Synthetic Metals 214 (2016) 30–34

Contents lists available at ScienceDirect

Synthetic Metals

journal homepage: www.elsevier.com/locate/synmet

Bioelectrocatalytic O_2 reduction with a laccase-bearing film of the copolymer of 3-methylthiophene and thiophene-3-acetic acid

Takashi Kuwahara, Kyogo Nakata, Mizuki Kondo, Masato Shimomura*

Department of Bioengineering, Graduate School of Engineering, Nagaoka University of Technology, 1603-1, Kamitomioka-machi, Nagaoka 940-2188, Japan

ARTICLE INFO

Article history:
Received 25 September 2015
Received in revised form 4 January 2016
Accepted 21 January 2016
Available online 3 February 2016

Keywords:
Biocathode
Biofuel cell
Conducting polymer
Laccase
Oxygen reduction
Thiophene

ABSTRACT

Biocathodes bearing laccase as an O_2-reducing enzyme have a problem involved in electrical wiring between the enzyme and the electrode. We report on efficient wiring of the enzyme molecules to the surface of a conducting polymer film for fabrication of a biocathode having high performance in O_2 reduction. Laccase was immobilized by adsorption on the conducting film prepared by electrochemical copolymerization of 3-methylthiophene and thiophene-3-acetic acid. The biocathode obtained thus with the copolymer film formed at an applied potential of +2.2 V vs. Ag/AgCl with passed charge of 1.0C cm^{-2} showed the onset potential of O_2 reduction corresponding to the redox potential of the T1 site of laccase (+0.62 V vs. Ag/AgCl at pH 4.5) and gave a large O_2 reduction current (87 $\mu A\,cm^{-2}$). This value of the O_2 reduction current is 1.7 times as large as that given by the biocathode fabricated with the film of 3-methylthiophene homopolymer (52 $\mu A\,cm^{-2}$). An increasing amount of passed charge through the electrochemical copolymerization led to an increase in the O_2 reduction current, which reached 158 $\mu A\,cm^{-2}$ when the biocathode was fabricated with the copolymer prepared with the passed charge of 2.0 C cm^{-2}. The O_2 reduction current was found to depend on the conductivity of the copolymer film, which was relevant to the composition of the copolymer.

1. Introduction

Biocathodes have been fabricated with O_2-reducing enzymes, such as laccase and bilirubin oxidase, and are attracting much attention due to potential application in biofuel cells [1–3]. These enzymes have been frequently applied to four-electron reduction of O_2 based on direct electron transfer from electrode materials to the enzyme without any redox mediators. In this way, a simple cathodic system can be provided for biofuel cells by the use of such enzymes. Carbonaceous materials have been often used for fabrication of biocathodes as conducting components which support enzyme and transfer electrons from the electrode to the enzyme. Carbon nanotubes, for example, are well known to give excellent electrochemical properties to the biocathodes [4,5]. Needless to say, it is an essential subject for developing a high-performance biofuel cell to achieve efficient electron transfer to the enzyme on the biocathode. Such electron transfer may be realized with novel conducting components.

Various methods of modifying carbonaceous materials have been investigated for the purpose of obtaining laccase-bearing biocathodes with increased performance in electrocatalytic O_2 reduction. Meredith et al. fabricated a biocathode with laccase and anthracene-modified carbon nanotubes and have reported that it shows excellent electron-transferring properties [6]. Karaskiewicz et al. [7] used analogues of natural substrates to bind and orient laccase on the surface of single-walled carbon nanotubes. Gutiérrez-Sánchez et al. [8] fabricated a biocathode by binding laccase molecules covalently to the composite of aminophenyl-modified carbon microfibers and carbon nanotubes via imino bond formation with aldehyde groups introduced by oxidation of sugar residues of laccase. They have demonstrated that the biocathode gives a large electrocatalytic O_2 reduction current. It should be pointed out that the performance of biocathodes bearing laccase is related to orientation of active sites of the enzyme molecules towards the electrode surface. Such orientation could be achieved by controlling the interaction between the surface and hydrophobic pockets surrounding the active sites to facilitate electron transfer from the surface to the active sites.

