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Factors affecting the interaction between carbon nanotubes and redox enzymes in direct electron transfer-type bioelectrocatalysis

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ABSTRACT

The effects of three types of water-soluble carbon nanotubes (CNTs) of different lengths on the direct electron transfer (DET)-type bioelectrocatalysis of redox enzymes were investigated. Bilirubin oxidase (BOD), copper efflux oxidase (CueO), and a membrane-bound NiFe hydrogenase (H_2ase) were used as model redox enzymes for four-electron dioxygen (O_2) reduction (in the case of BOD and CueO) and two-electron dihydrogen (H_2) oxidation (in the case of H_2ase). As a result, diffusion-controlled O_2 reduction in an O_2-saturated neutral buffer was realized by BOD on CNTs of a length of 1 μm, but the catalytic current densities decreased as the length of CNTs increased. However, almost opposite trends were obtained when CueO and H_2ase were utilized as the biocatalysts. Factors of the CNTs and the enzymes affecting the characteristics of the DET-type bioelectrocatalysis of the three enzymes were discussed. Finally, the electrostatic interaction between an enzyme (especially the portion near the redox active center) and CNTs is proposed as one of the most important factors governing the performance of DET-type bioelectrocatalysis.

1. Introduction

Direct electron transfer (DET)-type bioelectrocatalysis is an ideal process in which the electron is transferred directly from electrodes to the substrate molecule (or vice versa) via the redox active site of an enzyme [1,2]. Since the DET-type bioelectrocatalysis is constructed only with an enzyme and an electrode without any mediator, it is possible to miniaturize devices to extremely small sizes and to minimize the thermodynamic overpotential required in the electron transfer between an enzyme and an electrode, thereby making it very suitable for a variety of bioelectrochemical devices including biofuel cells [3–10] and biosensors [11–13]. However, the redox active sites of enzymes are usually deeply buried in peptides, and the interfacial electron transfer rate constant decreases exponentially with an increase in the distance between the electrode surface and the redox active site of the enzymes [2]. Therefore, the interfacial electron transfer between an enzyme and a solid electrode often has a high kinetic barrier.

Several redox enzymes are capable of direct electrochemical communication with meso-structured materials, although the number of DET-type enzymes is small [14–20]. Carbon nanotubes (CNTs) are nanowires constituted from one or more layers of seamlessly rolled graphene (single-walled and multi-walled CNTs, respectively) with large specific surface areas (in a precise sense, with large values for surface-to-weight ratio), and are expected to be great platforms for the immobilization of redox enzymes and DET-type bioelectrocatalysis ever since they were discovered in the 1990s [19–22]. CNTs often improve the performance of DET-type bioelectrocatalysis [23,24]. This fact is often accredited to their large specific surface areas, which are responsible for the increased amounts of adsorbed enzymes. However, non-faradaic current will also be proportional to the surface area, and the faradaic vs. non-faradaic current ratio should not increase with an increase in the surface area. Rather, the faradaic vs. non-faradaic current ratio may decrease because of the mass transfer barrier to the porous space. Actually, some enzymes show poorer catalytic activity when immobilized on CNTs with larger surface areas than those adsorbed onto CNTs with a smaller surface area [25]. On the other hand, when CNTs are modified onto a solid electrode surface, hydrophobic interactions between CNTs may converge to form three-dimensional meso-structures with different pore sizes [26]. Furthermore, the carboxy group (—COOH) on the surface of CNTs may be dissociated to provide a negatively charged surface [27]. To the best of the authors' knowledge, both the pore size [28,29] and the surface charge of the electrode [10,30–33] may affect DET-type bioelectrocatalysis.

