Electrochemical Study on A Heme-Histidine-Sodium Decyl Sulfate Nano Micelles Complex Artificial Peroxidase

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Abstract—A heme-histidine-sodium decyl sulfate nano micelles complex with peroxidase activity (MAP) was constructed self-assembly and immobilized on a hydroxy fullerene modified glassy carbon electrode. The cyclic voltammograms of the modified electrode with a pair of redox peaks were investigated. The formal potential of the MAP modified electrode was determined to be -0.312V , at a scan rate of 0.05V/s in 50 mmol/L sodium phosphate buffer solution ($\text{pH } 8.0$). The electron transfer rate constant (k_s) and cathodic transfer coefficient (α) were 1.96s^{-1} and 0.4 , respectively. The cathodic peak current of the MAP modified electrode responses linearly to hydrogen peroxide ranging from $300\mu\text{mol/L}$ to $1700\mu\text{mol/L}$. The detection limit was concluded to be $32\mu\text{mol/L}$. The apparent Michaelis-Menten constant (K_m^{app}) could be determined to be 1.08 mmol/L .

Keywords-heme; nano micelle; artificial peroxidase; electrochemistry

I. INTRODUCTION

It is an important aspect in the design of a heme protein through electrochemical investigation and simulation the functional or structural analogues. The electrochemical study of a heme protein and its mimic enzyme may reveal the electron transfer reaction principle and the energy conversion process of the heme protein. Moreover, it could provide theories foundation for the new type biosensors developments [1-2].

Heme is an important cofactor of a heme protein (horseradish peroxidase, cytochrome c, etc.) and the catalytic

activity center of a heme-containing enzyme. Sodium decyl sulfate, which has a hydrophobic tail and hydrophilic head, may form stable nano micelles in aqueous solution when its concentration is above CMC concentration. Because of the shorter chain length, the formed sodium decyl sulfate micelles have smaller diameter, stronger hydrophobic interaction and electrostatic effect [3-6], which may provide a hydrophobic environment and enhance the stability and activity of an artificial peroxidase [7-10].

Nano materials possess many excellent characters, such as large specific surface area, good conductivity, and biocompatibility, so it has an important role in electrochemical research and a wide range of applications. Hydroxy fullerenes are a product of functionalized fullerene, which is added with hydroxyl group on the surface of fullerene to increase its solubility in water and enhance the biocompatibility [11-12].

Chitosan is a linear polysaccharide, where β -(1-4)-linked D-glucosamine and N-acetyl-D-glucosamine are distributed stochastically. Its monomer molecular formula is $\text{C}_6\text{H}_{11}\text{NO}_4$. Chitosan has been used in immobilization technology, including immobilize enzyme on an electrode surface for its advantages of good biocompatibility, strong stability and simple structure [13].

In the present study, heme-histidine-sodium decyl sulfate nano micelles complex artificial peroxidase (MAP) was constructed self-assembly and immobilized on a glassy carbon electrode (GCE). Then the electrode was covered with a chitosan membrane. The MAP modified electrode shows a good response to hydrogen peroxide concentration.

II. EXPERIMENTAL

A. Reagents

Heme and sodium decyl sulfate (SDeS) are obtained from Sigma (Saint Louis, MO, USA). Hydroxy Fullerenes (HFs) is bought from Shenzhen Nanotech Port Ltd. Co. (China). Sodium dihydrogen phosphate, disodium hydrogen phosphate, chitosan (Chi) and hydrogen peroxide are purchased from Shanghai Chemicals Company, China. All chemicals used in these investigations are of analytical grade.

B. Artificial Peroxidase Preparation

Typical heme-histidine-SDeS nano micelles complex artificial peroxidase (MAP) was constructed by mixing heme, histidine (His) and SDeS in 50mmol/L, pH8.0 PBS to form a homogeneous colloidal solution [14-17]. The final concentrations of heme, SDeS and His were 2, 50 and 50mmol/L, respectively. The size of MAP nano micelle was 3.45nm, which was determined using a Zeta Plus Dynamic light scattering (DLS) instrument, Brookhaven Instruments Corporation, America. The chemical structures, preparation process and size of MAP nano-micelle could be seen in Scheme 1.

Scheme 1 Preparation process and size of MAP nano-micelle

C. Fabrication of Chi/MAP/Hfs/GCE

At first, a GCE was prepared similar to the procedure given in previous literature [18-19]. Then, 3 μ L HFs (2mg/mL) was dropped on the prepared GCE. After drying for 4 h at room temperature, 4 μ L MAP was dropped on the electrode and dried at 25°C. Finally, 4 μ L Chi (0.5%) was

covered on the surface of the electrode and dried at 25°C.

III. RESULTS AND DISCUSSION

A. Electrochemical Properties of Chi/MAP/Hfs/GCE

The cyclic voltammograms (CVs) of (a) bare GCE, (b) Chi/HFs/GCE, (c) Chi/His/HFs/GCE, (d) Chi/SDeS/HFs/GCE, (e) Chi/heme/HFs/GCE and (f) Chi/MAP/HFs/GCE were shown in Fig. 1A.

