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Carbon-nanotube-caged microbial electrodes for bioelectrocatalysis

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ABSTRACT

A method to stably immobilize microbes on electrodes was developed. Resting cells of *Methylobacterium extorquens* AM1(*Me*AM1) were caged within multiwalled carbon nanotubes (MWNTs)by adding the cells to a water dispersion of MWNTs then allowing the resulting mixture to dry on electrodes. The *Me*AM1-MWCNTs electrode thus obtained displayed excellent activities in the bidirectional bioelectrocatalysis due to formate dehydrogenase (s) in the resting cells; formate oxidation and carbon dioxide reduction proceeded at steady-state catalytic current densities of 0.6 ± 0.1 and −0.8 ± 0.1 mA cm^{-2}, respectively, using methyl viologen as mediator under very mild conditions (pH 7.0, atmospheric pressure, and 37 °C). In addition, the catalytic signal was stable for more than one week under continuous operation.

1. Introduction

Bioelectrocatalysis relies on biological entities to catalyze electrochemical processes, consisting, for example, in the transformation of the chemical energy of fuels into electrical energy, or *vice versa* [1,2]. Biological entities, including microorganisms and enzymes, are usually characterized by higher selectivity and catalytic efficiency than conventional catalysts under environmentally mild conditions.

On the other hand, reduction of carbon dioxide (CO_2), one of the most important greenhouse gases, to other carbon species contributes to the alleviation of global warming and produces valuable compounds that can be used as fuel or chemical feed stocks [3]. Formate ($HCOO^-$) as one of 2-electron reduced products of CO_2 is highly soluble in water at room temperature and has a formal redox potential close to that of dihydrogen (H_2). Formate salts have several additional advantages, like, for example, ease of storage and transportation, when used as fuel [4]. Therefore, the efficient interconversion between CO_2 and $HCOO^-$ is a process of great importance in the development of a C1-based environment-friendly economy. However, the high kinetic barrier associated with CO_2 reduction and the thermodynamic stability of this molecule are major challenges in the development of such an interconversion system. Although various organic and inorganic catalysts presiding to this interconversion have been synthesized to date, several disadvantages exist for their use, including their low selectivity, high cost, and need for extreme reaction conditions [5–8].

Resting cell suspension of *Methylobacterium extorquens* AM1 (*Me*AM1) has been used as whole-cell catalysts of the electrochemical reduction of CO_2 to $HCOO^-$, which proceeds through a mediated-electron-transfer (MET)-type mechanism [9,10]. Several formate dehydrogenases have been reported to preside to the bioelectrocatalytic oxidation of $HCOO^-$ and reduction of CO_2 under various conditions [11–16]. Tungsten-containing formate dehydrogenase (FoDH1) is the main enzyme involved in the oxidation of $HCOO^-$ by *Me*AM1. FoDH1 is composed of α- and β-subunits; the α-subunit contains the bis-tungstopretin guanine dinucleotide cofactor, three 4Fe-4S clusters, and one 2Fe2S cluster. This subunit acts as the catalytic center for the interconversion of the $HCOO^-/CO_2$ redox couple; by contrast, the β-subunit contains a flavin nucleotide and one 4Fe4S cluster, and it acts as the catalytic center for the interconversion of the NAD^+/NADH redox couple [17]. FoDH1 is a powerful electrocatalyst of the electrochemical interconversions between $HCOO^-$ and CO_2 and between NAD^+ and NADH, in reactions proceeding through both MET- and direct-electron-transfer-type mechanisms [18,19]. However, sophisticated procedures are required for the production and purification of highly active FoDH1. In addition, purified enzymes are often unstable under non-biological conditions, and their long-term durability may be difficult to achieve [20]. With these issues in mind, we explored the possibility of using resting whole-cells as electrocatalysts for the efficient $HCOO^-/CO_2$ interconversion.

A number of microbial electrochemical devices, including biofuel cells, bioreactors, and biosensors, have been reported over the past decades [9,10,13,14,21–26]. However, immobilizing microorganisms onto the surface of electrodes and constructing stable and highly efficient microbial electrodes are not easy objectives to achieve. In fact, in the reactions referred to above, *Me*AM1cells, used as whole-cell electrocatalysts, had not been immobilized [9,10].

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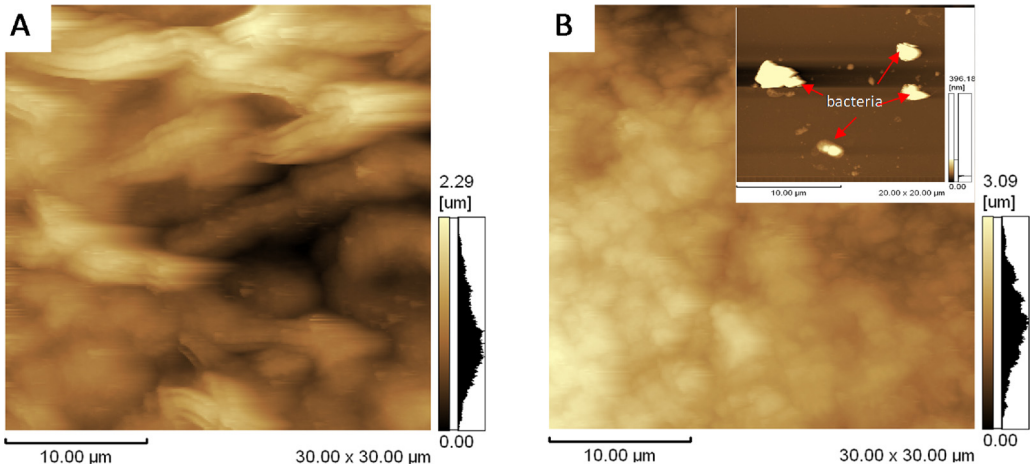

Fig. 1. AFM images of a MWCNT film in the absence (A) and presence (B) of *Me*AM1 cells. The inset in panel B shows *Me*AM1 cells in the absence of MWCNTs.

