Improved Performance of Gas-diffusion Biocathode for Oxygen Reduction

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ABSTRACT

The current density of gas-diffusion biocathodes for the oxygen reduction in biofuel cells was successfully increased by adjusting the hydrophobicity of porous carbon electrodes and by adding small amount of non-ionic surfactant to enzyme solution to be used in the enzyme adsorption process. Optimization of the hydrophobicity was performed with non-glycoprotein copper efflux oxidase as an electrocatalyst. Carbon slurry was prepared by mixing Ketjen black and polytetrafluoroethylene in 2-propanol at a weight ratio of 3:2. Carbon paper was modified with the slurry and dried at 60°C to remove the solvent. Addition of small amount of non-ionic surfactant such as Triton X-100 (about 0.01%) into the enzyme solution was very effective to adsorb the enzyme on the hydrophobic porous carbon surface. The proposed method is also effective for glycoprotein multi-copper oxidase to fabricate high performance gas-diffusion biocathode.

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1. Introduction

Biofuel cells are new electric devices which convert the energy of the redox reaction between oxygen and several organic compounds such as sugars and organic acids into the electric energy by utilizing enzymes as electrocatalysts.^{1–4} In order to catalyze four-electron reduction of dioxygen at cathode of biofuel cells, multi-copper oxidase (MCO) is frequently used as a powerful electrocatalyst. Several kinds of MCOs can communicate directly with electrodes in a way of direct electron transfer (DET).⁵ However, the current density at such biocathodes is limited due to low solubility of oxygen in aqueous electrolyte solutions as well as poor kinetics of the electron transfer from electrodes to enzymes. Some parts of the problems have been solved by creating a gas-diffusion biocathode, to which oxygen is supplied from the gas phase.⁶

In order to increase the current density at biocathodes, it would be important to construct a new gas-diffusion biocathode with three-phase boundary, which has the following properties in balance: hydrophobicity (or gas permeability) for oxygen transport and hydrophilicity (or suitable affinity between enzymes and electrode surface) for enzyme reaction and enzyme accumulation on electrode surface.

In this work, we first attempted to control the hydrophobicity of carbon electrodes by tuning a) the content of a hydrophobic polymer in carbon slurry and b) the temperature at which the electrode coated with the slurry was dried or heated. On the other hand, we also tried to increase the affinity between enzyme solution and the electrode surface by using a surfactant, which may assist the penetration of the enzyme solution into the pores of the hydrophobic carbon electrode and provide the interface suitable for DET-type electro-enzyme reaction. The current density of the catalytic reduction of oxygen was utilized as a measure to assess the properties and performance of the gas-diffusion biocathodes. The optimization of the hydrophobicity of the carbon electrodes and the advantages of the surfactant to increase the current density will be described.

2. Experimental

2.1 Enzymes and reagents

Copper efflux oxidase (CueO) from *Escherichia coli* and laccase from *Trametes* sp. were used as catalysts of biocathodes. CueO was purified according to the method in the literature,⁶ while laccase was donated from Daiwa Kasei Co. and used without further purification. Triton X-100 (Amersham Biosciences) was used as a non-ionic surfactant. All other chemicals used were of analytical reagent grade.

2.2 Preparation of electrodes

Ketjen black EC 300J (KB, Lion Co.) was used as carbon particle, while polytetrafluoroethylene (PTFE, Teflon 6-j, Du pont-Mitsui Fluorochemicals, melting temperature ca. 342°C) was utilized as a hydrophobic polymer.⁶ Both materials were dispersed in 2-propanol (Wako Pure Chemical) and mixed together with an ultrasonic disintegrator (Heat Systems) to prepare carbon slurry. The content of PTFE [PTFE/(PTFE + KB)] was changed in the range from 10 to 60%(w/w), where 3.5 mL of 2-propanol was used for 112.5 mg of PTFE + KB. The slurry was applied to the surface of a carbon paper (CP, TGP-H-120, thickness 0.37 mm, Toray) and dried at 60°C or heated at 400°C for 1 h in air. After drying or heating, an enzyme solution (2 mg mL⁻¹ of CueO or 10 mg mL⁻¹ of laccase; 300 μ L per 2.5 cm² CP) was spread over the KB/PTFE-modified CP surface and the enzyme solution was allowed to dry under reduced pressure.

