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Short communication

Direct electrochemistry and electrocatalysis of hemoglobin incorporated in composite film based on diblock weak polyelectrolyte PHAEMA-b-PDMAEMA and multi-walled carbon nanotubes

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ABSTRACT

The new composite films composed of diblock weak polyelectrolyte poly(2-hydroxyethyl methacrylate)-b-poly(2-(dimethylamino)ethyl methacrylate) (PHEMA-b-PDMAEMA, noted as PHD in the later content) and multi-walled carbon nanotubes (MWCNTs) were applied to immobilize hemoglobin (Hb) for biosensor fabrication. The characterization of Hb/PHD/MWCNTs films were demonstrated by ultraviolet–visible (UV–vis) spectra, scanning electron microscopy (SEM), electrochemical impedance spectroscopy (EIS), cyclic voltammetry (CV) and typical amperometric response (i–t) measurements. The immobilized Hb maintains its bioactivities and displays an excellent electrochemical behavior. The modified electrode exhibited good electrocatalytic activity to the reduction of hydrogen peroxide (H_2O_2). The linear response range of the H_2O_2 biosensor was from 1.0×10^{-6} to 1.5×10^{-3} M with a detection limit of 3.5×10^{-7} M. The apparent Michaelis–Menten constant of Hb on the PHD/MWCNTs film was estimated to be 0.51 mM. These results indicated that the composite films have potential applicability of new types third-generation biosensors or bioreactors based on direct electrochemistry of the proteins.

1. Introduction

It is well known that the direct electrochemistry of redox proteins and enzymes can establish a desirable model for studying the redox mechanism of proteins in biological systems and understanding the relationship between their structures and biological functions [1–4]. Meanwhile, studies on direct electron exchange between proteins and modifying electrodes can exploit the third-generation biosensors [5,6], biofuel cells [7,8], heterogeneous catalysts [9], biomedical devices [10], etc.

Recently, the development of hydrogen peroxide (H_2O_2) biosensors has received great interest. It is because that the determination of hydrogen peroxide is of considerable importance in chemical, biological, environmental and other fields [11,12]. H_2O_2 is not only a by-product of several highly selective oxidases, but also an essential mediator in food, pharmaceutical, clinical, industrial and environmental analyses. Hemoglobin (Hb) can catalyze the reduction of H_2O_2 and applied to develop the biosensor of H_2O_2 [13]. Hb is an important redox respiratory protein in red cells. It consists of four polypeptide chains, which each has one electroactive iron heme group [4]. Because of its commercial availability, moderate cost and known structure, Hb is considered as an ideal model protein for the study of the electron transfer of heme molecules. However, the denaturation of Hb on bare electrodes and its deeply buried electroactive center limit its direct electron transfer with electrodes. For this reason, a great amount of efforts have been devoted to composite films or matrix to immobilize Hb onto the electrode surface to obtain its direct electrochemical reactions. Various composite films or matrix, such as polymers [14–18], surfactants [19,20] and nanomaterials [21–28] have been exploited for immobilizing Hb and enhancing electron transfer between Hb and electrodes. In addition, the cast film technique is a very effective approach to realize the direct electrochemistry of the proteins incorporated into films [14]. It is because that these cast films have the structure similar to that of biological membranes. The biomembrane-like films provided a favorable microenvironment for proteins and enhance electron transfer between proteins and electrodes.

It is well known that Hb is a heme protein containing amino acids, which is polyampholytic molecule. Hence, Hb can interact with polyelectrolytes via electrostatic interactions. Polyelectrolytes with various charge densities, molecular weights and sizes, allow for the formation of a very efficient, tightly bound imprinting cover around the enzyme [29]. The low concentration polyelectrolytes can provide large improvements in the operational stability of the biosensors. However, these efforts are

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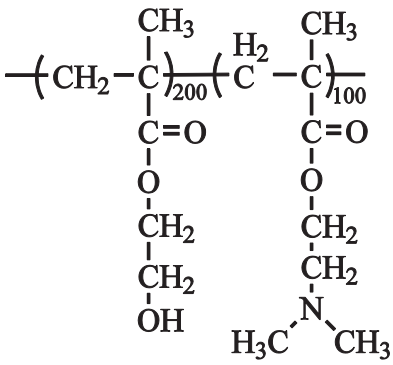

Scheme 1. Chemical structure of the diblock polymer PHD.

mainly focused on homopolyelectrolyte or random copolymer. Even though these polyelectrolytes have been used extensively for the stabilization of enzymes, their application to the development of biosensors is very limited [29]. With the development of the synthetic technology, block weak polyelectrolytes have attracted considerable attention in the biosensor field [30–32]. Similarly to natural amphiphiles (e.g., lipids), block weak polyelectrolytes comprised of two or more chemically incompatible blocks can microphase separate in solution into aggregates of multiple morphologies. In addition, block weak polyelectrolytes combine structural features of weak polyelectrolytes, block copolymers, and surfactants [33]. These quite unusual and unique properties play an important role in physico-chemical properties of biological cell structures. Block weak polyelectrolytes, as a film-forming material, which could not only provide a favorable microenvironment for the proteins but also demonstrate better stability. However, the understanding of the direct electrochemistry of enzyme based on block weak polyelectrolytes is still a question.

