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Preparation of poly(vinylpyrrolidone)-protected Prussian blue nanoparticles-modified electrode and its electrocatalytic reduction for hemoglobin

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Abstract

In this paper, a novel modified electrode was developed by using highly dispersed Prussian blue (PB) nanoparticles protected by poly(vinylpyrrolidone) (PVP). The size of the nanoparticles was controlled through adjusting the feed ratio of PVP/Fe^{2+}. Physical characteristics of the nanocomposite were studied by transmission electron microscopy (TEM), UV–vis, IR spectroscopy, and X-ray powder diffraction (XRPD) analysis. The electrocatalytic reduction of hemoglobin (Hb) at PVP-protected PB nanoparticles (PVP/PB NPs)-modified electrode had been investigated. In addition, the size effects and biocompatibility of PVP/PB NPs for the electrochemistry of Hb were also observed. Experimental results indicated that the reduction peak currents of Hb were linear with its concentrations over the range from 1.0×10^{-7} to 1.2×10^{-5} mol/L and the calculated detection limit ($S/N = 3$) was 4.0×10^{-8} mol/L.

Keywords: Prussian blue (PB) nanoparticles; Poly(vinylpyrrolidone) (PVP); Electrochemical catalysis; Hemoglobin (Hb)

1. Introduction

Due to the significance in fundamental understanding the mechanism of biological electron transfer (eT) and the potential application in the area of biosensors, the electrochemistry of redox proteins at a range of electrodes has been widely investigated [1–4]. Among the electroactive proteins, hemoglobin (Hb), which is an important respiratory heme protein in red cells, attracted many scientists' attention because of its specific biochemical roles involving electron transfer, oxygen transfer, storage and metabolism. Studies of the electrochemical behavior of Hb are essential for understanding of their biological activities and establishing a foundation for fabricating biosensors, bioreactors and biomedical devices. In addition, Hb is also a useful model for studying eT reactions of heme proteins because of its easy availability, inexpensive price and well-known structure. However, heterogeneous eT between Hb and conventional electrodes is very slow owing to its extended three-dimensional structure and inaccessibility of the electroactive center of Hb [5–9]. Furthermore, the adsorptive denaturation and conformational equilibria of protein at electrodes also have the negative influence on the eT between Hb and the electrodes. Great efforts have been made to enhance the eT of Hb by using mediators, promoters or special electrode materials [10–13]. The direct eT has been observed by incorporating Hb in organic membrane [14–22], inorganic materials [23–26] and composite films [27–30].

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Therefore, design and development of desired interfaces to accelerate the eT process between redox proteins and electrode surface are the most important steps for the research of electrochemistry of heme proteins. During the past decades, a burst of research activity is focus on the area of synthesis and functionalization of the nanoparticles because of their interesting size- and shape-dependent properties [31,32]. Rapid advances in nanotechnology are making contributions to help us understand the characteristics of protein and enzyme, stimulating much current interest in designing novel systems for detecting biomolecules or fabricating biosensors [33–39]. Nanoparticles may offer an excellent platform for the proteins and are becoming a new class of electrode materials for various biosensing applications.

In this paper, we developed a novel sensor modified with inorganic–organic hybrid nanocomposite, which was based on conjugation of Prussian blue (PB) nanoparticles with poly(vinylpyrrolidone) (PVP). We found that the PVP-protected PB nanoparticles (PVP/PB NPs) possessed some advantages, for example, well-defined structure, protein compatibility, resistance to protein dehydration and denaturation. The electrochemical characteristics of Hb were studied in this work with the novel PVP/PB NPs-modified electrode, which showed high catalytic activity and long-term stability for Hb measurement. In addition, an obvious size-dependent electrochemistry of Hb at PVP/PB NPs-modified electrode was also observed.

