Direct Electrochemistry of Hemoglobin in Layer-by-layer $\{PDDA/Hb\}_n$ Films Assembled on Pyrolytic Graphite Electrodes

Ping Li HE, Nai Fei HU*

Department of Chemistry, Beijing Normal University, Beijing, 100875

Abstract: Layer-by-layer $\{PDDA/Hb\}_n$ films were assembled by means of alternate adsorption of positively charged poly(diallyldimethyl ammonium) (PDDA) and negatively charged hemoglobin (Hb) at pH 9.2 from their aqueous solutions on pyrolytic graphite (PG) electrodes. Film growth during adsorption cycles was demonstrated by cyclic voltammetry and UV-Vis spectroscopy. Direct electrochemistry of Hb in $\{PDDA/Hb\}_n$ films on PG was studied.

Keywords: Hemoglobin, poly(diallyldimethylammonium), layer-by-layer films, direct electrochemistry, electrocatalysis.

The principle of alternate adsorption can be used to design and control specific molecular architectures[1]. Protein-polyion layer-by-layer assembly opens a possibility of organizing proteins with specific molecular architectural plan[1] and studying redox proteins with electrochemical methods[2]. Recently, we reported electrochemistry of layer-by-layer $\{PSS/Mb\}_n$ films grown on PG electrodes[1]. In this work, layer-by-layer $\{PDDA/Hb\}_n$ films were fabricated on PG electrodes. Electrochemistry and electrocatalysis with $\{PDDA/Hb\}_n$ films were characterized.

Hb was from Sigma. PDDA was from Aldrich. Cyclic voltammetry (CV) and square wave voltammetry (SWV) were run with a CHI660 electrochemical workstation (CHI). All potentials were *vs* SCE. PG electrodes were first immersed in polycationic PDDA solutions for 20 min. After being washed with water, the electrodes were then soaked in pH 9.2 buffers containing Hb for 20 min, where Hb had negative surface charges with its isoelectric point (pI) at 6.8. This cycle was repeated to make $\{PDDA/Hb\}_n$ multilayer films. Before running CV, an extra monolayer of PDDA was adsorbed as the outmost layer to protect the protein.

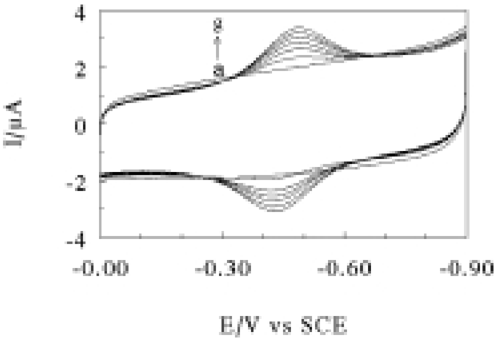
Cyclic voltammetry of $\{PDDA/Hb\}_n$ films showed a pair of reversible peaks for Hb Fe(III)/Fe(II) redox couple at –0.48 V in pH 9.2 buffers containing no Hb. The reduction and oxidation peak currents increased with the number of adsorbed {PDDA/Hb} bilayer (*n*) at first (**Figure 1**). After *n* exceeded 8, the peak currents showed little increase. This indicates that direct electron transfer processes are predominantly limited by the distance between Hb and the electrode surface. The reduction peak current of $\{PDDA/Hb\}_8$ films had a linear relationship with scan rate in the range of 0.05-2.0 V s^{-1}, suggesting the thin-layer electrochemical behavior. The $\{PDDA/Hb\}_8$ films showed good stability in the buffers for at least one month. The apparent heterogeneous electron transfer rate constant (k_s) and formal potentials ($E°'$) were estimated to be 65 s^{-1} and –0.342 V, respectively, at pH 7.0 by SWV from nonlinear regression analysis. $E°'$ of $\{PDDA/Hb\}_8$ films shifted linearly with pH between 5.0 and

12.0 with a slope of 50 mV pH^{-1}, indicating one-proton coupled single electron transfer in this pH range for Hb.

UV-Vis absorption spectroscopy was also used to monitor the process of layer-by-layer assembly. Soret absorption band of Hb at 405 nm increased linearly with *n*, indicating that the absorption process was completely reproducible from layer to layer. When $\{PDDA/Hb\}_8$ films were placed into buffers with different pH, the Soret band remained at 405 nm at pH 4.5 - 10.0. Reflective absorption infrared spectroscopy was used to monitor conformational changes for Hb in the films. The shapes of amide I and amide II bands at 1350–1800 cm^{-1} were almost the same for Hb and $\{PDDA/Hb\}_8$ films. All these results indicate that Hb in $\{PDDA/Hb\}_n$ films has a secondary structure similar to its native state.

The $\{PDDA/Hb\}_8$ films could catalyze reduction of trichloroacetic acid (TCA). When TCA was added into a pH 5.5 buffer, an increase in Hb Fe(III) reduction peak at –0.30 V was observed, accompanied by the decrease or disappearance of Hb Fe(II) oxidation peak. The reduction peak current increased with concentration of TCA. Compared to the reduction peak potential of TCA on a PDDA monolayer at –1.1 V, the $\{PDDA/Hb\}_8$ films lowered the reduction overpotential of TCA by 0.8 V. $\{PDDA/Hb\}_8$ films could also catalyze reduction of NO_2^- and oxygen, showing its potential application as biosensor to monitor some substrates.

Figure 1 Cyclic voltammograms for layer-by-layer $\{PDDA/Hb\}_n$ films at scan rate of 0.2 V s^{-1} in pH 9.2 buffer solutions with different numbers of bilayers.



(a) PDDA monolayer; (b) n=1; (c) n=2; (d) n=3; (e) n=4; (f) n=6; (g) n=8.

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