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Apoferritin as a bionanomaterial to facilitate the electron transfer reactivity of hemoglobin and the catalytic activity towards hydrogen peroxide

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Abstract

In this report, apoferritin as a stable bionanomaterial was modified with hemoglobin on pyrolytic graphite electrode. Rapid electron transfer reactions of hemoglobin were achieved with the help of apoferritin in a large pH range. Moreover, hemoglobin as an enzyme exhibits fine electrocatalytic activity towards the reaction of hydrogen peroxide, and a wide concentration range of linear relationship between the reduction peak current and the concentration of hydrogen peroxide has been obtained with a higher upper detection limit, which may be further developed for a hydrogen peroxide biosensor. Therefore, a new property of apoferritin is explored, in which apoferritin works as a bionanomaterial to be an accelerant of the electron transfer of Hb and a stabilizer to retain the catalytic ability of the protein under mal-condition.

Keywords: Apoferritin; Bionanomaterial; Hemoglobin; Hydrogen peroxide

1. Introduction

Bionanomaterials have just attracted the interest of researches. But they should be more interesting, since they have the character of both the nanomaterials and the biological molecules. One example is liposome, which has been used as liposomal drug targeting, release, and absorption [1,2]. Another example is a naturally existent bionanomaterial purple membrane, which contains the unique protein bacteriorhodopsin [3,4]. Among the possibly feasible bionanomaterials, a protein ferritin has attracted our attention due to not only its nano scale but also its high stability and special structure. Ferritin is composed of two parts: a nearly spherical shell of 24 protein subunits (~480 kDa) called apoferritin with an outer diameter of 12 nm and an inner cavity diameter of 8 nm in which Fe(III) is deposited as a stable hydrous ferric oxide mineral core containing several thousand atom of iron and several hundred molecular of inorganic phosphate [5–8]. As an iron-storage and detoxification protein, ferritin can withstand high temperatures (75–85 °C), a pH ranging from 2.0 to 12.0 [7,9]. So far, the structure of ferritin, the theory of iron in and out, and the usage to construct nanocrystal and to capture toxic micro-molecular have been studied [6–17]. However, these studies focus on the "inner" of ferritin, but have neglected the "outer", which might also have some special character.

Direct (i.e. unmediated) protein electrochemistry has attracted the interest of lots of scientists because of its importance in elucidating the intrinsic thermodynamic and kinetic properties of proteins and its potential application in bioelectronics fields [18]. Recently, some nanomaterials have been adopted to improve the electron transfer (ET) reactivity and the catalytic activity of redox proteins [19–22]. For example, direct electrochemical response of hemoglobin (Hb) has been obtained at a nano titanium dioxide modified electrode [23], and a nanocrystalline tin oxide modified electrode [24]. Although some inorganic and organic nanomaterials have been employed, there is no report that using bionanomaterials as matrix of redox protein to enhance the redox and enzymatic activities of proteins. In this work, Hb and apoferritin are co-modified onto PG electrode surface. Experimental results reveal that apoferritin as a useful bionanomaterial can act as an accelerant of the electron

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transfer reactivity of Hb and a stabilizer to retain the catalytic ability of the protein under mal-condition.

2. Experimental

2.1. Chemicals

Hb (from bovine blood), hemin, albumin from bovine serum (BSA), ferritin (type I: from horse spleen) and apoferritin (from horse spleen) were purchased from Sigma and used without further purification. H_2O_2 (30% (w/v) solution) was from Shanghai Chemical Reagent Company (China). All other reagents were of analytical grade. All solutions were prepared by double distilled water, which was purified with a Milli-Q purification system (Branstead, USA) to a specific resistance of > 16 MΩ cm. Solutions with different pH values were prepared as follows: pH 4.0–5.0, 0.1 M NaOAc–HOAc solution (ABS); pH 6.0–8.0, 0.1 M NaH_2PO_4–Na_2HPO_4 solution (PBS); pH 9.0–10.0, 0.1 M glycine–NaOH solution.

2.2. Preparation of modified electrodes

The substrate PG electrode was prepared by putting a PG rod into a glass tube with fixing it by epoxy resin. Electrical contact was made by adhering a copper wire to the rod with the help of Wood's alloy. The PG electrode was firstly polished on rough and fine sand papers. Then its surface was polished to mirror smoothness with an alumina powder (particle size of about 0.05 μm)/water slurry on silk. Eventually, the electrode was thoroughly washed by ultrasonicating in both double distilled water and ethanol for about 5 min.

20 μl of the mixture containing 50 μg Hb and 50 μg apoferritin was spread evenly onto the surfaces of the PG electrodes to prepare Hb–apoferritin co-modified electrodes. The electrodes surfaces were covered with Eppendorf tubes in the first 2 h to prepare uniform films, and dried overnight in the air. Then the modified electrodes were thoroughly rinsed with pure water and dried again.

The preparation of Hb–BSA and Hb–ferritin co-modified electrodes are the same as that of the Hb–apoferritin co-modified electrodes.

