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Layer-by-layer assembled hybrid film of carbon nanotubes/iron oxide nanocrystals for reagentless electrochemical detection of H_2O_2

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ABSTRACT

A new approach to construct a reagentless electrochemical H_2O_2 sensor is described. Iron oxide magnetic nanocystals (IOMNs), as peroxidase mimetics, were assembled to form a multilayer structure through the layer-by-layer (LBL) method. Polythionin (PTh) was first electrodeposited onto the glassy carbon electrode (GCE) surface to introduce amino groups. Carboxyl functionalized multi-walled carbon nanotubes (MWCNTs), amino functionalized IOMNs, and thionin monomers were alternatively anchored onto a polythionin-functionalized GCE surface in order by carbodiimide or glutaraldehyde chemistry. The resulting multilayer construction with three layers of IOMNs and thionin mediator exhibits excellent electrochemical response to the reduction of H_2O_2, whereas such a modified electrode with one layer construction only yields a slight response to H_2O_2 of the same concentration. The tethered MWCNTs enlarge the amount of immobilized IOMNs and effectively shuttle electrons between the electrode and the thionin. The calibration plot is linear over the wide H_2O_2 concentration range from 0.099 to 6.54 mM, with a detection limit of 53.6 μM.

1. Introduction

Due to their nanometer-scale size, biocompatibility and capability of being manipulated under an external magnetic field, iron oxide magnetic nanocystals (IOMNs) have shown a diverse range of applications in biomedicine areas [1–5]. Magnetic nanoparticles are generally considered to be chemically and biologically inert. Recently, Yan and coworkers made the surprising discovery that IOMNs exhibit an intrinsic enzyme mimetic activity similar to natural peroxidases [6]. In their study, IOMNs were demonstrated to be a highly effective catalyst, and their binding affinity for the substrate 3,3,5,5-tetramethylbenzidine is much higher than that of horseradish peroxidase (HRP). Also, IOMNs were found to remain stable over a wide range of pH and temperatures. More recently, Wang's group reported the colorimetric method for the assay of H_2O_2 by employing IOMNs to catalyze the oxidation of 2,2-azino-bis(3-ethylbenzo-thiazoline-6-sulfonic acid) diammonium salt into the colored product [7]. The detection for glucose was also verified by combining glucose oxidase with IOMNs. The detection platforms for H_2O_2 and glucose further confirmed that IOMNs possess intrinsic peroxidase-like activity, indicating great potential applications in various simple, robust, and easy-to-make analytical approaches in the future.

Various dye molecules have been used as electron-transfer mediators in fabricating of HRP-based H_2O_2 biosensors, among which thionin molecules are gaining the increasing interest. Thionin, a small planar molecule, has two $-NH_2$ groups symmetrically distributed on each side, which makes it a good candidate for covalent conjugation with other molecules or materials. It is well recognized that thionin exhibits excellent electrochemical redox properties toward increased electrocatalytical activity of enzymes in the reduction of H_2O_2 [8–10]. In addition, polythionin (PTh) could be electrochemically deposited onto various electrode surfaces and keeps an excellent efficiency of electron transfer between the HRP and the H_2O_2 electrode [11,12].

The layer-by-layer (LBL) deposition method for multilayer film is one of the techniques widely used to fabricate functional materials on a nanoscale, because of its simplicity, controllability, and versatility. In this work, multi-walled carbon nanotubes (MWCNTs) were modified onto PTh-electrodeposited glass carbon electrode (GCE), followed by the alternative anchoring IOMNs and thionin in LBL way, resulting in a new enzyme electrode for the reagentless electrochemical detection of H_2O_2.

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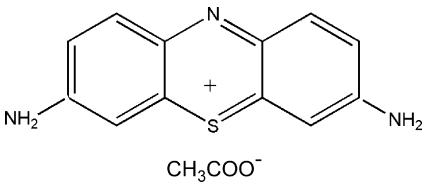


Scheme 1. The structure of thionin.

2. Experimental

2.1. Chemicals and materials

Thionin acetate (Scheme 1), 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride (EDC), and N-hydroxysulfosuccinimide (NHS) were purchased from Sigma–Aldrich (Milwaukee, WI). MWCNTs with a purity of 95% and a hollow structure (OD: 15 ± 5 nm; ID 7 ± 2 nm; length: 1–5 μm) were obtained from NanoLab (Brighton, MA). IOMNs (diameter 20 nm) terminated with carboxyl groups in water and those with amine groups were from Ocean NanoTech (Fayetteville, AR). pH 7.4 phosphate buffer solution (PBS) was prepared with 0.01 M phosphate buffer, 0.137 M NaCl, and 2.7 mM KCl unless otherwise noted. The water used throughout all experiments was purified with a Milli-Q system (Millipore, Bedford, MA). All experiments were carried out at room temperature.

2.2. Preparation of IOMN-modified GCE with LBL method

MWCNTs were carboxyl-functionalized and shortened by sonication in 3:1 HNO_3/H_2SO_4 for 4 h at 70 °C [13]. The dispersions were filtered, washed with water, dried and dispersed in water.