For these reasons, in this investigation, the direct electrochemistry of heme proteins using diblock weak polyelectrolyte copolymers PHEMA-b-PDMAEMA was studied. The poly(2-hydroxyethyl methacrylate)-b-poly(2-(dimethylamino)ethyl methacrylate) (PHEMA-b-PDMAEMA, abbreviated as PHD) diblock weak polyelectrolyte was synthesized by atom transfer radical polymerization (ATRP) [34], whose chemical structure is shown in Scheme 1. PHD can form a biomembrane-like film, which is a favorable microenvironment for proteins. It is a coexistence of a hydrophilic neutral block (PHEMA) and cationic weak polyelectrolyte block (PDMAEMA). PDMAEMA is the major types of the non-viral carriers for gene therapy, which was reported to be capable of affecting gene transfection [35]. PHEMA is a biocompatible polymer, which has been used as a potential carrier in drug delivery, dental, ophthalmic, scaffold, and neural tissue engineering applications [36–39]. In addition, it is well known that multi-walled carbon nanotubes (MWCNTs) has been used in third-generation electrochemical sensors because of its high conductivity properties, high chemical stability and extremely high mechanical strength [40]. Hence, in this work, Hb was immobilized in PHD/MWCNTs composite films on glassy carbon electrode (GCE) by simple cast method. The Hb/PHD/MWCNTs film modified GCE was sensitive to the electrocatalytic reduction of hydrogen peroxide (H_2O_2). These properties of Hb/PHD/MWCNTs films may open new possibilities to develop sensitive elements for mediator-free third-generation biosensors and biomedical device.

2. Experimental

2.1. Reagents and apparatus

$PHEMA_{200}$-b-$PDMAEMA_{100}$ (M_w = 49 kg/mol) was synthesized via atom transfer radical polymerization (ATRP) according to the procedures described in Ref. [34]. Bovine hemoglobin (M_w = 64,500, Sigma) was used without further purification. Multi-walled carbon nanotubes (MWCNTs) were obtained from Chengdu Organic Chemicals Co. Ltd. and were purified and functionalized as described in Ref. [41] Phosphate buffer solution (PBS) was prepared by mixing stock standard solution of Na_2HPO_4 and NaH_2PO_4. Stock solutions of H_2O_2 were freshly diluted from 30% solution (Xilong Chemical Co. Ltd.). All solutions were made up with twice-distilled water. Other reagents were analytical grade.

Scanning electron microscopy (SEM) image was recorded on a JSM-6610LV scanning electronic microscopy. Ultraviolet–visible (UV–vis) spectra were carried out by using a Perkin Elmer's LAMBDA25 UV–vis spectrophotometer. All electrochemical experiments were conducted on a CHI650D electrochemical workstation (Chenhua Instrument Company of Shanghai, China) in a conventional three-electrode system. The reference electrode was saturated calomel electrode (SCE), a platinum wire electrode was used as an auxiliary electrode and the working electrode was a modified GCE (with 3 mm diameter). Prior to each experiment, all solution was deoxygenated with high purity nitrogen and a nitrogen environment was then kept over the solution in the cell. All experiments were carried out at room temperature.

2.2. Preparation of Hb/PHD/MWCNTs/GCE

A bare glassy carbon electrode (GCE) was polished mirror-like surface with 0.05 and 0.03 μm alumina slurry followed by rinsing thoroughly with double-distilled water, and was successively sonicated in nitric acid (1:1), ethanol and double-distilled water, respectively. 5.0 mg MWCNTs were dispersed in 5.0 mL water solution by sonication for 60 min. Equivalent volume of 20 mg mL^{-1} PHD (in dimethylformamide) solution and 10 mg mL^{-1} Hb solution in PBS (pH 7.0) were hand-mixed thoroughly, and then 5 μL of 5.0 mg mL^{-1} MWCNTs solution and equivalent volume of the resulting mixture were dropped onto the GCE surface successively and dried in room temperature (noted as Hb/PHD/MWCNTs/GCE). For comparison, Hb/PHD/GCE and Hb/MWCNTs/GCE were fabricated with the similar procedures. All the modified electrodes were stored at 4 °C in a refrigerator.

3. Results and discussion

3.1. Characterization of Hb/PHD/MWCNTs film

The surface morphology of the Hb/PHD/MWCNTs film is an important factor affecting its performance. The surface morphology of MWCNTs and Hb/PHD/MWCNTs films were confirmed directly by SEM. As can be seen in Fig. 1A, many twisted MWNTs bundles can be observed. In the case of Hb/PHD/MWCNTS film (Fig. 1B), when Hb and PHD are introduced, the morphology of Hb/PHD/MWCNTS film is significantly changed, forming a porous structure (Fig. 1B). This unique porous structure could have the advantage of the small molecule transfer between the electrode and the solution. This is the same as the addition of protein to a polyelectrolyte–surfactant polymer matrix [42]. The interaction between PHD and protein might cause a change to form the porous structure.

UV–vis absorption spectroscopy is an effective means for monitoring the structure of heme protein at a modified electrode in electrochemical biosensor researches. It is well known that the position of the Soret absorption band of heme iron can provide information about conformation of heme proteins [43]. Fig. 1C exhibits the UV–vis spectroscopy of PHD/MWCNTs film, Hb film and Hb/PHD/MWCNTs film. As expected, Hb film gives a typical Soret band at about 410 nm (curve b). As seen curve c in Fig. 1C, the Soret band of Hb in the dry Hb/PHD/MWCNTs film is located