Figure 1A. The cyclic voltammograms (CVs) of (a) bare GCE, (b) Chi/HFs/GCE, (c) Chi/His/HFs/GCE, (d) Chi/SDeS/HFs/GCE, (e) Chi/Heme/HFs/GCE and (f) Chi/MAP/HFs/GCE. The tests were done in 50mmol/L, pH8.0 PBS at a scan rate of 0.05 Vs⁻¹

It could be seen that curve (f) shows a pair of stronger redox peaks respect to that of curve (e). The ratio of cathodic (I_{pc}) to anodic peak current (I_{pa}) was calculated to be 0.9. The difference between anodic (E_{pa}) and cathodic peak potential (E_{pc}) was determined to be 0.04V. Thus, the electrochemical process of Chi/MAP/HFs/GCE (f) was quasi-reversible [20]. Moreover, the formal potential ($E^0 = E_{pa}/2 + E_{pc}/2$) was determined to be -0.312V versus Ag/AgCl. In the meanwhile, no redox peaks could be seen in curve (a), curve (b), curve (c) and curve (d).

The CVs of the Chi/MAP/HFs/GCE in 50mmol/L PBS at different pH values were illustrated in Fig. 2B.

Figure 2B. CVs of Chi/MAP/HFs/GCE in 50mmol/L PBS at different pH values (from left to right): 7, 8, 9, 10, 11 and 12, respectively

The maximum cathodic current was obtained at pH 8.0, and so, this optimal pH value was chosen for the next set of

tests. The formal potential of the MAP modified GCE was pH dependent for the pH value ranging from 7 to 12. The slope (0.064 V/pH, Fig. 3C) was close to the Nernst's value (0.059 V/pH) for one-electron and one-proton process [21].

Based on the observed CVs of the Chi/MAP/HFs/GCE (Fig. 4D), the peak currents increased with increasing scan rate (ν) and were proportional to ν in the range of 0.01–1.2 V/s (Fig. 5E), indicating that the MAP was stably immobilized on the GCE. The cathodic peak potential altered linearly respect to $\ln \nu$ for the scan rates ranging from 0.5 to 1.0 V/s (Fig. 6F).

Figure 3C. Relationship between formal potential (E°) and pH value.

Figure 4D. CVs of the Chi/MAP/HFs/GCE in 50mmol/L, pH 8.0 PBS at the different scan rates (from inner to outer): 0.01, 0.02, 0.03 ... 1.2 V/s, respectively.

Figure 5E. Relationship between peak current (I_p) and scan rate (ν).

Figure 6F. Relationship between peak potential (E_p) and $\ln \nu$.

Then the number of electron (n), cathodic electron transfer coefficient (α), apparent heterogeneous electron transfer rate constant (k_s) and the average surface concentration of electroactive materials (MAP) on the surface of GCE (Γ) could be concluded to be 1, 0.4, $1.96s^{-1}$ and $9.10 \times 10^{-9} \text{ mol/cm}^2$, respectively [19-23]. Moreover, the size of MAP nano micelle was 3.45 nm (Scheme 1), then the theoretical concentration of one layer MAP could be calculated to be $1.40 \times 10^{-11} \text{ mol/cm}^2$ [12]. So the distribution of MAP on the surface of the modified electrode was very concentrated.

B. Electro-Catalytic Properties of Chi/MAP/HFs/GCE

The linear sweep voltammograms (LSVs) of Chi/MAP/HFs/GCE and Chi/Heme/HFs/GCE were presented in Fig. 7A and Fig. 8B.

Figure 7A. The linear sweep voltammograms (LSVs) of Chi/MAP/HFs/GCE.

Figure 8B. Chi/heme/HFs/GCE in the absence and presence of 0.3, 0.6 and 1.1mmol/L H_2O_2 in 50mmol/L PBS (pH8.0), at a scan rate of 0.05 Vs^{-1}

It could be seen that the MAP modified electrode show better response to H_2O_2 , respect to that of Chi/heme/HFs/GCE. In the meanwhile, either Chi/SDeS/HFs/GCE or Chi/His/HFs/GCE shows almost no response to H_2O_2 (data not shown). It seems that His and SDeS play important roles for the structure of MAP, and the heme acts as an essential role for the catalytical function of MAP. The MAP modified electrode shows more efficient catalytic activity respect to that of heme.

The possible electro-catalytic mechanism on Chi/MAP/HFs/GCE reaction could be expressed as the following [18]:

Fig. 9C shows the relationship between peak current (I_p) and H_2O_2 concentration. The linear range was from 300 to $1700 \mu\text{mol/L}$.

Figure 9C. The relationship between the peak current (I_p) and the concentration of H_2O_2 (with the typical linear range from 300 to $1700 \mu\text{mol/L}$).

The detection limit was $32 \mu\text{mol/L}$. Then, according to the electrochemical version of the Lineweaver-Burk Equation [19-24] and Fig. 10D,

Figure 10D. Lineweaver-Burk plot of MAP.

the apparent Michaelis-Menten constant (K_m^{app}) could be calculated to be 1.08 mmol/L . This value is less than that of HRP/thionine/GCE (1.6 mmol/L) [25], HRP/NF/MG/GCE

(2.1 mmol/L) [26]. Though it is larger than that of HRP/gelatin/RTILs/GCE (0.0684 mmol/L) [27], HRP/Au colloid/Cys/glutaraldehyde/Cys/GCE (0.094 mmol/L) [28], as an electrochemical system without protein, it may have important research significance and potential application value.

IV. CONCLUSIONS

A heme-histidine-sodium decyl sulfate nano micelles artificial peroxidase (MAP) was constructed and immobilized on a hydroxy fullerene modified glassy carbon electrode. The prepared MAP modified glassy carbon electrode could be used as a biosensor for H_2O_2 determination with low detection limit and high sensitivity. MAP could be used instead of native horseradish peroxidase to construct a sensitive H_2O_2 biosensor without any protein.

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