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Glucose oxidation catalyzed by FAD-dependent glucose dehydrogenase within Os complex-tethered redox polymer hydrogel

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ABSTRACT

FAD-dependent glucose dehydrogenase (FAD-GDH) from *Aspergillus terreus* was co-immobilized on a glassy carbon (GC) electrode surface with a poly(1-vinylimidazole)-tethered Os(2,2′-bipyridine)$_2$Cl complex as a redox mediator. The steady-state catalytic current for glucose oxidation was 2.6 mA cm^{-2} at pH 7 and 25 °C. This value increased 1.6-fold after oxidative deglycosylation of the enzyme, which is the highest value so far reported for a GC-electrode-based glucose anode. The deglycosylation process did not decrease the stability of the FAD-GDH dissolved in the buffer solution and immobilized within the hydrogel. A 10% decrease in the catalytic current was observed after 24 h continuous operation.

1. Introduction

Three glucose-oxidizing enzymes have been used for glucose sensors (i.e., self-monitoring blood glucose (SMBG) sensors) and anodes of biofuel cells [1–4]: flavin adenine dinucleotide (FAD)-dependent glucose oxidase (GOx), nicotinamide adenine dinucleotide (NAD)-dependent glucose dehydrogenase, and pyrroloquinoline quinone (PQQ)-dependent glucose dehydrogenase. Recently, FAD-dependent glucose dehydrogenase (FAD-GDH) has garnered significant attention as an electrocatalyst for glucose-oxidizing electrodes, because it displays highly selective oxidation activity toward β-D-glucose, as well as thermal durability, and does not use O_2 as an electron acceptor [5–9]. FAD-GDH from *Aspergillus terreus* was first isolated and purified in 2003 [5], and was later characterized for SMBG sensor applications [6]. The enzyme has already been employed in commercial SMBG sensor strips [1].

Previous studies disclosed that FAD-GDH from *A. terreus* does not display direct electrochemical communication between the enzyme active site (FAD) and the electrode surface, and requires a redox mediator to shuttle electrons [6]. Ferricyanide anion was initially used as such a mediator [6]. However, the reactivity (affinity) between the enzyme and this mediator was unsatisfactory, requiring a high concentration of the mediator to obtain an acceptable glucose oxidation current density. This would likely be due to electrostatic repulsive forces or hydrophobic/hydrophilic interactions between the negatively charged mediator and the substrate-binding pocket of the enzyme.

In this study, we focused on an Os complex as a redox mediator, and fabricated an enzyme-modified electrode based on a redox "wiring" technique pioneered by A. Heller [10]. To this end, poly(1-vinylimidazole), tethering an Os complex (Os(2,2′-bipyridine)$_2$Cl) as a redox mediator, and the enzyme were cross-linked by a diepoxy-type cross-linking agent to form a redox hydrogel on the carbonaceous electrode surface. Electrons generated during enzymatic glucose oxidation are transferred to the electrode via the Os complexes. This "wiring" technique allows the electrode to generate a high catalytic current density because the enzyme and mediator are co-immobilized on the electrode surface in high concentrations.

We characterized the FAD-GDH from the *A. terreus*-"wired" redox hydrogel electrode under neutral pH conditions at 25 °C. Additionally, deglycosylated FAD-GDH was used to prepare a hydrogel electrode to increase the glucose oxidation current efficiency [9,11,12]. At the resulting optimal composition, a steady-state catalytic current density for glucose oxidation of 4.1 mA cm^{-2} at pH 7 and 25 °C was obtained at a loading of 200 μg cm^{-2} hydrogel.

2. Experimental

2.1. Reagents and materials

All reagents were of analytical grade or higher and purchased from Wako Pure Chemicals (Osaka, Japan) unless otherwise

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specified. Native GOx from *A. niger* (350 units (U) mg^{-1}) was also purchased from Wako Pure Chemicals. FAD-GDH from *A. terreus* (2290 U mg^{-1}) was a generous gift from Ikeda Tohka Industries Co., Ltd. (Fukuyama, Japan) [5]. Polyethylene glycol diglycidyl ether (PEGDGE) with an average molecular weight of 500 was purchased from Sigma-Aldrich (Japan). The Os polymer, poly(1-vinylimidazole)-(Os(2,2′-bipyridine)$_2$Cl), was synthesized in our laboratory according to a previous report [10]. The polymer was quaternized by treatment with bromoethylamine, also according to a literature procedure [13].

2.2. Deglycosylation process

Carbohydrate chains on the peripheral surfaces of the GOx and FAD-GDH molecules were oxidized and removed with periodate: GOx or FAD-GDH (1 mg) was reacted with sodium periodate (0.044 mg) for 1 h at 25 °C in the dark in sodium hydrogen carbonate solution (0.053 mg/25 μL) [14]. The reaction solution was replaced by distilled water using an Amicon Ultra centrifugal filter. The carbohydrate content was determined by the phenol-sulfuric acid reaction [15]. Briefly, a glucose solution (0.5 mL) mixed with 5% phenol solution (0.5 mL) and sulfuric acid (2.5 mL) was boiled for 20 min, and then the absorbance at 490 nm was measured. A calibration curve was generated using a standard glucose solution, for comparison against determinations of diluted enzyme solutions in the same manner. FAD-GDH had a carbohydrate content of 26 kDa, which was reduced to 6.5 kDa for the deglycosylated FAD-GDH.

