

Direct Electrochemistry of Myoglobin in DDAB-Clay Composite Films

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Abstract: Ordered films were made by casting a mixture of aqueous dispersions of didodecyldimethylammonium bromide (DDAB)-clay composite and myoglobin (Mb) solution on pyrolytic graphite (PG) electrodes. The Mb-DDAB-clay film electrodes showed stable and reversible cyclic voltammetric responses in buffers and can catalyze the reduction of trichloroacetic acid (TCA).

Keywords: Myoglobin, didodecyldimethylammonium bromide, clay, composite film electrode, electrochemistry, electrocatalysis.

Direct electrochemistry of redox proteins can provide a good model for the studies of redox process in biological system. Electron transfer between redox metalloproteins and underlying electrodes was greatly enhanced in lamellar liquid crystal surfactant films compared to that on bare electrodes with the proteins in solution¹, because these films provide a favorable biomembrane-like microenvironment for the proteins². Ordered surfactant-clay composite films in which anionic clay platelet layers sandwich bilayers of cationic surfactants have similar structure and properties to the surfactant films and can also be made to contain redox centers or proteins³⁻⁵. The present work evaluates DDAB-clay films with incorporated Mb in order to achieve reversible redox transformation and electrochemical catalysis in stable films.

Mb was from Sigma. DDAB was from Eastman Kodak. Bentonite clay was from Southern Clay Products. Cyclic voltammetry (CV) was done with a model 173 potentiostat (PARC), Square wave voltammetry (SWV) was done with a CHI660 electrochemical workstation (CHI). DDAB-clay precipitate was prepared by reacting clay colloids dispersed in water with aqueous DDAB dispersion as described previously⁴. The films were prepared by casting a few microliters of mixture of DDAB-clay aqueous dispersion and Mb solution onto pyrolytic graphite (PG) electrodes. The water was then allowed to evaporate overnight. All the potentials reported are *vs.* SCE.

When a Mb-DDAB-clay film electrode was placed into a pH 5.5 buffer containing no Mb, a pair of well-defined and quite reversible CV peaks was observed at about -0.25V, characteristic of Mb heme Fe(III)/Fe(II) redox couple (Figure 1a). The reduction peak current at the steady state has a linear relationship with scan rate between 0.1 and 2.0 V s⁻¹, indicating the thin-layer electrochemical behavior. The films showed very good stability for at least 4-5 days. The average apparent heterogeneous electron transfer rate constant (k_s) was estimated to be 75 s⁻¹ at pH 7.0 by SWV from nonlinear regression analysis as described previously⁶.

The formal potential (E°) of Mb-DDAB-clay films, estimated as the midpoint of cathodic and anodic CV peak potentials, shifted linearly with pH between pH 5.5-11.0 with a slope of 53 mV pH⁻¹, close to the theoretical value of 58 mV pH⁻¹ at 20 °C,

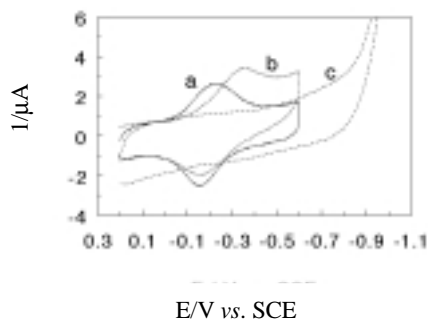
indicating one proton was coupled with one electron in the reversible electron transfer in this pH range.

Positions of the heme Soret absorption band of Mb provides information about possible denaturation of Mb. Both dry films cast from Mb alone and Mb-DDAB-clay on transparent indium tin oxide glasses showed Soret bands at 408 nm. When Mb-DDAB-clay films were placed into buffer solutions with different pH, the Soret band was found to remain at 408 nm between pH 4.5 and 9. All these results indicate that Mb in DDAB-clay films has a secondary structure similar to the native state of Mb in medium pH range.

The results of differential scanning calorimetry (DSC) and X-ray diffraction (XRD) suggest that DDAB-clay films have an ordered multilayer structure similar to the "all organic" DDAB films and the incorporation of Mb does not disturb this structure.

The Mb-DDAB-clay films can catalyze the reduction of TCA (Figure 1). When TCA was added into a pH 5.5 buffer, an increase in the MbFe(III) reduction peak was observed, accompanied by the decrease or disappearance of MbFe(II) oxidation peak (Figure 1b). Comparing to the direct reduction of TCA on DDAB-clay films (Figure 1c), the Mb-DDAB-clay films lowered the overpotential by about 0.7 V. Thus, Mb-DDAB-clay films may have the potential application as sensors to monitor some environmental pollutants.

Figure 1. Cyclic voltammograms at 0.08 V s^{-1} in pH 5.5 buffers: (a) Mb-DDAB-clay film; (b) Mb-DDAB-clay film in buffers containing 0.07 mol/L TCA; (c) DDAB-clay film in buffers containing 0.07 mol/L TCA.



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