Electrochemistry of Self-assembled Ultra-thin Films Composed of Chloroperoxidase and Polyions

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Direct electron transfer between chloroperoxidase (CPO) from Caldariomyces fumago and gold electrode has been achieved by layer-by-layer adsorption of CPO and polydimethyldiallylammonium chloride (PDDA) monolayers on a gold electrode. QCM frequency shift demonstrated monolayer formed during each adsorption. Electrochemical characterization studies show thin layer properties of these PDDA–CPO–PDDA films. The PDDA–CPO–PDDA ultra-thin films were stable, up to 97% electrochemical activity remained after two days storage in phosphate buffer pH 5.

Chloroperoxidase excreted by the filamentous fungus Caldariomyces fumago (EC1.11.1.10) is a versatile monomeric hemoenzyme with a molecular weight of 42,000 g mol^{-1}. Hybrid structure of two different significant enzyme families, peroxidase and cytochrome P450s, offers CPO fascinating features cross over the catalytic boundaries of other oxidative hemoproteins and perform multiple functions, such as heteroatom oxidation, epoxidations, hydroxylation, and oxidation of alcohols and indole, even metabolism of hydrophobic drugs, carcinogens, and other foreign compounds like cytochrome P450 enzymes.[1–3]

Iler proposed a novel technique for film assembly by sequential adsorption of oppositely charged colloidal particles in 1966.[4] 25 years later, Decher et al. extended this pioneering work to a combination of linear polycations and polyanions as a novel preparative technique of molecular multilayers.[5,6] This method features excessive adsorption (more than neutralization) at every stage of the polycation/polyanion assembly, which led to the recharging of the outermost surface at every step of the film formation. This method has been successfully used for mimics of direct electron transfers in biological systems, which is usually inhibited by following factors which includes protein surface denaturation, lack of electrical access to prosthetic groups, or unfavorable orientation of proteins at electrode. To our knowledge, no direct electron transfer on the CPO from Caldariomyces fumago has been reported previously.

Layered polycation–CPO films were grown on Au treated with 3-mercaptopropanesulfonic acids, sodium salt (MPS) ethanol solution, to provide an even negatively charged surface, which took approx 16 h. The MPS/Au modified electrodes were immersed into 3 mg mL^{-1} polydimethyldiallylammonium chloride (PDDA, MW 90,000) for adsorption of PDDA layer. Subsequent washing with deionized water was taken to remove excessive PDDA on the electrode surface. The PDDA–MPS/Au electrodes were immersed into pH 5 buffer containing CPO (0.2 mg mL^{-1}), and a layer of negatively charged CPO was adsorbed. After washing, an outer PDDA was added. Adsorption times were 30 min for PDDA at ambient temperature and 15 h for CPO at 4 °C. For comparison, a bare Au electrode was immersed into CPO solution (pH 5) for 15 h at 4 °C to obtain a CPO modified Au electrode.

Mass and thickness of individual layers were estimated for dry films with a quartz crystal microbalance (QCM, SEIKO EG&G with a 9 MHz AT cut resonator, Japan) and averaged layer thicknesses were 0.7 nm for MPS, 0.9 nm for PDDA, 1.9 nm for CPO.[7] Compared to the molecular dimensions of PDDA and crystallized CPO enzyme molecules, formation of monolayer at each step was confirmed,[8] a monolayer of CPO enzyme lies on the monolayer of PDDA and the surface concentration of CPO was 1.05×10^{-10} mol cm^{-2} obtained by QCM.

A Model 273 Potentiostat/Galvanostat (EG&G PARC, U.S.A.) was employed for cyclic voltammetric studies. A three-electrode system used for cyclic voltammetry consists of a nanofilm-coated gold resonator working electrode, a platinum plate counter electrode, and a saturated calomel reference electrode (SCE). In order to obtain oxygen-free condition, all the buffer solutions were purged thoroughly with pure nitrogen prior to electrochemical measurements and maintained under the nitrogen atmosphere during the experiments. Temperature was 25 ± 0.5 °C.

Figure 1 shows cyclic voltammograms of PDDA–CPO–PDDA, PDDA on the MPS-modified Au electrodes and the CPO/Au electrode in phosphate buffer solution, pH 5. A pair of well-defined redox peaks was observed for PDDA–CPO–PDDA film at a scan rate of 100 mV s^{-1}. The oxidation–reduction peaks with equal height but with around 138 mV peak separation suggest quasi-reversible electron transfer in the

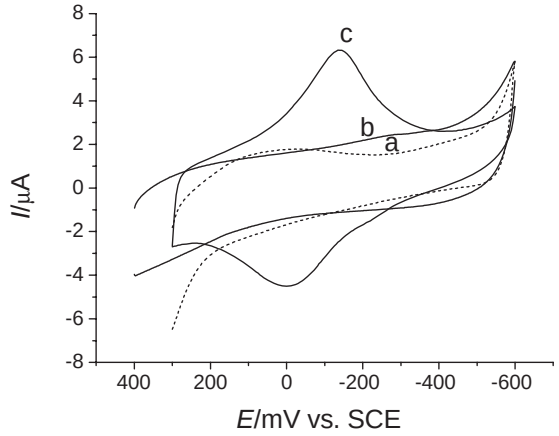

Figure 1. Cyclic voltammograms at the scan rate of 100 mV s^{-1}: (a) CPO/Au electrode, (b) PDDA–MPS/Au, (c) PDDA–CPO–PDDA–MPS/Au in anaerobic 50 mmol L^{-1} phosphate buffer solution containing 0.1 mol L^{-1} KCl, pH 5.