2. Experimental

2.1. Chemicals and reagents

Hemoglobin from bovine blood (MW 66,000) was obtained from Sigma Co. Ltd., (USA); Nafion (5 wt.% solution in mixture of alcohol and 10% water) was purchased from Aldrich Chemical Company Inc.; poly(vinylpyrrolidone) (PVP, K30) was obtained from Shanghai Chemical Reagent Company (China). All chemicals were at least analytical reagent grade and all solutions were prepared by double-distilled deionized water.

2.2. Apparatus

Transmission electron microscopy (TEM, JEOL-100C-II instrument) was used for the measurement of the size of PVP/PB NPs. The sample was prepared by placing suspension of PVP/PB NPs (being dispersed in H_2O with ultrasonic wave) onto a copper grid coated with a layer of amorphous carbon.

All the electrochemical experiments were carried out with a CHI-830 Electrochemical workstation (CH Instruments, USA). A conventional three-electrode cell, with a bare GC electrode (2 mm diameter, BAS Co., Japan) or PVP/PB NPs-modified GC electrode as the working electrode, a saturated calomel electrode (SCE) (Jiangsu Electroanalytical Instruments Factory, China) as the reference electrode, and a platinum electrode as the auxiliary electrode, was adopted.

2.3. Preparation of PVP/PB NPs

A series of PVP/PB NPs with different diameters were prepared by mixing equimolar amount of aqueous $FeCl_2$ and $K_3Fe(CN)_6$ solutions in the presence of PVP [40]. Briefly, 2 ml $K_3Fe(CN)_6$ (0.1 mmol) solution was slowly added into 8 ml homogenous mixture of $FeCl_2{\cdot}4H_2O$ (0.1 mmol) and PVP (A: 2 mmol, B: 5 mmol, or C: 10 mmol). Under vigorous stirring, a blue solution was readily formed with the final concentrations of $[Fe^{2+}] = [Fe^{3+}] = 10$ mmol/L, and [PVP] = 200, 500 or 1000 mmol/L, respectively. Acetone was added into the resulting PB solutions to remove the residual KCl. After being cleaned with acetone for several times, the PB naoparticles were left and dried in the room temperature.

The physical characteristics of the PVP/PB NPs were studied by transmission electron microcopy (TEM, JEM-100C-II, Japan), UV–vis (Cary 50, Varian, Australia), IR spectroscopy (Nexus 670, USA), and X-ray powder diffraction (XRPD, Bruker, D8 Advance, Germany) analysis.

2.4. Preparation of PVP/PB NPs-modified electrode

The resulting PVP/PB NPs (5 mg) were dispersed in 1 ml PBS (0.05 M, pH 5.05) and the mixture was stirred for half an hour to achieve a well-dispersed suspension.

Prior to use, the GC electrode was mechanically polished with alumina paste (0.5 μm) until a mirror finish was obtained, followed by extensive rinsing with doubly distilled water. The electrode was further sonicated in 1:1 HNO_3 (v_{HNO_3}: v_{H_2O}), 1 mol/L NaOH and doubly distilled water, respectively. Suspension of PVP/PB NPs (5 μl) was cast on the surface of GC electrode and the electrode was allowed to dry at room temperature. Then, 5 μl Nafion solution (3 wt.%, diluted with ethanol) was dispersed on the surface of PVP/PB NPs film to improve the stability of the modified electrode as well as to get rid of the interferences from anions. Finally, the electrode was dried in room temperature so that a well-distributed PVP/PB NPs film was immobilized on the surface of GC electrode.

2.5. Electrochemical experiments

All electrochemical measurements were done in a three-electrode system. The electrochemical responses of PVP/PB NPs-modified GC electrodes were evaluated in an electrochemical cell containing 5.0 ml 0.05 mol/L PBS (pH 5.05) at room temperature ($20 \pm 2\,^{\circ}C$). All solutions were deoxygenated by bubbling highly pure nitrogen for at least 15 min and maintained under nitrogen atmosphere during the experiments.