2.3. Preparation of glassy carbon electrode

Glassy carbon (GC) rotating disk electrodes (3 mm diameter, BAS Japan, Tokyo) were used for all experiments. Prior to modification, the electrodes were polished with an alumina suspension (particle size 0.3 μm, Buehler), sonicated, rinsed with water, and then dried.

2.4. Electrode modification

The hydrogel was prepared as previously reported with some modification [10]. The GOx-hydrogel was made of 36% weight GOx (1 mg in 50 μL distilled water, 4 μL), 53 wt% redox polymer (12 mg mL^{-1}, 10 μL), and 11 wt% PEGDGE (5 mg mL^{-1}, 4.8 μL). A total hydrogel loading of 200 μg cm^{-2} was deposited on the electrodes. The resulting electrodes were cured for 12 h in a desiccator at 25 °C and 20% relative humidity. The FAD-GDH-hydrogel was prepared by the same procedure as for the GOx electrode, using FAD-GDH (1 mg in 40 μL distilled water).

2.5. Electrochemical experiments

The measurements were performed using a potentiostat (BAS 50 W). A spiral platinum wire was used as the counter electrode, and all potentials were referred to a Ag/AgCl (saturated KCl) electrode. The working electrodes were rotated using a RDE-2 (BAS Japan, Tokyo). All electrochemical measurements were performed in a water-jacketed electrochemical cell in 0.1 M phosphate buffer at pH 7. The temperature was maintained at 25 °C through the use of an isothermal circulator.

3. Results and discussion

3.1. Glucose oxidation on GOx- and FAD-GDH-modified GC electrodes

Fig. 1(A) shows the cyclic voltammograms (CVs) for glucose oxidation on GOx- and FAD-GDH-modified GC electrodes in pH 7 phosphate buffer solution containing 200 mM glucose. The GOx-modified electrode showed a steady-state catalytic current of 2.2 mA cm^{-2} (gray line, Fig. 1(A)), which is ca. 5.5-fold higher than that reported earlier by Heller [10], although the hydrogel loading (200 μg cm^{-2}) and composition (GOx/polymer/cross linker = 36:53:11) are almost identical. In this study, we optimized the curing conditions (constant temperature, 25 °C, and constant humidity, 20%) and the curing time (12 h). Additionally, the backbone of the redox polymer was partially quaternized with 2-bromoethylamine at over ~17% of the imidazole sites to impart water solubility and to form strong electrostatic adducts between the redox polymer and the polyanionic enzyme.

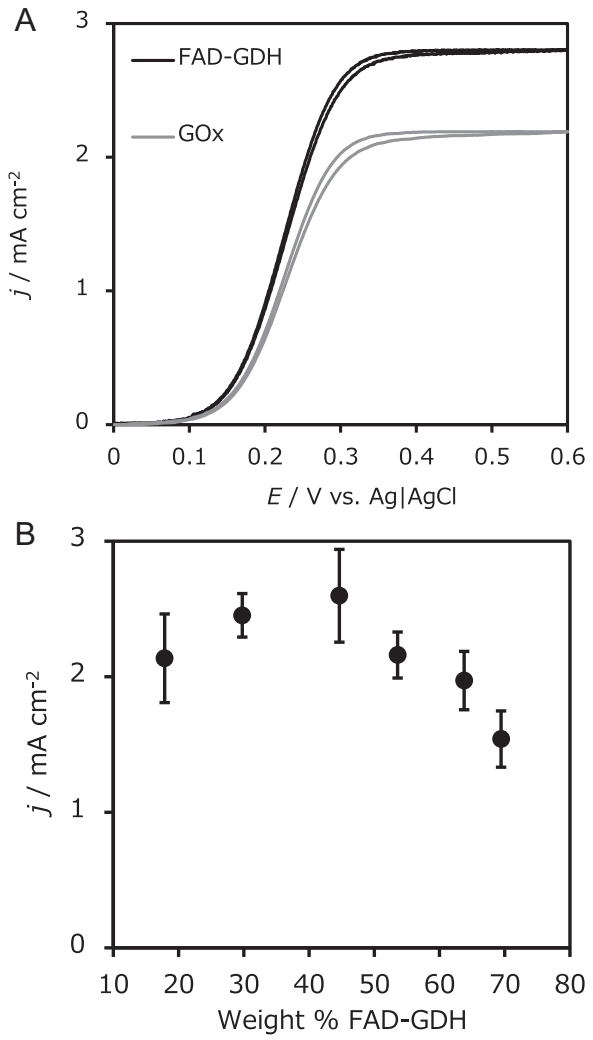

Fig. 1. (A) Cyclic voltammograms for glucose oxidation on FAD-GDH electrode (black line) and GOx electrode (gray line); 200 mM glucose, 100 mM phosphate buffer, pH 7.0, 25 °C, 5000 rpm, Ar atmosphere, 10 mV s^{-1}. (B) Dependence of the steady-state catalytic current density on the FAD-GDH weight fraction for a total hydrogel loading of 200 μg cm^{-2}.

The FAD-GDH-modified GC electrode afforded a 2.6 mA cm^{-2} steady-state catalytic current for glucose oxidation (black line, Fig. 1(A)), which was 20% higher than that of the GOx-modified electrode. It is noted that the voltammogram of the steady-state catalytic glucose oxidation depends on the hydrogel composition, as shown in Fig. 1(B); at a constant cross-linker weight percentage (11%) for a total hydrogel loading of 200 μg cm^{-2}, the weight ratio of FAD-GDH to the total hydrogel was varied. The CV as shown in Fig. 1(A) was obtained at the hydrogel composition FAD-GDH/polymer/cross linker = 44.5:44.5:11.