Direct Electron Transfer of Hemoglobin Molecules on Bare ITO Electrodes

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Direct electron transfer of hemoglobin (Hb) molecules was found at bare indium–tin–oxide (ITO) electrodes without promoters and mediators or modifications of electrodes by cyclic voltammetry. The quantity of electroactive Hb molecules was close to that of monolayer deposition on the electrode surface. The peak current, indicating redox reaction of a heme Fe^{III}/Fe^{II} couple, changed in proportion to sweep rate.

The redox-active protein molecules on electrodes are one of the important materials for the development of biosensors, bioelectrocatalytic systems, and further bioanalytical devices.[1] However, directly adsorbed protein molecules on metal electrodes have been known to show a slow electron transfer because electroactive groups should be embedded deeply in the protein structure or strong adsorption onto the metal surfaces should bring about their denaturation.[1] Thus, the improvement of electron-transfer characteristics of proteins on the electrode surfaces is most important subjects in this field. Currently, there are a number of reports for retaining the ability of heme proteins by using promoters and mediators or modifications of electrodes.[2]

Hemoglobin (Hb) has been widely investigated in protein electrochemistry.[2,3] Hb, contained in a red blood cell, has four iron-bearing heme groups, consisting of two α and two β subunits with polypeptide chains. The heme sites in Hb molecules are known to act as the electron-transfer centers. Recently, Durrant and co-workers reported that nanoporous metal oxide electrodes, such as titanium oxide, TiO_2, and tin oxide, SnO_2, allow the direct electron transfer of Hb without the addition of any promoters and mediators.[3] In other heme protein, cytochrome *c* has been known to show the electrochemical response at bare indium–tin–oxide (ITO) electrodes.[4,5] However, so little report included for the Hb electrochemistry at ITO electrodes even in recent years.[6] In this study, we investigated the electrochemical response of Hb molecules on bare ITO electrodes, which were formed by vapor deposition on a glass substrate ($0.05 \times 20 \times 50\,mm^3$, MatsunamiGlass, Japan),[7,8] by using cyclic voltammetric techniques.

The ITO film surface was cleaned with acetone and ethanol and then washed with Milli-Q water (resistivity $> 18\,M\Omega\,cm$). The surface area of the bare ITO working electrode was standardized with leak-proof silicone rubber solution cell to be $1.23\,cm^2$. A bare gold (Au) electrode sputtered on the slide glass substrate was also prepared to confirm whether the Hb molecules exhibit any electrochemical response or not. The Au electrode was soaked in 50/50 sulfuric/nitric acid solution for 1 h, then cleaned with water and ethanol, and then stored in water. The electrode potential was controlled with a potentiostat (EG&G Princeton Applied Research, Model 273). A platinum wire and an Ag/AgCl electrode were used as a counter and a reference electrode, respectively. All the potentials in this paper are quoted against Ag/AgCl sat. KCl.

Bovine Hb ($M_W = 64{,}500$) and $1 \times 10^{-2}\,mol\,dm^{-3}$ phosphate buffered saline (PBS, pH 7.4) were purchased from Sigma and Wako Pure Chemical Industries, Ltd., respectively, and were used without further purification. The concentration of Hb was adjusted to $1 \times 10^{-4}\,mol\,dm^{-3}$ using the PBS solution. All experimental solutions were deaerated by bubbling nitrogen for at least 0.5 h, and a nitrogen atmosphere was kept over the solutions during measurements.

Figure 1 shows the cyclic voltammograms (CVs) of bare ITO electrodes in Hb absent and present solutions. A curve recorded in PBS solution, i.e., in Figure 1a, indicates the non-Faradaic current. A pair of redox peaks, at around $-0.2\,V$ in the cathodic sweep and at around $-0.1\,V$ in the anodic sweep, was found in Hb-containing solution. It indicates that Faradaic current attributed to the redox couple of heme Fe(III)/Fe(II) in Hb molecules flowed at the non treated bare ITO electrode.

The quantity of electrochemically active Hb molecules can be estimated from the integration of the charge, Q, in CVs according to the equation: $Q = nFA\Gamma^*$, where n is the number of electron transferred per protein molecule, F is Faraday constant, A is the surface area of electrode, and Γ^* is concentration of the protein molecule.[9] The concentration of electrochemically active Hb molecules was estimated to be about $3.1 \times 10^{-12}\,mol\,cm^{-2}$, assuming $n = 4$.[2,9] On the other hand, the monolayer coverage on the electrode surface can be calculated to be $8.0 \times 10^{-12}\,mol\,cm^{-2}$, assuming one protein molecule occupied $20\,nm^2$.[10] These results suggested that the directly adsorbed Hb

