures.

![Scheme 1. Electrode reaction mechanism.](image-url)
which indicates that Th has been incorporated onto the MoSI modified ITO substrate. Furthermore, characteristic peaks of thionin are mainly based on the strongly electrostatic interaction between negatively charged MoSI NWs and positively charged Th as well as reduced state of Th [8].

Stability of the Th-NWs/GCE was tested, more than 50 complete CV cycles were performed at a scan rate of 0.1 V/s, and the experimental results showed that redox peak currents decreased a little in the first 3 scans while the peak currents remained at 90% of the initial values after 50 cycles. In contrast, Th can be adsorbed on the surface of the GCE after the bare GCE was immersed in 0.5 mM thionin solution for a period of time, and one pair of redox peaks corresponding to the reduction of thionin can also be observed. Nevertheless, voltammetric responses disappeared rapidly after several cyclic scans due to dissolution of adsorbed thionin into the solution. Moreover, a series of six Th-NWs/GCEs prepared in the same manner and immersed in 0.1 M pH 6.70 PBS, with a scan rate of 0.1 V/s, gave average cathodic peak currents of 3.2 μA with a RSD of 6.5% and anodic peak currents of 2.1 μA with a RSD of 5.5%. These results suggest that the Th-NWs/GCE has good reproducibility and stability.

To address the analytical applicability of the Th-NWs/GCE, electrocatalytic activity of the modified electrode towards the reduction of hydrogen peroxide was investigated. The cyclic voltammograms of Th-NWs/GCE in the absence and presence of H₂O₂ are shown in Figure 3 (curves b and d). The voltammograms obtained upon addition of H₂O₂ showed an increase in the cathodic peak current and a decrease in the anodic peak current, with no change in the peak potentials of the two electrode processes, which indicated a typical electrocatalytic reduction of hydrogen peroxide. In contrast, the reduction of H₂O₂ did not show a clearly defined wave at the bare GCE (Fig. 3 curve a) and NWs/GCE (Fig. 3 curve c) under the same experimental conditions, whereas the reduction of H₂O₂ at the NWs/GCE occurred at much more positive potentials than that for the bare GCE, which suggest that MoSI NWs seem to act as an electron-transfer promoter. Having a catalytically active surface and very high aspect ratio (length over diameter), MoSI NWs can increase the surface area of the electrode, so the background voltammetric response for the NWs-coated surface was larger than that for the bare surface (Fig. 1 curves A and B). Moreover, the voltammetric response of Th at the NWs/GCE was larger than that at the bare GCE. These positively suggest that the reduction of H₂O₂ is promoted by MoSI NWs, which also provide a base for electrocatalysis of H₂O₂ at the Th-NWs/GCE with Th as a redox mediator.

Figure 4 displays amperometric responses of successive addition of H₂O₂ in the 0.1 M pH 6.70 phosphate buffer solution at the Th-NWs/GCE. Well-defined steady-state current responses with an increase in the H₂O₂ concentration from 0.8 μM to 2.8 mM were recorded at an applied potential of −0.27 V and the steady-state current responses were obtained within 4 – 7 s. From this figure, we can see that the measurable lowest limit is 0.8 μM, comparing favorably with other studies [8, 15 – 16]. The linear ranges from 5 μM to 0.1 mM ($R^2 = 0.998$) and also from 0.1 mM to 2.8 mM ($R^2 = 0.999$) were obtained. The linear regression equation was $I(\mu A) = 0.0019[H_2O_2](\mu M) + 0.398$ (Fig. 7 Inset a) and $I(\mu A) = 0.639[H_2O_2](mM) + 1.08$ (Fig. 7 Inset b) respectively. The reason for the different calibration slope at the low and high concentration ranges might be different changes of the film structure in the process of electrocatalysis, which needs further study. In addition, the relative standard derivation is found to be 5.8% by five replicative measurements of 2.0 mM H₂O₂ solution. These results indicate that the present sensor exhibits a wide linear concentration range, low detection limit, good stability and repeatability for the determination of H₂O₂.