2.3 Electrochemical measurements

The gas-diffusion cell used was identical with that reported in a previous paper.⁶ A titan mesh, platinum wire, and Ag|AgCl|KCl (sat.) were utilized as a current collector, counter electrode, and reference electrode, respectively. All potentials are referred to the reference electrode. Cyclic voltammetry was done on an electrochemical analyzer (BAS 100B) at a scan rate of 20 mV s⁻¹. All electrochemical measurements were performed in 1.0 M citrate

Figure 1. The current density at 0 V on cyclic voltammograms as a function of the content of PTFE. The gas-diffusion electrode used was pretreated at 400°C (closed circle) or at 60°C (open circle). The voltammetric experiments were performed in air atmosphere. The error bar indicate the standard deviation of the experiments ($n = 3$).

buffer solution of pH 5.0 at room temperature ($23 \pm 3^\circ\text{C}$) under quiescent and passive mode.

3. Results and Discussion

3.1 Effects of the PTFE content and the temperature of the heat treatment on the current density

KB/PTFE-modified CP is frequently pre-treated at 400°C to disperse PTFE homogeneously among KB particles. The increase in the PTFE ratio increases the hydrophobicity of the carbon electrodes. Figure 1 shows the current density (j) at 0 V in the voltammetric measurements under the passive mode in air atmosphere at CueO-adsorbed KB/PTFE-modified CP electrodes, which was fabricated at various content of PTFE. As shown by the closed circles, when the KB/PTFE-modified CP was pre-treated at 400°C, the maximum value was observed at 20%(w/w) of PTFE. The increase in the j value with an increase in the PTFE content seems to be ascribed to an increase in the oxygen transport in the gas-diffusion biocathode, while the decrease in j at further increase in PTFE seems to be ascribed to the hindrance in the DET-type kinetics (since the onset potential shifted to the negative potential) and the over-accumulation of CueO at the electrode surface due to increased hydrophobicity.

As shown by the open circles in Fig. 1, similar dependence of the j value on the PTFE content was observed at CueO-adsorbed KB/PTFE-modified CP electrodes, when the KB/PTFE-modified CP electrodes were pre-treated at 60°C. The optimum PTFE content was 40% and the maximum j value was slightly larger than that observed for the 400°C-treatment. In the 60°C-treatment, the PTFE would be somewhat heterogeneously dispersed among the KB particles. This situation seems to be suitable to construct bioelectrochemical three-phase boundary. In addition, the 60°C-treatment is more convenient in the electrode fabrication than the 400°C-treatment. Therefore, KB/PTFE slurry was prepared at 40%(w/w) of PTFE and KB/PTFE-modified CP electrodes were dried at 60°C in the following experiments.

3.2 Effects of surfactant on the current response

The KB/PTFE-modified CP electrodes are still very hydrophobic, and the enzyme solution was labile to be repelled on the electrode during the enzyme adsorption process. This situation seems to be inconvenient to draw out the performance of the porous carbon electrode. Therefore, we attempted to add small amount of Triton X-100 in the enzyme solution to decrease the surface tension. The surfactant-containing enzyme solution spread quickly on the hydrophobic KB/PTFE-modified CP electrodes.

Figure 2. Cyclic voltammograms of CueO-adsorbed KB/PTFE-modified CP electrodes under (A) argon and (B and C) oxygen atmosphere. The electrode for the voltammogram (A and C) was prepared with a CueO solution containing 0.015% TritonX-100, while the enzyme solution for (B) was prepared without surfactant.

Figure 3. Chronoamperometric responses at CueO-adsorbed KB/PTFE-modified CP electrodes. The enzyme solution used for the enzyme adsorption process contained (A) no surfactant and (B) 0.015% Triton X-100. The current was recorded at 0 V in oxygen atmosphere.

