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The Electrochemical Characteristics of Multilayer Assembly of Hemoglobin and Polystyrene Sulfonate at Self-assembled Monolayer Surface

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Abstract: A multilayer film of hemoglobin (Hb) molecules and polyelectrolyte sulfonate were fabricated on a thiol self-assembled monolayers (SAMs) by electrostatic force. The Hb maintains electroactive property in the multilayer film, methylene blue (MB) incorporated into the multilayer can enhance the electron transfer rate between the Hb and the electrode surface.

Keywords: Multilayer film, hemoglobin, methylene blue.

It was well known that protein interacted strongly with natural and synthetic polyelectrolytes mainly through electrostatic forces to form a complex. These forces may lead to the formation of amorphous precipitates, protein assembly by the alternately electrostatic adsorption immobilize protein can be formed a multilayer film[1,2]. By preparation of anisotropic protein layer and precise control of the distance of active layer, sequential reaction and vectorial transfer of electron and energy become feasible target. In this work, we have prepared a multilayer assembled by the alternate electrostatic adsorption of hemoglobin (Hb) and ploystyrene sulfonate (PSS) at s thiol self-assembled monolayers surface. Hb in the multilayer film showed a well electrochemical response. Furthermore, electrochemical active dye molecule-methylene blue (MB) could be incorporated into the multilayer, it can enhance the electron transfer rate between the protein and electrode surface.

Experimental

All electrochemical measurements were performed on model 370 electrochemistry system (EG&G, USA) at 25 + 1℃ using three electrode system, a saturated calomel electrode (SCE) as the reference electrode, a platinum plate as counter electrode and a gold electrode (d=0.5mm) as working electrode.

The self-assembled monolayers of 2-aminoethanethiol (AET) on gold surface were formed by immersing gold electrode in a 2 mmol/L AET ethanol solution for 24 h. The AET SAMs electrode was immersed in 5% glutaric dialdehyde solution for 30 min, then immersed in 5 mg/ml Hb HAc-NaAc solution (pH=5.5, in this condition, the molecule of

Hb is positively charged ions) for 30 min, whereupon, Hb layers were attached to the SAM surface by glutaric dialdehyde, and the Hb layers were positively charged. The SAM–Hb electrode was immersed in HAc-NaAc buffer (pH=5.5) containing 5 mg/ml PSS for 30 min, the negatively charged PSS was formed. Subsequently, the multilayers of Hb/PSS could be formed by alternately immersing the electrode into aqueous solutions of Hb and PSS. Incorporating MB into Hb/PSS multilayer was prepared by immersing of the electrode in 0.01mol/L solution of MB for about 30min.

Result and Discussion

Figure 1a shows the cyclic voltammograms of SAMS/Hb/PSS electrode in HAc-NaAc buffer solution containing no Hb, a pair of redox peak is obtained. At sweep rate 20 mV/s, E_{pa}=-0.08V, E_{pc}=-0.30V, $E_{1/2}$ is -0.19V, which is the same as its formal potential[3], ΔE_p is 240 mV, indicates that the redox process is irreversible. **Figure 1b** shows the cyclic voltammograms of SAMs/PDDA/PSS electrode in the same solution, no redox peaks are obtained, this can confirm that the redox peaks are the redox reaction of Hb in the multilayer film, indicates that the Hb in the multilayer has electrochemical activity.`

At various scan rates, the peak currents are proportional to the scan rates over the range of 10 to 50 mV/s, indicated a surface adsorption property. The formal electron transfer rate constant heterogeneous (Ks) and the electrochemical transfer coefficients (α) could be calculated from the variations of the peak potential as a function of the sweep rates (υ)[4]. From the equation:

$E_a=E_0+RT/(1-\alpha)nF\bullet\ln[(1-\alpha)nF\upsilon/RTK_s]$ and $E_c=E_0-RT/\alpha nF\bullet\ln[\alpha nF\upsilon/RTK_s]$

Ks and α were found to be $8.47\times10^{-2}S^{-1}$ and 0.31, respectively. These results show an irreversible electron transfer kinetics of Hb in the multilayer film.