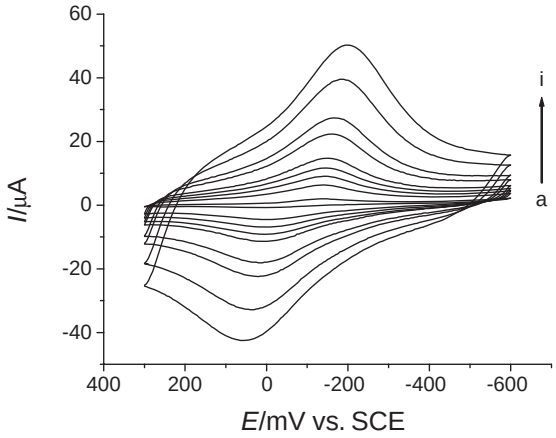

Figure 2. Cyclic voltammograms of PDDA–CPO–PDDA–MPS/Au at the scan rates of 25, 100, 150, 200, 250, 400, 500, 750, and 1000 mV s^{-1} (a–i) in anaerobic 50 mmol L^{-1} phosphate buffer solution containing 0.1 mol L^{-1} KCl, pH 5.

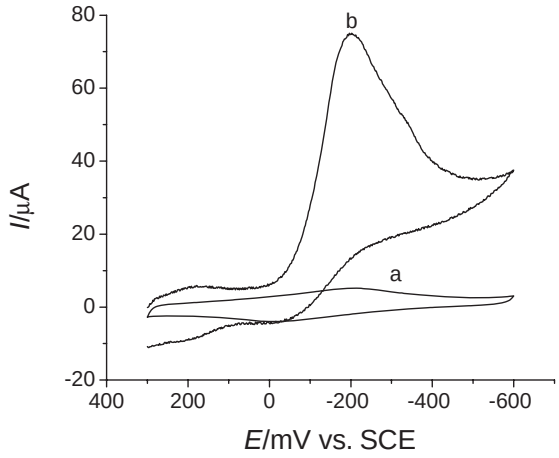

Figure 3. Cyclic voltammograms of PDDA–CPO–PDDA–MPS/Au electrode at the scan rate of 100 mV s^{-1} in 50 mmol L^{-1} phosphate buffer solutions containing 0.1 mol L^{-1} KCl, pH 5 (a) O_2 free, (b) O_2-saturated.

ultra-thin PDDA–CPO–PDDA films. Midpoint potential based on the averaged oxidation and reduction peak potentials was estimated to be −68 mV. Compared to −30 mV, the midpoint potential obtained from mediated optical redox titration,[9] the redox potential difference may be contributed to the environmental variation around the electroactive heme of CPO. In contrast, no reduction and oxidation peaks were observed for bare Au electrode in CPO solution, implying no or extremely slow electron transfer between the gold electrode and the CPO. Also, no electron transfer was observed for PDDA–MPS/Au modified electrodes. Integration of the Fe^{III} reduction peaks of PDDA–CPO–PDDA films was used with Faraday's law to estimate surface concentrations of electroactive CPO in the films. The CPO surface concentration was 1.04×10^{-10} mol cm^{-2}, close to 1.05×10^{-10} mol cm^{-2} obtained by QCM, which strongly suggests that almost all of the CPO molecules adsorbed are electroactive.

Figure 2 shows cyclic voltammograms of PDDA–CPO–PDDA at an MPS-modified Au electrode at different potential sweep rates. The plot of logarithm reduction peak currents vs logarithm scan rates from 0.025 to 1.0 V s^{-1} give a slope of 0.92, close to 1, characteristic of diffusionless voltammetric behavior within monolayer films of PDDA–CPO–PDDA formed on the MPS-modified Au electrode. The stability of PDDA–CPO–PDDA on the MPS-modified Au electrode was also evaluated by cyclic voltammetry. The results obtained show that after two days storage in the pH 5 phosphate buffers, the cyclic voltammetry of the ultra-thin films still provided 97% of the original peak area as well as the stable peak potentials, suggesting that successful CPO immobilization was achieved.

Figure 3 shows the typical cyclic voltammograms of PDDA–CPO–PDDA films in buffer solutions with and without oxygen. In a volume of 8 mL of O_2-saturated phosphate buffer solution, the PDDA–CPO–PDDA films gave greatly increased Fe^{III} reduction peaks accompanied by the disappearance of Fe^{II} oxidation peaks due to the CPO–Fe^{II} reaction with O_2. This is consistent with catalytic reduction of oxygen to hydrogen peroxide as reported previously.[10] Hydrogen peroxide formed at the electrode further oxidizes the CPO–Fe^{III} into the high valent oxo-ferryl species.[11] At the end of the first cycle of the catalytic reaction, the high valent oxo-ferryl species returns to the CPO–Fe^{III} for the next cycle reaction. Thus, if the potential is held at −0.6 V, H_2O_2 will be continuously generated at the electrode as long as there is oxygen in the phosphate buffer solution. This interesting discovery could provide a unique approach for CPO-catalyzed bioconversions. Compared to traditional design, our reaction system is simple and cost-effective. Because CPO enzymes are immobilized on the electrode, the enzymes can be reused many times for catalytic reactions. More significantly, the in-situ continuous generation of H_2O_2 completely replaces the external addition of H_2O_2 for conventional method, which eliminates the inactivation of CPO due to the excessive addition of H_2O_2 reported previously.[12,13] Therefore, enzyme stability and total turnover number (TTN) for CPO-catalyzed reactions would be improved significantly.

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