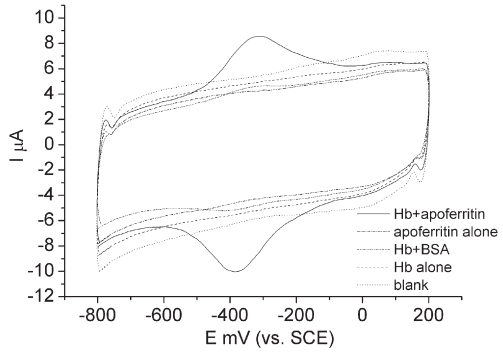

Fig. 1. Cyclic voltammograms obtained at an Hb and apoferritin co-modified PG electrode for 0.1 M PBS with pH 7.0. Scan rate: 200 mV s^{-1}.

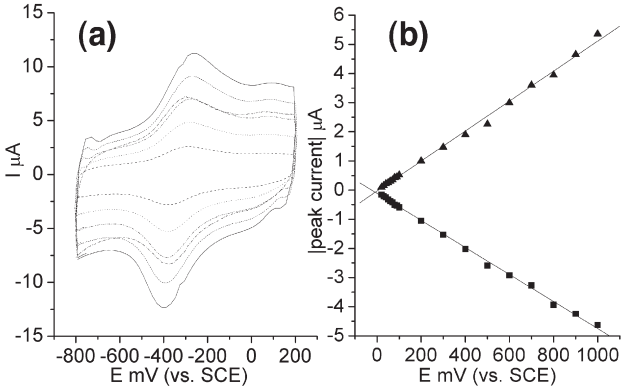

Fig. 2. a) Cyclic voltammograms obtained at an Hb and apoferritin co-modified PG electrode at different scan rate. Others same as in Fig. 1. b) The relationship between peaks potentials and scan rate.

2.3. Apparatus and electrochemical measurements

Electrochemical experiments were carried out on a VMP Potentiostat (PerkinElmer, USA) with a three-electrode system. In a one-compartment volume (10 ml) glass cell, the modified PG electrode as the working electrode, a saturated calomel reference electrode (SCE) and a platinum wire auxiliary electrode were used for the measurements. All the potentials reported in this work were versus SCE.

3. Results and discussion

As shown in Fig. 1, no voltammetric peak can be observed at a bare PG electrode or at an apoferritin alone modified PG electrode. And certainly, Hb alone modified electrode cannot exhibit any peak according to the previous studies [25]. However, obviously, a pair of peaks at −382.9 mV and −315.1 mV, which is characteristic of heme Fe^{III}/Fe^{II} redox couple, is observed with a Hb–apoferritin co-modified electrode. And, a much smaller pair of peaks can be observed with a Hb–BSA co-modified electrode, which works as a control. Therefore, with the help of apoferritin, Hb can take redox reactions at the PG electrode surface, and the electron transfer rates are greatly enhanced.

A pair of peaks can be also observed with a ferritin and Hb co-modified electrode (data not shown). And, the cyclic

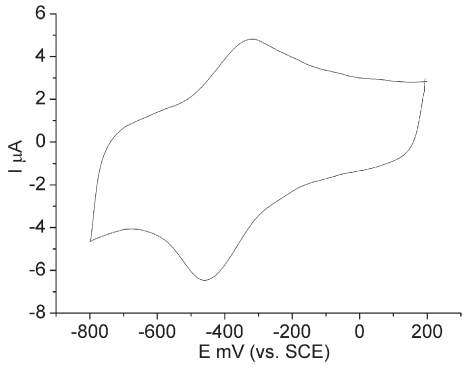

Fig. 3. Cyclic voltammograms obtained at a hemin and apoferritin co-modified PG electrode. Others same as in Fig. 1.

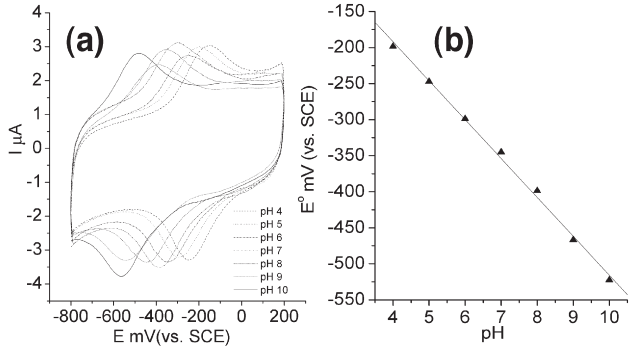

Fig. 4. a) Effect of pH on the cyclic voltammograms obtained at an Hb and apoferritin co-modified PG electrode. Others same as in Fig. 1. b) The relationship between $E^{0\prime}$ and pH.

