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Electrochemistry and Electrocatalysis with Hemoglobin in DHP-PDDA Surfactant-Polymer Multibilayer Composite Films

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Abstract: Polyionic complex DHP-PDDA was prepared by reacting anionic surfactant dihexadecyl phosphate (DHP) with polycationic poly(diallyldimethyl ammonium) (PDDA). Thin films made from DHP-PDDA with incorporated hemoglobin (Hb) on pyrolytic graphite (PG) electrodes were characterized by electrochemistry and other techniques.

Keywords: Hemoglobin, dihexadecyl phosphate, poly(diallyldimethyl ammonium), multibilayer composite films, electrochemistry, electrocatalysis.

Some synthetic surfactants can form ordered films featuring stacked bilayer by self-assembling. Direct electrochemistry of redox proteins in these biomembrane-like films can provide a good model for the study of redox process in biological system[1]. Surfactant-polymer composite films possess similar multibilayer structure to surfactant films alone, and have better stability over the latter because of introduction of polymer backbones in the films[2-4]. In this work, a new type of polyionic complex DHP-PDDA was prepared by reacting anionic double-chain surfactant DHP with polycationic PDDA. Hb-DHP-PDDA films on PG electrodes were characterized by various techniques.

Hb and DHP were from Sigma. PDDA was from Aldrich. Cyclic voltammetry (CV) and square wave voltammetry (SWV) were done with a CHI 660 electrochemical workstation (CH Instruments). The precipitate of DHP-PDDA was formed after mixing a PDDA solution with an aqueous dispersion of DHP at room temperature. The Hb-DHP-PDDA films were prepared by casting a few microliters of mixture of DHP-PDDA aqueous dispersion and Hb solution onto PG electrodes. All potentials were *vs.* SCE.

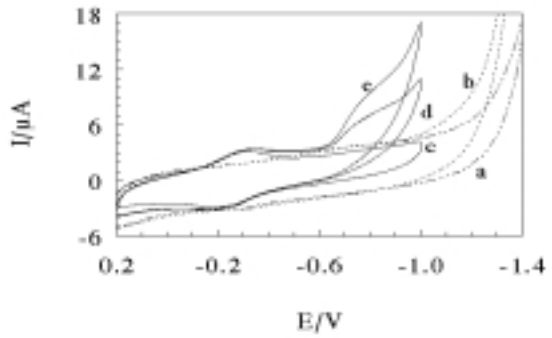
A pair of well-defined and quite reversible CV peaks was observed at about –0.26 V when a Hb-DHP-PDDA film electrode was immersed into a pH 5.5 buffer containing no Hb, characteristic of Hb heme Fe(III)/Fe(II) redox couple (**Figure 1c**). This indicates that direct electron transfer between Hb and PG electrode in DHP-PDDA film microenvironment was much fast than that for Hb in solution at bare PG. The reduction peak current had a linear relationship with scan rate between 0.05–2.0 V s^{-1}, suggesting the thin-layer electrochemical behavior. The films showed very good stability for at least 2 weeks. The apparent heterogeneous electron transfer rate constant (k_s) was estimated to be 30 s^{-1} at pH 7.0 by SWV from nonlinear regression analysis as described

previously[1]. The formal potential (E°′) of Hb-DHP-PDDA films shifted linearly with pH between pH 4.0 and 11.0, suggesting proton transformation is coupled with the reversible electron transfer in this pH range.

Both dry films cast from Hb and Hb-DHP-PDDA on transparent indium tin oxide coated slides showed spectroscopic Soret bands at 410 nm. When Hb-DHP-PDDA films were placed into buffers with different pH, the Soret band remained at 410 nm at pH 4.5–9.0, indicating that Hb in DHP-PDDA films retains its secondary structure similar to its native state in the medium pH range. The results of DSC and XRD suggest that DHP-PDDA films have an ordered multilayer structure in which surfactant DHP forms tail-to-tail bilayer sandwiched between PDDA backbones.

The Hb-DHP-PDDA films could catalyze reduction of nitrite. When NO_2^- was added into a pH 5.5 buffer, a new reduction peak at about –0.8V was observed (**Figure 1d**). The peak current increased with increasing the concentration of NO_2^- (**Figure 1e**). Compared to the reduction of NO_2^- on DHP-PDDA films without Hb (**Figure 1b**), the reduction overpotential of nitrite was lowered by Hb-DHP-PDDA films by at least 0.4 V. Hb-DHP-PDDA films could also catalyze reduction of trichloroacetic acid and oxygen, showing its potential application as biosensor to monitor some substrates.

Figure 1 Cyclic voltammograms at 0.1 s^{-1} in pH 5.5 buffers:



(a) DHP-PDDA film; (b) DHP-PDDA film in buffers containing 0.1 mmol L^{-1} NO_2^-; (c) Hb-DHP-PDDA film; (d) Hb-DHP-PDDA film in buffers containing 0.1 mmol L^{-1} NO_2^-; (e) Hb-DHP-PDDA film in buffers containing 0.2 mmol L^{-1} NO_2^-.

Acknowledgments

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References

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