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Research Paper

Highly Permeable Gas Diffusion Electrodes with Hollow Carbon Nanotubes for Bilirubin Oxidase-Catalyzed Dioxygen Reduction

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ABSTRACT

A gas diffusion electrode system that can supply gaseous substrates to enzymes from the gas phase in bioelectrocatalysis is essential to increase the mass-transfer of gaseous substances; such a system can also address the problem of mass-transfer-limited reactions for gaseous substrates with low solubility in the dissolved system. We have constructed gas diffusion dioxygen (O_2)-reducing biocathodes with bilirubin oxidase (BOD) as an electrocatalyst. Novel carbon nanotubes (CNTs) with hollow structures were used as a carbon material to increase the gas permeability. We achieved a steady-state current density of more than 30 mA cm^{-2} at pH 5 in direct electron transfer- (DET-) type bioelectrocatalytic O_2 reduction under quiescent conditions. The significance of the structural characteristics of the hollow CNTs was discussed based on microscopic observations. The hollow CNTs also provided platforms for mediated electron transfer-(MET-) type bioelectrocatalysis of BOD (not only in monolayer but in multilayers). MET-type reaction of BOD proceeded effectively at pH 7.0, at which the DET-type activity decreased down to one third of that at pH 5.0, and can cover a weak point of BOD as a decrease in the enzyme activity at neutral pH.

1. Introduction

Bioelectrocatalysis, in which an enzyme reaction and an electrode reaction are coupled, is a fundamental process in biofuel cells [1–8]. The reactions can be classified into two types: mediated electron transfer (MET) and direct electron transfer (DET) [1–8]. In a MET-type system, an artificial redox mediator shuttles electrons between an electrode and an enzyme. In a DET-type system, an enzyme directly communicates with an electrode.

Biofuel cells are energy conversion devices in which enzymes are utilized as bioelectrocatalysts [1–8]. Thus, the device can operate under very mild and safe conditions (at neutral pH, room temperature, and atmospheric pressure). Bioanodes and biocathodes are essential to construct biofuel cells [1–8]. At the bioanode, enzymes catalyze the oxidation of several biologically related reductants, such as sugars, alcohols, amines, organic acids, or dihydrogen (H_2). In contrast, at the biocathode, enzymes catalyze the reduction of oxidants. Because the formal potential of the water (H_2O)/dioxygen (O_2) redox couple is relatively high and O_2 is abundant in the air, O_2 is the most desirable oxidant.

Numerous studies to develop O_2-reducing biocathodes have been conducted and are summarized below. There are two main considerations to improve the performance of a biocathode: the selection of suitable enzymes and the arrangement of the electrode structures. Concerning the enzymes, multi-copper oxidases (MCOs) are well-known enzymes that catalyze 4e$^-$-reduction of O_2 to H_2O [9]. Among MCOs, laccase [10,11], Cu efflux oxidase [12,13], and bilirubin oxidase (BOD) [14–16] are often utilized to construct both MET- and DET-type biocathodes. Recently, BOD has generated interest as a highly promising enzyme for biocathodes because it exhibits high bioelectrocatalytic activity, even at neutral pH; also, the formal potential of its electron donating site (type 1 copper site) is relatively close to that of the H_2O/O_2 redox couple [14–19]. Concerning the electrode structures, mesoporous materials such as carbons and metal nanoparticles [13,20–23] or carbon

Abbreviations: BOD, bilirubin oxidase; MET, mediated electron transfer; DET, direct electron transfer; MCO, multi-copper oxidase; CNT, carbon nanotube; KB, Ketjen black; PTFE, poly(1,1,2,2-tetrafluoroethylene); H-CNT, hollow CNT; WPCC, water-proof carbon cloth; ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid).

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nanotubes (CNTs) [19,24–34] have been utilized as electrode materials. Additionally, gas diffusion systems have been employed to increase mass-transfer of O_2 [34–39]. Because the solubility of O_2 is low (approximately 1.3 mM under 100% O_2 atmospheric conditions at room temperature) [11,13,16], O_2-reducing bioelectrocatalysis in a dissolved system is governed by mass-transfer of O_2 to the electrode surface; this may limit the power of biofuel cells. Gas diffusion electrodes that supply gaseous substrates to enzymes from the gas phase can realize high-speed mass-transfer of O_2 and overcome this problem [34–39]. Therefore, a gas diffusion system is required to improve the performance of biocathodes and biofuel cells.

Gas diffusion electrodes have been developed using several different electrode materials and have been utilized to conduct bioelectrocatalysis reactions with gaseous substrates such as O_2 [34–39], H_2 [39–41], and carbon dioxide [42]. In the case of O_2 reduction, steady-state O_2-reducing catalytic current densities of more than 10 mA cm^{-2} have been achieved under quiescent conditions [34–39]. The electrodes balance the hydrophilic/hydrophobic properties for the bio-three-phase interface where the gas diffusion bioelectrocatalysis occurs. Carbon materials such as Ketjen black (KB), carbon black, and CNTs are often mixed with a hydrophobic binder such as poly(1,1,2,2-tetrafluoroethylene) (PTFE) to construct gas diffusion electrodes [34–39]. Binders are known to act as insulators; thus, they decrease the electroactive surface area. Surfactants that are normally contained in CNT suspensions as dispersing agents are also believed to adsorb onto the electrode surface and interfere with the bioelectrocatalysis. Our group published a study concerning a new type of gas diffusion electrode in 2016 as a short communication [34]. We utilized BOD as the bioelectrocatalyst and constructed a gas diffusion electrode by coating water-proof carbon cloth (WPCC) with water-dispersed CNTs without any binders or surfactants [34]. The water-dispersed CNTs were confirmed to be a highly suitable electrode material for DET-type bioelectrocatalysis with BOD because they can provide a surface on which BOD adsorbs with favorable orientation [19]. Because the space of the bio-three-phase interface is limited, it is very important to realize favorable orientation and to effectively utilize the enzyme monolayer. The electrode achieved a high O_2-reducing catalytic current density of more than 15 mA cm^{-2} under quiescent conditions [34]. However, the catalytic current density was still governed by gas permeation at high reduction current densities and reached a plateau as the amount of water-dispersed CNTs increased [34]. We confirmed that excess water-dispersed CNTs accumulated on the surface of the electrode and were not involved in bioelectrocatalysis in the gas diffusion system [34].

