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Short communication

Micro-cubic monolithic carbon cryogel electrode for direct electron transfer reaction of fructose dehydrogenase

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ABSTRACT

Micro-cubic monolithic carbon cryogel (CCG) electrode was fabricated for the electrochemical fructose dehydrogenase (FDH) reaction. The CCG can adsorb a sufficient amount of FDH even inside the CCG structure. FDH inside the CCG worked well as an electrocatalyst for D-fructose oxidation. An extremely high catalytic current density as large as 0.6 A/cm^3 was achieved under stirring condition.

1. Introduction

Enzyme-based biofuel cells (BFCs) are fuel cells that utilize redox enzymes as electrocatalysts [1–4]. Simple and small BFCs can be formatted into portable power sources and miniature power sources for power-on-chip or microelectromechanical systems [5]. A number of implantable medical devices might also benefit from implanted BFCs. Heller's group has reported miniature biofuel cells that consist of two 7-μm-diameter and 2-cm-long carbon fibers, each coated with "wired" (electrically connected) glucose oxidase and "wired" multicopper oxidase, respectively [6,7]. "Wired" enzymes are immobilized in an electron-conducting hydrogel that contains Os complexes as the redox mediator. A porous electrode with large surface area is indispensable for increasing the current density. Carbon nanotube modifications on carbon fiber by chemical vapor deposition (CVD) successfully increased the efficient specific surface area [8]. Recently, Mano et al. developed a porous carbon nanotube fiber electrode to increase efficient surface area for "wired" enzymes [9]. Their miniature BFC exhibits more powerful and stable performance than conventional BFCs using nonporous carbon fiber electrodes.

On the other hand, direct electron transfer (DET) reactions between redox enzymes and electrodes have received considerable attention for the construction of more practical, simplified, and downscaled biofuel cells. It would be possible to increase the catalyst concentration per electrode volume by removing redox mediator and other related materials, such as backbone polymers and linkers. Carbon cryogel (CCG) is a suitable electrode material for DET reactions because it has a large specific surface area and the mesopore size is easily controlled, resulting in the high loading capacity of electro-active enzymes [10,11]. We have demonstrated that fructose dehydrogenase (FDH) adsorbed on an electrode made of CCG with the larger average pore radius (r_p) (>>20 nm) exhibited high catalytic current density for fructose oxidation without redox mediators. Interestingly, the catalytic current depended on the pore size; low catalytic current density was observed when the r_p of CCG was small compared with the molecular size of FDH [11].

Increasing the amount of adsorbed FDH (Γ_{FDH}) is a key to increase the catalytic current density. Nevertheless, careful comparison between electrochemical activity and Γ_{FDH} should be made. In our previous study, a CCG-coated electrode was fabricated as follows. A CCG block was broken into small particles (<1 μm in diameter) and mixed with a binder and organic solvent, and the carbon slurry was put on a carbon support [10,11]. In this configuration, this CCG-coated electrode has two kinds of pores: the mesopores inside the CCG and the crannies that formed between CCG particles. It is difficult to distinguish the bioelectrocatalytic reactions proceeding at these two places. Therefore, in this study, we represent a FDH reaction on a micro-cubic monolithic electrode to make it clear whether the FDH inside the carbon is able to catalyze the DET reaction.

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2. Experimental

2.1. Materials

FDH (169 U mg^{-1}) was purchased from Toyobo Co., Ltd. (Japan) and used without further purification. Poly(vinylidene difluoride) (PVDF, #9305) was kindly donated from Kureha (Japan). Resorcinol, formaldehyde solution (36.0–38.0 wt.% in water, stabilized by 5–10 wt.% methanol), Na_2CO_3, *t*-butanol, *N*-methyl-2-pyrrolidone (NMP), and D-fructose were purchased from Wako Pure Chemical Industries, Ltd. (Japan) and used as received. The synthesis of CCG with 30 nm r_p was reported earlier [11–13]. The texture of the CCGs was evaluated from N_2 adsorption and desorption isotherms at −196 °C using an adsorption apparatus (BELSORP-mini II, BEL Japan Inc.). The BET surface area, average peak radius, mesopore volume, and total pore volume of CCGs were 620 m^2/g, 30 nm, 0.23 m^2/g, and 2.7 cm^3/g, respectively. A block of CCG was crushed into micro-cubic CCG with a mortar and pestle.

2.2. Enzyme adsorption on CCG particles

The CCG particles were heated at 300 °C in vacuum for 3 h to complete dryness. One mL of FDH solution (25 μM) in pH 5.0 McIlvaine buffer (ionic strength 0.3 M with Na_2SO_4) was added to 10 mg of prepared CCG particles in a sealed vessel. The mixture was shaken at 4 °C for 16 h, and the supernatant was obtained by centrifugation at 17,400 *g* for 10 min. The amount of immobilized FDH was calculated from the the concentration change of the supernatant before and after the adsorption procedure. The concentration of the FDH solution was determined spectrophotometrically using a molar extinction coefficient for FDH of 23,000/M/cm at 550 nm [14].