In this work, three kinds of multi-walled CNTs of different lengths (referred to as CNT1, CNT2, and CNT3) were utilized as platforms for the adsorption of bilirubin oxidase (BOD), copper efflux oxidase (CueO), and a membrane-bound NiFe hydrogenase (H_2ase), respectively. All three enzymes are reported as DET-type enzymes at suitable electrodes [15–17]. Both BOD and CueO belong to the family of multicopper

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oxidases that contain four copper atoms in the active site and catalyze a four-electron reduction of dioxygen (O_2) [34]. One of the four copper atoms (known as the "blue" type I (T1) copper atom) is responsible for accepting electrons directly from an electrode and then donating the electrons to the other three copper atoms (in the T2/T3 cluster) wherein O_2 is reduced to water [15,16]. On the other hand, H_2ase contains Ni and Fe atoms in the catalytic center for the bidirectional reaction of H_2 oxidation and evolution and a series of FeS clusters for the electron transfer directly from or to an electrode [35]. We investigated and discussed several factors of the CNTs and the enzymes affecting the interaction between the CNTs and the redox enzymes in the DET-type bioelectrocatalysis.

2. Experimental

2.1. Materials, enzymes, and reagents

Three types of water-dispersed multi-walled CNTs (0.1%, w/w) (an average length of CNT1 (L) = 1 μm), CNT2 (L = 3 μm), and CNT3 (L = 10 μm)) were obtained from Nitta Corp. (Japan) (Fig. S1). BOD (EC 1.3.3.5) from *Myrothecium verrucaria* was donated by Amano Enzyme Inc. (Japan) and used without further purification. CueO from *Escherichia coli* was expressed and purified as previously described [36]. O_2-sensitive hydrogenase (H_2ase, EC 1.12.2.1) from *Desulfovibrio vulgaris* Miyazaki F was purified according to the literature [37]. All other chemicals were of analytical grade, unless otherwise specified, and all solutions were prepared with distilled water.

2.2. Preparation of enzyme/CNT-modified electrodes

A glassy carbon disk electrode (GCE; diameter, 3 mm) was utilized as a working electrode and was polished and washed before modification. A 10 μL aliquot of a water-dispersed CNT suspension was applied onto the GCE surface and dried at 60 °C for 10 min. This operation was repeated six times and the final amount of CNTs was about 60 μg without any binder. The prepared CNT-modified GCE is referred to as CNT/GCE and was cooled down to room temperature. A 30 μL aliquot of a BOD solution (10 mg mL^{-1}) was dropped onto the CNT/GCEs and dried at 4 °C for 2 h. The BOD-adsorbed CNT/GCE was referred to as BOD/CNT/GCE and washed with a fresh buffer solution before electrochemical measurements were carried out. CueO/CNT/GCEs and H_2ase/CNT/GCEs were similarly prepared.

2.3. Electrochemical measurements

All electrochemical measurements were performed using an electrochemical analyzer (ALS 701E, ALS Co. Ltd., Japan) with the prepared CNT/GCE as a working electrode, a Pt wire as a counter electrode, and an Ag|AgCl|sat. KCl electrode as a reference electrode. All potentials were referred against the reference electrode. The current densities in this study were calculated based on the projected surface area of the working electrode (0.07 cm^2).

2.4. Zeta potential measurements

Scanning electron microscopy (SEM) was performed using a Hitachi S-4300 instrument. Atomic force microscopy (AFM) was performed using a Shimadzu SPM-9600 instrument. Average zeta potentials (ζ) of the three kinds of CNT dispersions were measured at 25 °C using a Zeta-potential and Particle size Analyzer (ELS-Z2, Otsuka Electronics Co. Ltd., Japan). It should be noted that all CNT dispersions were sonicated before measurements in order to work with fully dispersed CNT samples, of which the pH value was 6.4.

3. Results and discussions

3.1. Four-electron reduction of O_2 at BOD/CNT/GCEs and CueO/CNT/GCEs

Fig. 1 shows rotating disk linear scan voltammograms (LSVs) of the three kinds of BOD/CNT/GCEs under an O_2-saturated phosphate buffer (0.1 M, pH 7.0, and 25 °C) at a rotating rate (ω) of 4000 rpm and a scan rate (υ) of 5 mV s^{-1}. Well-defined reduction waves were observed with an onset potential of ~0.55 V for all of the BOD/CNT/GCEs examined. No reduction wave was detected in the absence of BOD. These catalytic waves are ascribed to the DET-type bioelectrocatalytic reduction of O_2 by BOD on the electrodes [25,31].