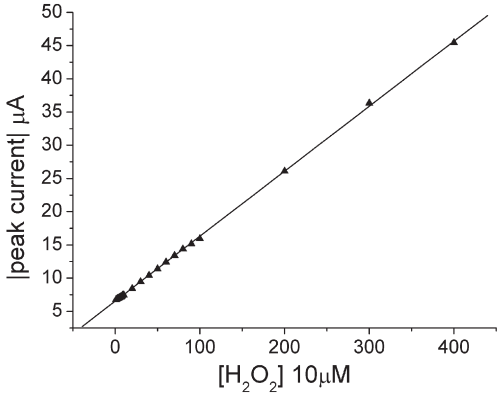

Fig. 6. Relationship between the catalytic peaks currents and the concentration of H_2O_2.

voltametric waves in the ferritin case are the same as that of the apoferritin, indicating that the enhancement of the redox reactivity of Hb by ferritin is not through the inner core but the protein shell.

When the scan rate is 200 mV s^{-1}, the redox peaks separation is about 68 mV, which is a little more than the peak separation of reversible process (59 mV), indicating a quasi-reversible redox process [26]. And the apparent formal potential ($E^{0\prime}$) is −349 mV in the pH 7.0 buffer solution. Fig. 2a shows the cyclic voltammograms at different scan rate. The peaks currents are linearly proportional to the scan rate in the range of 50 to 1000 mV s^{-1} (Fig. 2b), indicating a thin-layer electrochemical behavior [26].

The electrochemical response of Hb observed as above coincides with the previous reports by immobilizing the protein with some other materials on electrode [27–29]. Meanwhile, it should be mentioned that the peaks separation and the $E^{0\prime}$ will be 126 mV and −395 mV respectively, if hemin instead of Hb is employed to be co-modified with apoferritin as control (Fig. 3), indicating that heme does not dissociate in this study [28–31].

Since ferritin can withstand a wide range of pH, we have examined the pH effect on the electrochemical behavior of Hb with variable pH values. As is shown in Fig. 4a, fine redox waves of the protein can be observed in all the test pH range, although an increase of pH in the test solution will lead to a negative shift of $E^{0\prime}$ of Hb. In the pH range of 4.0–10.0, the slope of −54.0 mV pH^{-1} can be obtained (Fig. 4b), with a linear regression equation of −0.9983. The slope value is close to the theoretical value of −57.6 mV pH^{-1}, which suggests a one proton-coupled single electron transfer [32]. However, as is reported by the previous studies, the peak currents will be changed even if the protein can show electrochemical response in a wide pH range [25,29,33]. It can also be observed that the peaks currents of Hb keep stable of about 1.4 μA in all the pH range, indicating that the electron transfer reactivity of Hb is balanced in a wide pH range with help of apoferritin.

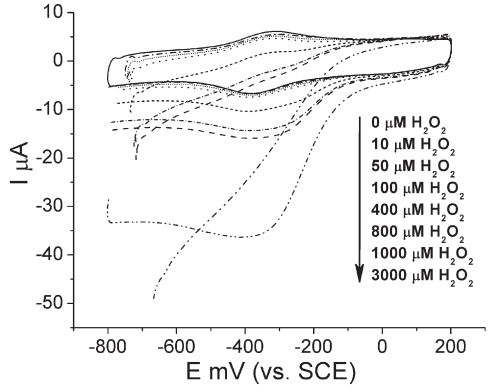

Fig. 5. Cyclic voltammograms obtained at an Hb and apoferritin co-modified PG electrode with the H_2O_2 concentration of 0, 10, 50, 100, 400, 800, 1000, 3000 μM (from inner to outer) in the test solution. Others same as in Fig. 1.

Fig. 5 shows that the cathodic peak of Hb will increase and the anodic peak will decrease if H_2O_2 is added to the test solution. It is a characteristic of an electrochemically catalytic reaction. No similar phenomena happen when the bare PG electrode or the electrode modified alone with Hb or apoferritin are employed. So the catalytic reaction of H_2O_2 is due to the Hb co-modified onto the PG electrode surface, and apoferritin can make the protein exhibit this catalytic activity.

The relationship between the H_2O_2 concentration and the increase of the catalytic peak current has been shown in Fig. 6. It is linear in the range of the H_2O_2 concentration from 1.0×10^{-5} M to 4.0×10^{-3} M (the linear regression equation is $Y=6.533+0.09781\times X$, $R=0.9999$). It is observed that the upper limit of this linear range is much higher than the previous reports [25,28,29,34]. Usually, Hb cannot bear this high concentration of H_2O_2, and will lose most of its enzymatic activity. However, under the condition in our experiments, Hb finely retains its activity, indicating that Hb modified with apoferritin has a high stability. In other words, apoferritin can work as a stabilizer to retain Hb's catalytic ability under mal-condition.

4. Conclusion

In conclusion, rapid electron transfer reactions of Hb can be achieved with the help of apoferritin, as a bionanomaterial. Moreover, Hb as an enzyme exhibits fine electrocatalytic activity towards the reaction of H_2O_2, and a wide concentration range of linear relationship between the reduction peak current and the concentration of H_2O_2 has been obtained with a higher upper detection limit, which may be further developed for a

H_2O_2 biosensor. And this sensor can be used in a large pH range. Therefore, a new property of apoferritin has been explored, in which apoferritin works as a bionanomaterial to be an accelerant of the electron transfer of Hb and a stabilizer to retain the catalytic ability of the protein under mal-condition.

Acknowledgments

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