In summary, we have developed a novel amperometric sensor for the detection of H₂O₂ based on a new kind of nanomaterial MoSI NWs, which act as not only a good substrate for the immobilization of redox mediator Th but also a promoter for electrocatalysis of H₂O₂. The construction of the sensing platform is based on strong electrostatic interactions between negatively charged MoSI NWs and positively charged Th molecules. The method is simple and enzymeless. A measurable lowest detection of 0.8 μM was achieved, which is superior to previous work on electrochemical biosensors based on Th as an electron transfer mediator [8, 15 – 16]. The sensor shows possible applications in fabrication of a H₂O₂ detector and other chemical or biological sensors.

**Experimental**

MoSI NWs were fabricated by direct synthesis from elemental material that had been mixed in the desired
stoichiometries, as described elsewhere [25–32]. Powders composed of aggregates of individual nanowires were obtained with two different stoichiometries Mo₆S₃I₆ and Mo₆S₄.₅I₄.₅. In this work, all studies were carried out on Mo₆S₃I₆ nanowires. Thionin chloride of analytical grade was from Aldrich. H₂O₂ of guaranteed grade was from Beijing Chemical Reagent Company. A 0.1 M, pH 6.70 phosphate buffer solution (PBS) was used as the electrolyte. All other reagents were of analytical grade. Water was triply distilled with a quartz apparatus. All electrochemical experiments were conducted at the ambient temperature (20–28°C).

In 2-propanol, 1 mg mL⁻¹ MoSI dispersion was made. The dispersion was initially sonicated for 2 min using a high-power ultrasonic tip (120W, 60 kHz) followed by a mild sonication for 2 h using a low-power ultrasonic bath. Prior to use, the dispersion was resonicated for 20 min to obtain uniform suspension, which was denoted as MoSI NWs suspension.

A GCE was first polished with 0.05 µm alumina slurry, and then washed ultrasonically in triply distilled water and ethanol for a few minutes, respectively. Subsequently, the GCE was coated by casting 20 µL of 1 mg/mL MoSI NWs suspension and dried under an infrared lamp (this modified electrode is denoted as NWs/GCE). Finally, the NWs/GCE was immersed in 0.5 mM Th solution of ethanol for some time, resulting in the incorporation of Th. The experimental results indicated that the current responses increased with increasing dipping time in the Th solution, and then reached a constant value when the dipping time was longer than 2 h. So a dipping time of 2 h was chosen. The modified electrode was then soaked in ethanol for 5 min, and then thoroughly rinsed with water to remove loosely bound Th, subsequently were air-dried (this modified electrode is denoted as Th-NWs/GCE).

A model CHI 660A electrochemical workstation (Chenhua Instrument Co. Ltd., Shanghai, P. R. China) with a conventional three-electrode cell was used to perform electrochemical measurements. The working electrode was a modified or bare GCE with a diameter of 4 mm. A KCl saturated calomel electrode was used as the reference electrode and a platinum electrode as the auxiliary electrode. Prior to electrochemical experiments, the solutions were routinely deaerated by purging with high purity nitrogen.

In steady-state amperometric measurements, the potential was set at –0.27 V in a magnetically stirred solution, and the current-time curves were recorded after a constant background current had been established.

Transparent indium tin oxide (ITO) glasses were used as the substrate for Raman spectral characterization. ITO glass were immersed in 3.0 M nitric acid solution for 2.5 hours, followed by rinsing ultrasonically with water and ethanol for 10 minutes, respectively, and then dried with high purity nitrogen stream. An ITO glass was coated by casting 20 µL of 1 mg/mL MoSI NWs suspension and dried under an infrared lamp. Another ITO glass coated with MoSI NWs was immersed in the 0.5 mM Th solution of ethanol overnight, and then rinsed with ethanol. Raman spectra for MoSI NWs, Th and Th-NWs, which were prepared on substrates separately, were recorded on a Renishaw 1000 micro-Raman system with an excitation wavelength of 632.8 nm.

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References