Figure 2 indicated that as the number of the multilayer increasing, the peak currents and the ΔEp increased. Integration under low sweep rates of the cathodic peaks provides that the amounts of Hb in the multilayer increase with addition of Hb layers. The redox peak currents of Hb increase with addition of Hb layer, this proves that Hb layer has been formed on the multilayer and the Hb at different layer can be reduced and oxidized during cyclic voltammetry. And the inreasing of the ΔEp proved that the electron transfer rate becomes slower with increasing distance between the Hb and the electrode surface. But there is no obvious changing of K_s and α when the number of the multilayers is lower than 10.

The cyclic voltammograms of MB-SAMs/Hb/PSS electrode is illustrated in **Figure 3**, a redox peak was obtained, at a sweep rate of 20 mV/s, E_{pa}=-0.16V, E_{pc}=-0.24V. The anodic peak potential has shifted to positive direction and the cathodic to negative direction, the anodic and cathodic currents increase, compared with **Figure 1**. These results prove that the catalytic reaction between the Hb and Mb has changed the electron self exchange rate of Hb in the multilayer and electron transfer of Hb at electrode. Ks and α were found to be $0.19S^{-1}$ and 0.39, respectively, these results shows a semi-reversible electron transfer kinetics of Hb in the multilayer film.

Figure 1 The cyclic voltammograms of SAMs/Hb/PSS(**a**) and SAMs/PSS/PDDA (**b**) electrode in HAc-NaA buffer solution scan rate 20mV/s.

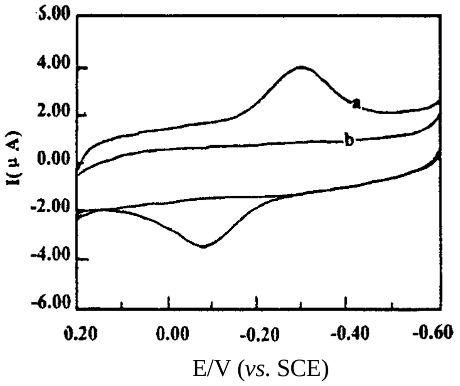
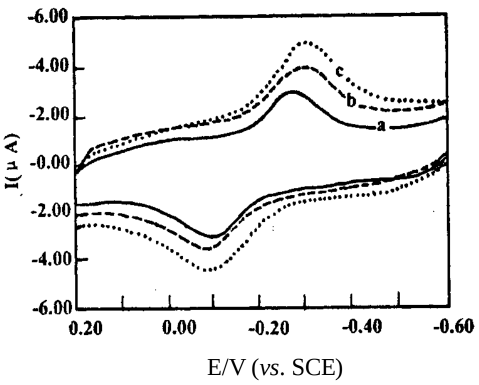
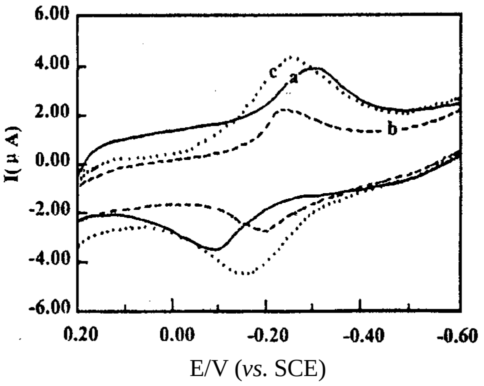


Figure 2 The cyclic voltammograms of SAMs/Hb/PSS electrode in HAc-NaAc buffer solution with various Hb layers: **a**: 3, **b**: 5, **c**: 10, scan rate 20mV/s



MB incorporated in PSS layer had a possibility to move in the multilayer film, and the electron self-exchange of MB in multilayers was easy. MB in PSS layer could be interacted with Hb, it could enhance the electron transfer rate of Hb at the electrode surface, or could promote the self-exchange rate of electron between the Hb at different layers. This result is similar to the Nafion modified electrode incorporated with MB had catalysis on Hb in solution[5,6]. MB can be used as a mediator to promote the electron transfer of Hb at the MB modified electrode surface [7].

Figure 3 The cyclic voltammograms of SAMs/Hb/PSS (**a**), MB-SAMs/PSS/PDDA (**b**) and MB-SAMs/Hb/PSS (**c**) electrode in HAc- NaAc buffer, scan rate 20 mV/S.



It is possible to use this method to design biosensor and to study the interaction between two or more protein species. MB incorporated in multilayer can enhance the electron transfer rate. This is another interesting way to study the electron transfer promotion.

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