On the other hand, hydrophobic interactions are known to drive highly conductive carbon nanotubes (CNTs) to converge with each other to form three-dimensional structures on the surface of electrodes. Consequently, CNTs are considered promising scaffolds for the immobilization of biocatalysts [27]. A method frequently reported to develop bacterial CNT-electrodes relies on the cloning of bacteria on the surface of CNTs over extended periods of time. However, the process to obtain electrodes with optimal performance is quite long, as it may take anywhere from days to weeks [28,29]. In addition, the mentioned method may suffer from several problems: the bacteria only grow in multilayers on the electrode surface, and they frequently detach from it in stirred (or convected) solutions.

In this work, we attempted to cage the *Me*AM1 cells inside water-dispersed multiwalled CNTs (MWCNTs) and, subsequently, to immobilize the MWCNT-caged *Me*AM1 cells onto the surface of an electrode. Nafion® was also used as a support. The electrodes thus obtained, which contained immobilized MWCNT-caged *Me*AM1 cells, were characterized by a high enough level of activity in the bidirectional interconversionof the CO_2/$HCOO^-$ redox couple.

2. Experimental

2.1. Bacteria and reagents

*Me*AM1 cells (NCIMB 9133) were purchased from NCIMB (Aberdeen, Scotland, UK) and cultured as described in a previous article [15]. The cultured cells were harvested and centrifuged at 10,000 *g* and 4 °C. The precipitated cells were stored at − 30 °C before use. Water-dispersed MWCNTs with lengths in the 1–4 µm range and outer diameters in the 10–15 nm range were kindly donated by Nitta Corp. (Japan). Sodium formate and Nafion® dispersion (5% in 2-propanol) were obtained from Wako Pure Chemical (Japan). Methylviologen (MV,4,4′-dimethyl-1,1′-bipyridinium dichloride hydrate) was obtained from Tokyo Chemical Industry (Japan). All the other chemicals were of analytical grade, unless otherwise specified, and used as received. All the solutions in this study were prepared with distilled water.

2.2. Preparation and characterization of electrodes containing immobilized, MWCNT-caged MeAM1 cells

The resting cells of *Me*AM1 were suspended in 0.1 M phosphate buffer (pH 7) and to the resulting suspension was added a MWCNT suspension (0.15%) at a weight ratio of 160:1. Nafion® was added to the mixture thus obtained to a final concentration of 0.2%. A 10-µL aliquot of the resulting suspension was transferred onto the surface of a polished glassy carbon electrode (GCE, diameter: 3 mm) and, there, allowed to dry at room temperature for approximately 2 h. The morphology of the MWCNT film containing Nafion® was examined by atom force microscopy (AFM, Shimadzu SPM-9600).

2.3. Bioelectrochemical measurements

Cyclic voltammetry and chronoamperometry experiments were carried out using an electrochemical analyzer (ALS 701E, ALS Co. Ltd., Japan) comprising the GCE on whose surface were immobilized MWCNT-caged *Me*AM1 cells (*Me*AM1-MWCNT/GCE) as the working electrode, a platinum wire as the counter electrode, and an Ag|AgCl|sat.KCl electrode as the reference electrode. All the potentials in this study were referred against the reference electrode. The current densities were calculated based on the projected surface area of the working electrode (0.07 cm^2). For experiments under CO_2-saturated conditions, the pH value of 0.05 M (M = mol dm^{-3}) sodium hydrogen carbonate-containing phosphate buffer was adjusted to 7.0, and measurements were performed in CO_2 atmosphere.

3. Results and discussions

3.1. Surfaces morphology of theMeAM1-MWCNT film

In Fig. 1(A) is reported an AFM image of the MWCNT film containing Nafion® in the absence of *Me*AM1 cells; the MWCNT film displays a three-dimensional structure comprising mesopores of various sizes. By contrast, the image of the MWCNT film in the presence of *Me*AM1 cells shows a smooth surface on which are distributed small, circular grains (Fig. 1(B)).The size of these grains is of 2–3 µm, which is very close to that of *Me*AM1 cells (see inset in Fig. 1(B)). These results indicate that the *Me*AM1 cells were caged within MWCNTs.

3.2. $HCOO^-$ oxidation and CO_2 reduction with MeAM1-MWCNT/GCE

No redox waves were observed at a *Me*AM1-MWCNT/GCE and in the absence of MV^{2+}, and no catalytic current was measured in the presence of $HCOO^-$ (data not shown), indicating that no direct electron transfer occurred between *Me*AM1 cells and the electrode. MV^{2+} is often used as a redox mediator in MET-type bioelectrocatalysis of the reactions involving the redox couples of CO_2/$HCOO^-$and $2H^+$/H_2 [9,10,15,16,30]. In Fig. 2(A) are reported cyclic voltammograms (CVs) of *Me*AM1-MWCNT/GCE in the absence and presence of $HCOO^-$ (0.1 M) in argon (Ar)-saturated phosphate buffer solution (0.1 M, pH 7.0) containing 50 mM MV^{2+}. A pair of non-catalytic reversible redox waves due to the redox reaction of MV^{2+} were observed. The mid-point potential (E_m) was − 0.60 V(Fig. 2(A), dashed line). An increase in the