A Novel Biofuel Cell Based on Direct Electron Transfer Utilizing Enzymatic Activity of Hemoglobin at Indium–Tin–Oxide Electrodes in Cathodic Process

Yusuke Ayato and Naoki Matsuda*

National Institute of Advanced Industrial Science and Technology, AIST, 807-1 Shuku-machi, Tosu 841-0052

(Received January 21, 2009; CL-090073; E-mail: naoki.matsuda@aist.go.jp)

A novel direct electron-transfer (DET)-based biofuel cell was established using indium–tin–oxide (ITO) substrates and hemoglobin (Hb) as the electrocatalyst for hydrogen peroxide (H_2O_2) in cathodic reaction systems. The anodic reaction utilized the oxidation of hydrogen (H_2) at platinum (Pt), and a phosphate buffered saline (PBS) with pH 7.4 solution was employed as the electrolyte in this study. The observed open-circuit voltage (OCV) and the maximum power density in the assembled single cell at room temperature were 0.34 V and 0.15 $\mu W\,cm^{-2}$, respectively.

Biofuel cells are candidates for clean and renewable energy conversion systems typically utilizing the electrocatalytic activity of microorganisms or enzyme proteins.[1] A number of biofuel cells have been established; however studies of them have been mainly conducted with mediated electron transfer (MET) at the electrodes, especially at the anode, even in recent years.[2] It is believed that most of the enzyme proteins, which have the electrocatalytic activity of fuel cell reactions, show a slow electron transfer between the reaction site in the protein and the electrode. Thus, research has mainly focused on mediators or promoters carrying the electron between proteins and the electrodes and the modification or immobilization of proteins on the electrode surfaces. Hence, few reports include the development of direct electron-transfer (DET)-based biofuel cells.[3]

Hemoglobin (Hb) has been widely investigated in protein electrochemistry.[4–7] The heme sites in Hb molecules are known to act as the electron-transfer centers. In the early stages of investigation, heme protein molecules, such as Hb, have also been believed to show slow electron transfer at electrode/electrolyte interfaces. However, recently we demonstrated that bare indium–tin–oxide (ITO) electrodes allow the DET of Hb without the addition of any promoters or mediators.[8,9] In addition, the electrocatalytic activity of Hb for hydrogen peroxide (H_2O_2) has been observed at several modified or immobilized electrodes.[5] In this study, we developed a novel DET-based biofuel cell reducing H_2O_2 to water at the cathode using Hb–ITO systems.

Bovine Hb ($M_W = 64500$), 39 mM phosphate buffered saline (PBS, pH 7.4), and 30% H_2O_2 were purchased from Sigma, Kanto Chemical Co., Inc., and Wako Pure Chemical Industries, Ltd., respectively, and were used without further purification. The concentrations of Hb and H_2O_2 solutions were adjusted using the PBS solution to 5 μM and 1.8 mM, respectively. 5 μM Hb and 1.8 mM H_2O_2 PBS solution was prepared by mixing the same volume of 10 μM Hb and 3.6 mM H_2O_2 containing PBS solutions. All experimental solutions were deaerated by bubbling nitrogen for at least 0.5 h. The ITO film surface was cleaned with acetone and ethanol and then washed with Milli-Q water (resistivity >18 MΩ cm). The electrocatalytic activity of Hb for H_2O_2 was investigated by a three-electrode system using ITO, platinum (Pt) wire, and silver–silver chloride (Ag/AgCl) saturated KCl electrodes as the working, counter, and reference electrodes. The electrode potential was controlled by a potentiostat (ALS/CHI Model 720C, BAS). A single biofuel cell was assembled and the cell performance was tested using current sweep on a Solartron SI 1287 Electrochemical Interface.

We have demonstrated that the bare ITO electrodes induced the direct electron transfer of Hb molecules in PBS (pH 7.4) solutions.[8,9] We confirmed a pair of redox peak current due to heme Fe^{III}/Fe^{II} of Hb in CV with 0.5 $V\,s^{-1}$ at around −0.19 V in the cathodic sweep and at around −0.12 V in the anodic sweep regions in a 5 μM Hb containing solution (data not shown). We performed further CV measurements in Hb and H_2O_2 PBS solutions. Figure 1 shows the CVs at the ITO electrode in the presence and absence of Hb and H_2O_2 in PBS solution. The reduction current of H_2O_2 was observed at the ITO electrode only; however, remarkably large reduction current was observed in the negative potential region from around 0.05 V in the Hb containing PBS solution. These results revealed that the Hb molecules exhibited electrocatalytic activity for H_2O_2 in the PBS solution at the ITO electrode.