Figure 2 shows cyclic voltammograms at the CueO-adsorbed KB/PTFE-modified CP electrodes. As curve (C) is compared with curve (B) in Fig. 2, the current density increased by the addition of 0.015% TritonX-100 and reached to 45 mA cm^{-2} at the peak potential in the oxygen atmosphere (35 mA cm^{-2} of the peak current in air). The performance is drastically improved compared with the previous one (19 mA cm^{-2}),⁶ and the density is the highest one in the world as DET-type gas-diffusion biocathodes. However, the addition of the increased amount of the surfactant (for example at 0.05%) caused a drastic decrease in the catalytic current density. Similar effects were observed when Tween-20 (ICN Biomedicals, Inc.) or Brij-35 (Sigma-Aldrich) was used as non-ionic surfactant.

The appearance of the peak in the voltammograms (Fig. 2) indicates the depression of oxygen in the vicinity of the three-phase boundary and the oxygen transport is a limiting factor in the biocathode reaction. In order to verify this point, chronoamperometry was done at 0 V. As shown by line (B) in Fig. 3, the current density decreased from 45 to 20 mA cm^{-2} within 50 s due to the limitation of the oxygen transport in the KB/PTFE-modified CP electrode. However, the steady-state current density of 20 mA cm^{-2} is substantially high as biocathodes under quiescent conditions. In addition, the surfactant effect is also evidenced in the steady-state current as judged from the comparison between lines (A) and (B) in Fig. 3.

Figure 4. Cyclic voltammograms of laccase-adsorbed KB/PTFE-modified CP electrodes under (A) argon and (B and C) oxygen atmosphere. The enzyme solutions used for the enzyme adsorption process contained (B) no surfactant and (A and C) 0.01% Triton X-100.

3.3 Application to glycoprotein MCO

CueO is not glycoprotein, but most of MCOs are modified with sugar chains in a process of eukaryotic post-translation and more hydrophilic than CueO. One of the drawbacks of glycoprotein MCOs as electrocatalysts is that the current density of glycoprotein MCO-based reaction was much lower than that of CueO-based one in DET-type biocathodes. Therefore it is very important to check whether the surfactant treatment is effective for glycoprotein MCO adsorption on electrodes and to increase the current density at the electrodes. Laccase was chosen here as a model of polysaccharide-modified MCO, since the overpotential of oxygen reaction is minimized at laccase-modified electrodes.⁴ As shown by Fig. 4, the TritonX-100 addition (0.01%) drastically increased the current density about 4.2 times at -0.1 V, which is compared with the value of 1.5 times (Fig. 2). Thus we can conclude that the Triton effect is more effective for laccase than for CueO. The current density reached to 2.5 mA cm^{-2} even at 0.4 V. The onset potential is about 0.6 V, which is almost identical with that observed at carbon aerogel-

modified CP without surfactant.⁴ This result suggests that such small amount of surfactant does not interfere the DET reaction. Such surfactant effects were beyond our expectation and were very useful to construct gas-diffusion biocathodes.

4. Conclusion

This work clearly demonstrates the importance of the optimization hydrophobicity for oxygen transport and hydrophilicity for enzyme adsorption and enzyme reaction. We have concluded that the KB/PTFE slurry is prepared at a PTFE content of 40% and the slurry-modified CP is dried at 60°C to get the best performance of air-diffusion biocathodes in this work. In addition, TritonX-100 is a very effective additive of the enzyme solution to adsorb the enzyme on the hydrophobic porous carbon electrodes. The proposed method was effective to glycoprotein (such as laccase) as well as non-glycoprotein (CueO).

The gas-diffusion biocathodes prepared in the proposed manner show very nice performance for the oxygen reduction. However, the important rate-determining factor is the oxygen transport in the biocathodes. This problem may be solved in part by using some additive during the construction of the carbon electrodes. Details will be reported in the near future.

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