2.3. Micro-cubic CCG electrode and FDH adsorption

Micro-cubic electrodes were produced on glassy carbon (GC) disk electrodes of 1-mm diameter (BAS Inc.). The GC disks were polished to a mirror finish by use of a 1 μm alumina slurry, followed by sonication in distilled water for a few minutes to remove any residual alumina. Micro-cubic CCG particles suspended in NMP solution were spread over a grass laboratory dish, and one particle was picked up for microscopic observation (KEYENCE, VHX-100). Micro-cubic CCG electrodes were fabricated by placing, with a paint brush, a small piece of CCG on the GC electrode coated thinly with PVDF solution (1%, dissolved in NMP) to minimize the FDH adsorption on the GC disk and fix the CCG on GC. After drying for 1 h, the electrodes were submerged in 40 μM FDH solution for 24 h at 4 °C under reduced pressure.

2.4. Electrochemical measurements

Cyclic voltammetry was performed on an electrochemical analyzer (ALS 1000A) using a platinum wire and an Ag|AgCl|KCl (sat.) electrode as the counter and reference electrodes, respectively. Scan rate was 20 mV/s. All electrochemical measurements were performed in a McIlvaine buffer (pH 5, an ionic strength 0.3 M with Na_2SO_4) at room temperature (25 ± 2 °C).

3. Result and discussion

3.1. Enzyme adsorption on CCG particle

We first investigated the total amount of adsorbed FDH (Γ_{FDH}) on CCG particles because it is reasonable to contemplate that the catalytic current density would be related to enzyme loading. While Γ_{FDH} on CCG particles of 5 nm r_p was less than 0.01 mg per 1.0 mg CCG, Γ_{FDH} was as large as 0.23 mg per 1.0 mg of CCG particles of 30 nm r_p, and is close to the amount of enzyme loading on other mesoporous silica materials, such as SBA-15 and MCM-41 [15–17]. These results suggest that FDH exists inside CCG when the mesopore size is sufficiently larger than the FDH molecule.

3.2. Electrochemical response of micro-cubic CCG electrode

We prepared two micro-cubic CCG electrodes with different particle sizes of cubic CCG (r_p = 30 nm): $CCG_{180}E$ and $CCG_{260}E$ with side lengths of 0.18 and 0.26 mm, respectively. After the immobilization of the FDH on the CCG, cyclic voltammograms in Fig. 1 were observed in the absence (dotted curve) and presence of 200 mM of D-fructose under quiescent (dashed curve) and stirring (solid curve) conditions at a scan rate of 10 mV/s. Both electrodes showed large charging current due to the large geometric surface area of CCG. The electrochemical capacitance per volume of $CCG_{180}E$, 1.8 F/cm^3, was almost

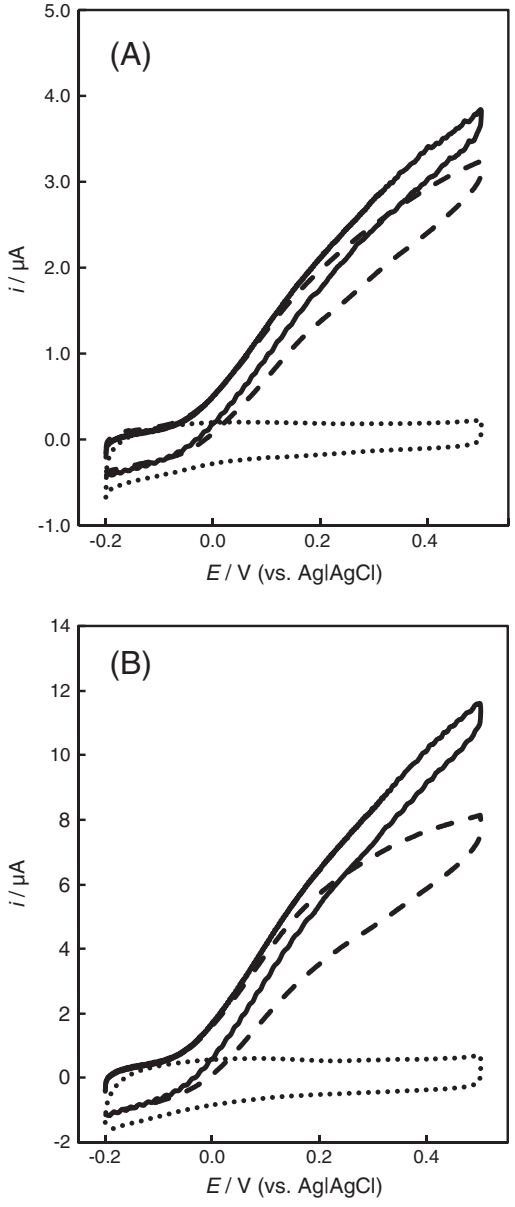

Fig. 1. Cyclic voltammograms of FDH adsorbed in the CCG matrix of the electrode, at pH 5 McIlvaine buffer in the absence (dotted curve) and presence of 200 mM of D-fructose under quiescent (dashed curve) and stirring (solid curve) conditions. Upper panel (A), $CCG_{180}E$; lower panel (B), $CCG_{260}E$. Scan rate was 10 mV/s.