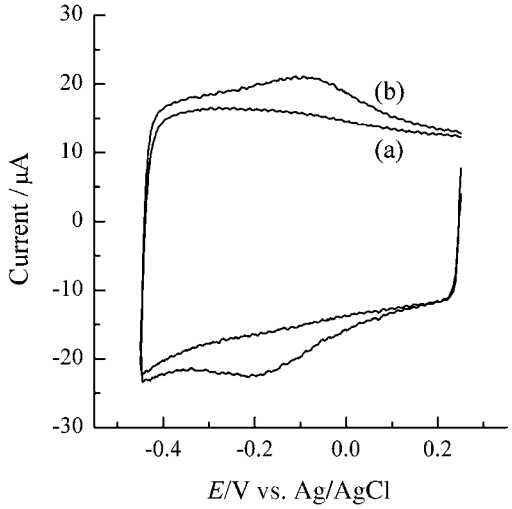

Figure 1. CVs of bare ITO electrodes in: (a) $1 \times 10^{-2}\,mol\,dm^{-3}$ PBS solution (pH 7.4) and (b) $1 \times 10^{-2}\,mol\,dm^{-3}$ PBS solution (pH 7.4) containing $1 \times 10^{-4}\,mol\,dm^{-3}$ Hb. Sweep rate: $1.0\,V\,s^{-1}$.

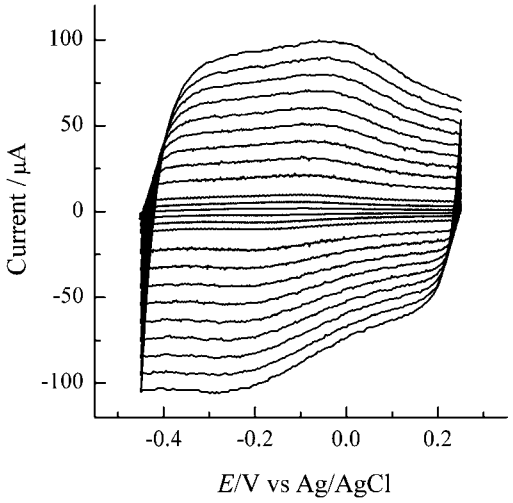

Figure 2. CVs of bare ITO electrodes with different sweep rates in 1×10^{-2} mol dm^{-3} PBS solution (pH 7.4) containing 1×10^{-4} mol dm^{-3} Hb. Sweep rate: 0.1, 0.3, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0 V s^{-1}.

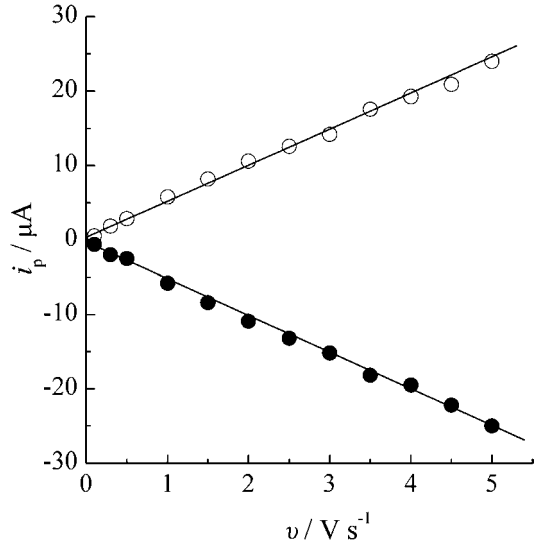

Figure 3. Dependence of anodic (○) and cathodic (●) peak current on sweep rate. Experimental conditions are the same as in Figure 2.

molecules on the bare ITO electrode surface were electrochemically active species.

Figure 2 shows the CVs recorded with different sweep rates from 0.1 to 5.0 V s^{-1}. At lowest sweep rate, i.e., 0.1 V s^{-1}, the smallest redox peak current is observed in Figure 2, and the CVs curve with the largest redox peak current corresponds to that observed at fastest sweep rate, i.e., 5.0 V s^{-1}. The formal potential, $E^{\prime 0}$, taken by the mid-point of the anodic and cathodic peak potentials in CVs with 0.1 V s^{-1}, was -0.157 V. The peak separation was -0.076 V, indicating a fast electron-transfer process.

The anodic and cathodic peak currents, i_{pa} and i_{pc}, increased with increasing sweep rates as seen in Figure 2. When the peak currents were plotted against the sweep rate, a direct linear relationship was obtained as shown in Figure 3, indicating a surface-controlled electrode process. These results prove that the nontreated bare ITO electrode generates the electron transfer of heme Fe(III)/Fe(II) in Hb molecules and indicate that the electrochemically active species were Hb molecules adsorbed on the ITO electrode surface.

On the other hand, no oxidation and reduction peak currents due to the redox couple of Fe(III)/Fe(II) in Hb were observed in CVs with any sweep rates on bare Au electrodes.

We demonstrated that Hb molecules on the non-treated bare Au electrode surface were electrochemically inactive, whereas CVs clearly displayed a pair of redox peaks when Hb molecules were adsorbed on the nontreated bare ITO electrode surface. The origin of the electrochemical activity of Hb molecules on the electrode surfaces will be presented elsewhere. This finding was an indication of the great advantages for the field of bioelectronics and widespread use of ITO electrodes.

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