Chinese Chemical Letters Vol. 11, No. 8, pp. 719–720, 2000

Enhanced Electron Transfer for Hemoglobin in $2C_{12}N^{+}PVS^{-}$ Composite Films Modified on Pyrolytic Graphite Electrodes

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Abstract: Stable thin films made from polyionic complex $2C_{12}N^{+}PVS^{-}$ with incorporated Hb on PG electrodes were characterized by electrochemistry. The electron transfer between Hb and PG electrodes was greatly facilitated in microenvironment of $2C_{12}N^{+}PVS^{-}$ films. The Hb-$2C_{12}N^{+}PVS^{-}$ films could be used to catalytically reduce trichloroacetic acid.

Keywords: Hemoglobin, poly(vinyl sulfate), didodecyldimethylammonium, surfactant-polymer composite films, electrochemistry, electrocatalysis.

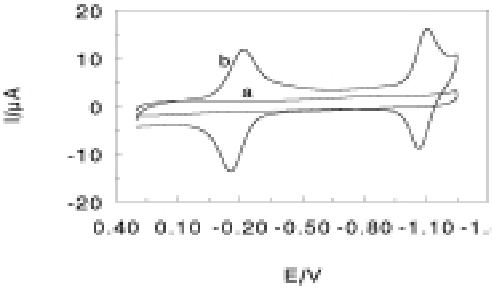
Some water-insoluble surfactants can be introduced onto surface of electrodes and form ordered bilayer structure by self-assembling. The films provide a biomembrane-like microenvironment which is favorable to electron transfer for some redox proteins on electrodes [1]. Surfactant-polymer multibilayer composite films show similar biomembrane-mimetic bilayer structure to surfactant films alone, but demonstrate advantages over the latter for their physically stronger solidity and stability because of introduction of polymer backbones[2]. In this paper, polyionic complex $2C_{12}N^{+}PVS^{-}$ was synthesized by reacting polyanionic poly(vinyl sulfate) with cationic surfactant didodecyldimethylammonium. Hemoglobin (Hb) incorporated in $2C_{12}N^{+}PVS^{-}$ films on pyrolytic graphite (PG) electrodes was characterized by electrochemistry.

Hb and poly(potassium vinyl sulfate) ($K^{+}PVS^{-}$) was from Sigma. Didodecyldimethylammonium bromide ($2C_{12}N^{+}Br^{-}$) was from Eastman Kodak. A CHI 660 electrochemical workstation (CH Instruments) was used for cyclic voltammetry (CV) and square wave voltammetry (SWV). A three-electrode cell was used with a saturated calomel electrode (SCE) as reference. The precipitate of $2C_{12}N^{+}PVS^{-}$ was formed immediately after mixing $K^{+}PVS^{-}$ solution with an aqueous dispersion of $2C_{12}N^{+}Br^{-}$ at room temperature. After centrifuging, washing and drying, the pure and dry solid powder of $2C_{12}N^{+}PVS^{-}$ was collected. The Hb-$2C_{12}N^{+}PVS^{-}$ films were prepared by first casting a few microliters of $2C_{12}N^{+}PVS^{-}$ in chloroform onto PG electrodes, after drying, a few microliters of Hb solution was then spread onto the $2C_{12}N^{+}PVS^{-}$ film surface. The water was then allowed to evaporate overnight.

Steady state CVs for Hb-$2C_{12}N^{+}PVS^{-}$ films in pH 5.5 buffers containing no Hb showed two pairs of well defined and nearly reversible redox peaks (**Figure 1**), indicating much faster direct electron transfer between Hb and PG electrode in $2C_{12}N^{+}PVS^{-}$ films than for Hb in solution at bare PG. The first pair of peaks centered at -0.19 V, characteristic of HbFe(III)/Fe(II) redox couple. The second peak pair at -1.08 V was most probably attributed to HbFe(II)/Fe(I) couple[3]. Since the first couple of peaks is better understood in Hb electrochemistry, it would be studied in more detail. The first peak pair showed approximately symmetric peak shape and nearly equal heights of cathodic and anodic peaks. The cathodic peak current increased linearly with scan rate from 0.1 to 2 V s^{-1}, characteristic of thin layer electrochemical behavior. Integration of the cathodic peaks at different scan rates gave nearly constant charge values, from which the surface concentration of electroactive Hb in the films, Γ^*, was estimated to be 1.22×10^{-10} mol cm^{-2}. The films showed very good stability for at least 10 days.

SWV was used to estimate formal potential (E°') and apparent heterogeneous electron transfer rate constant (k_s) for Hb-$2C_{12}N^+PVS^-$ films. The model used for nonlinear regression was a combination of thin-layer SWV theory and assumption of dispersion of formal potentials[4]. At pH 7.0, average k_s and E°' were estimated to be 29 s^{-1} and -0.19 V, respectively. CV peak potentials of Hb-$2C_{12}N^+PVS^-$ films shifted linearly with increasing pH between 4.0 and 8.0 with a negative slope, showing that the electron transfer of Hb is accompanied by proton transportation. When trichloroacetic acid (TCA) was added into pH 5.5 buffers, an increase in the HbFe(II) reduction peak at -1.08 V for the films was observed. This increase in reduction peak for HbFe(II) was accompanied by the disappearance of the HbFe(I) oxidation peak, indicating reaction of HbFe(I) with TCA in a catalytic cycle. Thus, Hb-$2C_{12}N^+PVS^-$ films may have the potential application as sensors to monitor some organohalides.

Figure 1. Cyclic voltammograms at 0.2 V s^{-1} in pH 5.5 buffers: (a) $2C_{12}N^+PVS^-$ films; (b) Hb-$2C_{12}N^+PVS^-$ films.



Acknowledgment

This work was supported by the National Natural Science Foundation of China (29975003).

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Received 18 February 2000