In this study, we attempted to improve the gas permeability of the gas diffusion electrode reported in 2016 [34]. Water-dispersed hollow CNTs (H-CNTs) fabricated from the aforementioned water-dispersed CNTs (termed "pristine CNTs" in this study) were used as novel electrode materials. We expected that the hollow structures of the H-CNTs would increase the gas permeability. We will discuss the performance and characteristics of the H-CNTs based on microscopic observation from the viewpoint of bioelectrochemistry.

2. Experimental

2.1. Materials

Two types of CNTs were obtained as follows. Water-dispersed multi-walled pristine CNTs (outer diameter: 10 – 15 nm, length: 1 – 4 μm, without surfactant) and water-dispersed H-CNTs (without surfactant) fabricated from pristine CNTs were kindly donated by Nitta Corp. (Japan). BOD (EC 1.3.3.5) from *Myrothecium verrucaria* was donated by Amano Enzyme Inc. (Japan) and was used without further purification. WPCC (EC-CC1-060T) was purchased from Toyo Corp. (Japan). 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) diammonium salt was purchased from Wako Pure Chemical (Japan). The PTFE membrane filter (T050A025A; pore size: 0.5 μm, thickness: 75 μm) was obtained from Advantec (Japan). All other chemicals used in this study were of analytical grade unless otherwise specified, and all solutions/suspensions were prepared with distilled water.

2.2. Electrode preparation

A CNT suspension (pristine CNTs or H-CNTs) was applied to one side of WPCC sheets and dried at 55 °C to prepare the CNT-modified WPCC electrodes. A PTFE sheet was attached to the opposite side (without CNTs) of the CNT-modified WPCC electrode by pressure bonding. These electrodes are denoted as pristine CNT/WPCC and H-CNT/WPCC. A 160 μL (1 L = 1 dm^3) aliquot of an ABTS solution (50 mM, 1 M = 1 mol dm^{-3}) in 10 mM phosphate buffer (pH 7.0) was applied onto the H-CNT-mounted electrode surface, which was then completely dried to prepare ABTS-adsorbed H-CNT/WPCC (ABTS/H-CNT/WPCC). Subsequently, a 300 μL aliquot of a BOD solution (20 mg mL^{-1}) dissolved in 10 mM phosphate buffer (pH 7.0) was applied onto the CNT-mounted electrode surfaces (pristine CNT/WPCC, H-CNT/WPCC, or ABTS/H-CNT/WPCC); the electrodes were then dried for 2 h under reduced pressure at room temperature.

2.3. Microscopic and electrochemical measurements

Scanning electron microscopy (SEM) was performed using a Hitachi S-4300 instrument. Charge coupled device (CCD) imaging was performed on a Keyence digital microscope VHX-5000. Cyclic voltammetry and chronoamperometry were conducted using BAS CV 50W and ALS 714C electrochemical analyzers. A handmade gas diffusion-type electrolysis cell identical to that reported in a previous paper [34] was used for electrochemical measurements. The projected surface area of the working electrode was set to 1.0 cm^2. The electrode was set with the PTFE sheet-attached side facing the gas phase. The current collector was a Ti mesh that was attached to the BOD-adsorbed and CNT-mounted sides of the working electrode. A Pt mesh and an Ag|AgCl|sat. KCl electrode were used as the counter and reference electrodes, respectively. All potentials in this study are referenced to the reference electrode. The measurements were performed in 1.5 M citrate buffer (pH 5.0) or 1.0 M phosphate buffer (pH 7.0) at 40 ± 2 °C under quiescent and O_2 atmospheric conditions.

3. Results and Discussion

3.1. Optimization of H-CNT/WPCC in DET-type bioelectrocatalysis with BOD

Fig. 1 (A) shows linear sweep voltammograms (LSVs) at the BOD-adsorbed H-CNT/WPCC electrodes. Clear reduction waves with a linearly increasing part were observed. The non-faradaic background current was sufficiently small compared with the observed ones and the wave showed no-hysteresis in cyclic voltammetric mode (Fig. S1). This means that the faradaic current was in steady-state. These steady-state waves are ascribed to O_2-reducing DET-type bioelectrocatalysis with BOD, in agreement with previous reports [14–19]. Linearly increasing property seems to be ascribed to random orientation of BOD and exponential decay of the direct electron transfer kinetics of the protein with an increase in the distance between the redox center of the randomly oriented enzymes and electrode surface [4]. Interestingly, the O_2-reducing catalytic current increased as the amount of H-CNTs