The GCE was first carefully polished with alumina on polishing cloth. The electrode was then placed in ethanol and subjected to vibration to remove adsorbed particles. Finally, the electrode was rinsed with distilled water.

To introduce the NH_2– functional group on the GCE surface, the electrode was first scanned with cyclic voltammogram (CV) of 2.5 cycles between −0.4 and 1.2 V at a scan rate of 100 mV/s in 1:1 HAc solution containing 2 μM thionin. A stable film of polythionin was deposited on the electrode surface.

MWCNTs with carboxylic groups are linked with the amino groups of polythionin using well-known carbodiimide chemistry, which has been reported extremely [14,15]. To attach MWCNTs onto the polythionin modified GCE, 2 μl of 1.4 mg/ml MWCNTT water solution was cast on the electrode and dried in air. Then, 2 μl of freshly prepared 0.2 M EDC and 0.1 M NHSS in water were dropped onto the MWCNT-covered electrode, washed off after 0.5 h and dried. This was immediately followed by 15 min incubation with 2 μl of 4 mg/ml IOMNs in water with an amine group, washed off and dried. Then the amino groups of IOMNs were activated by 2 μl of 2.5% glutaraldehyde for 15 min, washed thoroughly and dried. Later, the electrode was incubated for 15 min with 2 μl of saturated thionin solution in water. The obtained electrode was carefully washed with water to remove the physically absorbed chemicals after each reaction step and were blown over the film surface with an N_2 stream until the adhering water was completely removed. The multilayer films were deposited by alternately covering the electrode surface with glutaraldehyde, IOMNs, glutaraldehyde and thionin in order. Repetition of the above procedures led to the formation of alternating IOMN/Th multilayer films with a desired number of bilayers $\{IOMN/Th\}_n$. The multilayer films were referred to as $\{IOMN/Th\}_n$ (Scheme 2). IOMNs mean those with amino groups unless otherwise noted.

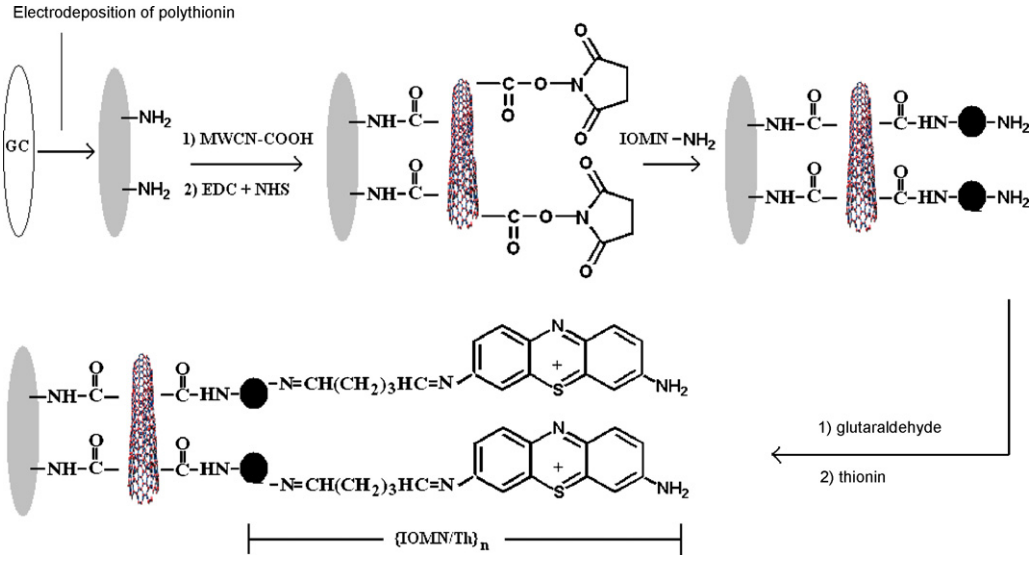
For comparison, IOMNs with carboxyl and those with amine groups were deposited onto the polythionin-modified GCE without MWCNTs. The former was done with the help of EDC and NHSS, and the latter with the help of glutaraldehyde. Both were modified with a layer of thionin by glutaraldehyde again.

2.3. Apparatus

Electrochemical experiments were performed with the CHI 660 electrochemical workstation. A conventional three-electrode cell was used with an Ag/AgCl as the reference electrode, a Pt wire as the counter electrode and a GCE disk (2 mm diameter, modified or unmodified) as the working electrode.

3. Results and discussion

Thionin is a phenothiazine redox dye with two –NH_2 groups symmetrically distributed on each side. Both thionin monomer and the electrogenerated polythionin have excellent electrocatalytic activity toward the redox of small molecular compounds. Here,



Scheme 2. Illustrations of GCE/PTh/MWCNT/$\{IOMN/Th\}_1$ assembled in LBL way using amino functionalized IOMNs and thionin.