However, the limiting catalytic current densities (at 0.1 V) depended on the CNTs used; the density reached as high as 8.6 ± 0.3 mA cm^{-2} at BOD/CNT1/GCE and 6.3 ± 0.5 mA cm^{-2} at BOD/CNT2/GCE, while only 1.6 ± 0.4 mA cm^{-2} was recorded at BOD/CNT3/GCE. The total amount of CNTs used was almost the same for the three electrodes.

Fig. S2 shows Levich plots of the catalytic reduction of O_2 at the three kinds of BOD/CNT/GCEs. The plot for the current density at BOD/CNT1/GCE was almost linear against the square root of the rotating speed. Note here that, according to the Levich equation [38], the diffusion-controlled current density under O_2-saturated conditions at 25 °C and at ω = 4000 rpm is expected to be 8.1 mA cm^{-2} with a diffusion coefficient of 1.7 × 10^{-5} cm^2 s^{-1} [25]. The detected O_2 reduction current density at BOD/CNT1/GCE was close to the theoretical value. Therefore, it can be concluded that BOD/CNT1/GCE can almost realize a diffusion-controlled O_2 reduction in the O_2-saturated pH 7.0 buffer. Furthermore, compared to BOD/CNT2/GCE and BOD/CNT3/GCE, BOD/CNT1/GCE provided a much sharper sigmoidal curve, as judged from Fig. S3, indicating a significantly better performance in the interfacial electron transfer kinetics at CNT1/GCE than those at CNT2/GCE and CNT3/GCE.

Fig. 2 shows rotating disk LSVs of the catalytic O_2 reduction at the three kinds of CueO/CNT/GCEs under an O_2-saturated phosphate buffer (0.1 M, pH 7.0, and 25 °C). As shown in Fig. 2, clear reduction waves were observed for all of the CueO/CNT/GCEs with an onset potential of ~0.35 V. The waves are ascribed to the DET-type bioelectrocatalytic reduction of O_2 by CueO. However, in contrast to BOD, the catalytic current density at CueO/CNT1/GCE was much smaller than those at CueO/CNT2/GCE and CueO/CNT3/GCE. In addition, the sharper sigmoidal curve (Fig. S4) at CueO/CNT3/GCE indicates a better interfacial electron transfer kinetic performance at CNT3/GCE than at CNT2/GCE and CNT1/GCE. The effects of CNTs on the DET-type catalysis of CueO seem to show an opposite trend compared to those of BOD.

3.2. Two-electron oxidation of H_2 at H_2ase/CNT/GCEs

Fig. 3 shows rotating disk cyclic voltammograms (CVs) obtained for the three kinds of H_2ase/CNT/GCEs in an H_2-saturated buffer solution

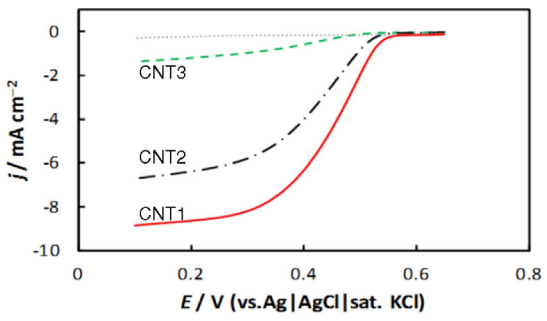

Fig. 1. Rotating disk LSVs of O_2 reduction for BOD/CNT1/GCE (solid line), BOD/CNT2/GCE (dashed–dotted line), and BOD/CNT3/GCE (broken line). The dotted line represents an LSV on a CNT1/GCE without BOD. All measurements were carried out in O_2-saturated phosphate buffer (0.1 M, pH 7.0, and 25 °C) at ω = 4000 rpm and υ = 5 mV s^{-1}.