The observed high electrocatalytic activity of Hb for H_2O_2 allowed us to develop a novel DET-based biofuel cell consisting of a hydrogen (H_2) oxidation anode and a H_2O_2 reduction cathode utilizing a Hb–ITO electrode system. The open-circuit voltage (OCV) and the power density can be expected around 0.7 V and 10^{-6} $W\,cm^{-2}$, respectively, because the oxidation potential of H_2 should be −0.64 V vs. Ag/AgCl in pH 7.4 solution and

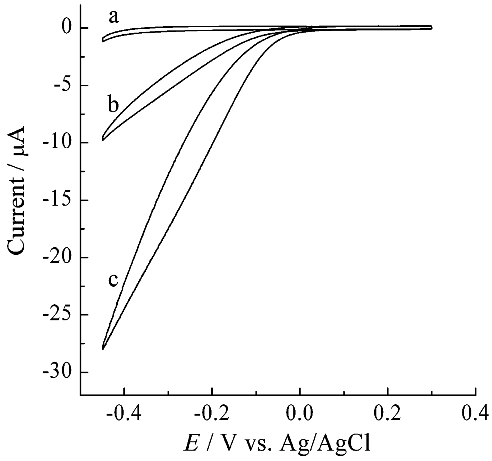

Figure 1. CVs of ITO electrodes in: (a) 39 mM PBS solution (pH 7.4), (b) 39 mM PBS solution (pH 7.4) containing 1.8 mM H_2O_2, and (c) 39 mM PBS solution (pH 7.4) containing 1.8 mM H_2O_2 and 5 μM Hb. Sweep rate and geometric surface area are 0.01 $V\,s^{-1}$ and 1.13 cm^2, respectively.

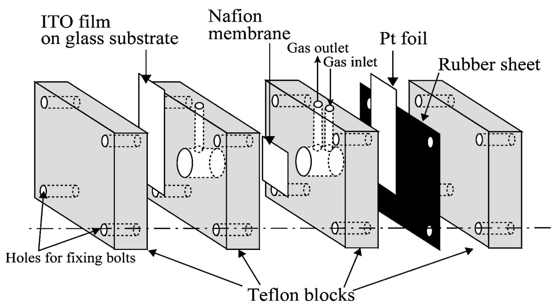

Figure 2. Illustration of single cell components.

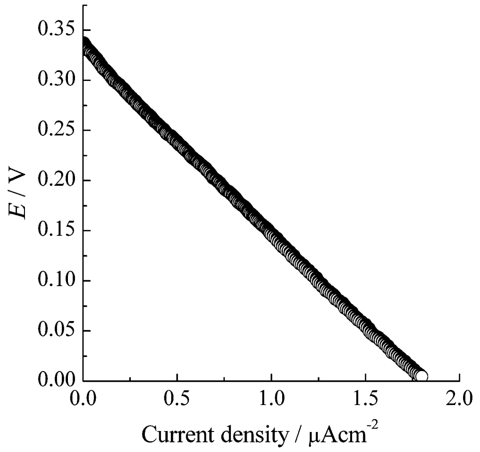

Figure 3. The current–potential curve observed using current sweep in the assembled single cell. Sweep rate is 1 nA s^{-1}.

the H_2O_2 reduction at the biofuel cell cathode will be the rate-determining step.

The single cell of biofuel cell was assembled as shown in Figure 2. A Pt foil and the ITO film were used as the anode and cathode electrodes, respectively. Nafion® membrane (DuPont) is a well-known cation-exchange membrane in polymer electrolyte fuel cells,[10,11] and was employed to maintain ion transport and prevent the incorporation of Hb molecules into the anode. The Nafion® membrane was cut into 20 × 20 mm^2 pieces and was placed between two central Teflon blocks as shown in Figure 2. After assembling the cell, the 10-mm diameter cylindrical center holes in the central two Teflon blocks were filled with PBS solution at the anode and 5 μM Hb and 1.8 mM H_2O_2 PBS solution at the cathode. H_2 gas was produced in a water electrolysis cell and provided to the anode by bubbling the solution.

Figure 3 shows a current–potential curve recorded with a sweep rate of 1 nA s^{-1} in a homemade single cell. The overvoltage from the expected OCV might be caused by the cross-over phenomena of H_2 and H_2O_2; however, the OCV of 0.34 V was obtained in the single cell assembled in this study, as seen from the zero intercept in Figure 3. The power density was calculated from the data of the current–potential curve and plotted in Figure 4. The maximum power density of 0.15 μW cm^{-2} was obtained at around 0.9 μA cm^{-2}. These results proved that this type single cell worked as a biofuel cell.

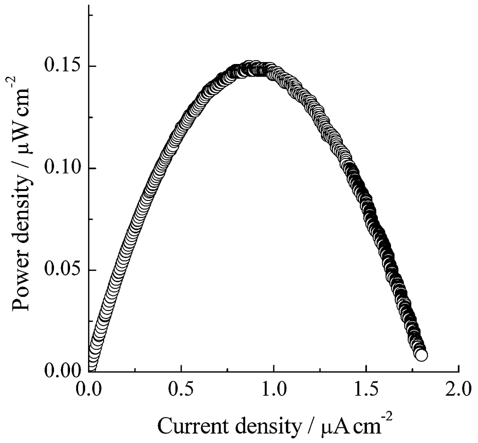

Figure 4. Characteristics of the power density for the assembled single cell.

We successfully demonstrated that Hb molecules retain electrochemical and electrocatalytic activity at the bare ITO electrode without any promoters or mediators. The novel DET-based biofuel cell was developed, and the practical potential of this type of cell was predicted in this study. Further work will improve the cell performance elsewhere; however, we demonstrated that this type biofuel cell operated with simple electrode structure in neutral pH at room temperature.

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