Conducting polymers have greatly contributed to the progress of organic electronics [9,10]. They have also played an important role in bioelectronics and bioelectrochemistry. We have recently reported on effective direct electron transfer from the film of a conducting polymer, poly(3-metylthiophene) (P3MT), to the laccase adsorbed on the film without any redox mediators [11]. However, no marked electron transfer was observed with common conducting polymers, such as polyaniline and polypyrrole [12]. In

* Corresponding author.
E-mail address: smasato@vos.nagaokaut.ac.jp (M. Shimomura).

http://dx.doi.org/10.1016/j.synthmet.2016.01.014
0379-6779/

addition, polythiophenes except for the derivatives having P3MT-like chemical structure gave similar results to those with the common polymers. These findings suggest the surface properties of the conducting components are important factors to determine the performance of the laccase-bearing biocathodes.

A wide variety of conducting polymers have been synthesized with many kinds of monomers including newly-designed ones. Moreover, the physical and chemical properties of conducting polymers can be easily controlled by copolymerization of different monomers. For the purpose of developing the laccase-bearing biocathode with an enhanced O_2-reducing ability, we examined the usability of these conducting polymers as a component of the biocathode. Among them, a copolymer of 3-methylthiophene (3MT) and thiophene-3-acetic acid (T3A) was found to have a marked aptitude as the component. In the present paper, we report on a simple and convenient approach to fabrication of the biocathode with the copolymer of 3MT and T3A that allows direct electrical wiring to the laccase on the cathode. The film of the copolymer was prepared electrochemically. Laccase was immobilized on the film by adsorption, where the redox sites of laccase was considered to be brought into contact with the surface of the film. It was demonstrated that the biocathode fabricated thus showed good performance to give a larger O_2 reduction current than that fabricated with the P3MT film.

2. Experimental

2.1. Materials

Laccase from *Trametes versicolor* (EC 1.10.3.2, 108 U mg^{-1}) was supplied by Amano Enzyme Inc. 3MT and T3A were purchased from Tokyo Chemical Industry Co., Ltd. Tetraethylammonium perchlorate was purchased from Nacalai Tesque, Inc. Other reagents were of analytical grade, which were used as received. All aqueous solutions were prepared with distilled water passed through a purification system. Au electrodes (0.50 cm × 2.0 cm) deposited on an alumina plates were purchased from Sunrise Industrial Co., Ltd. The working area of the electrodes was adjusted to 0.5 cm × 0.5 cm by masking with Kapton film prior to use in electrochemical polymerization.

2.2. Fabrication of biocathodes

As shown in Fig. 1, biocathodes were fabricated through following simple two steps: (1) preparation of a conducting copolymer electrode by electrochemical copolymerization of 3MT and T3A; (2) adsorption of laccase on the surface of the electrode by immersing the electrode in laccase solution. The biocathodes were named according to the T3A content in the conducting copolymer film employed. Thus, Lac-T3A[50%] means the biocathode fabricated with the film containing 50% T3A (T3A[50%]).

Lac-T3A[7%], for example, was fabricated in the following manner: Prior to the electrochemical polymerization, it was confirmed that the oxidation potentials of 3MT and T3A were +1.5 and +1.6 V vs. Ag/AgCl, respectively. The copolymer film (T3A [7%]) was formed on the Au electrode by electrochemical polymerization at an applied potential of +2.2 V vs. Ag/AgCl in 10 mL acetonitrile solution containing 3MT (4.5 mmol), T3A (0.5 mmol) and tetraethylammonium perchlorate (1.0 mmol). Prior to the polymerization, for removal of dissolved O_2, the solution was saturated with N_2 by babbling via an external source. The polymerization was continued until 1.0 C cm^{-2} of charge was passed through. The copolymer film obtained was rinsed with distilled water to remove residual chemicals. Subsequently, the film was immersed in acetate buffer solution (0.050 M, pH 4.5) containing laccase (1.0 mg mL^{-1}) for 18 h at 4 °C to adsorb laccase on the film. The biocathode fabricated thus was rinsed several times with distilled water and stored in acetate buffer solution (0.050 M, pH 4.5) at 4 °C.

For comparison, a biocathode was fabricated with the conducting copolymer film in a dedoped state. The film was dedoped by applying a potential at 0 V vs. Ag/AgCl for 30 min in acetonitrile containing tetraethylammonium perchlorate (0.10 M). The dedoped film was rinsed thoroughly with distilled water and employed for biocathode fabrication. The doping level (molar ratio of the dopant to thiophene rings) was evaluated for the copolymer and P3MT with a quartz crystal microbalance based on the mass decrease due to dedoping. It was estimated that both T3A[7%] and P3MT were in the same doping level of 0.21.

2.3. Characterization of biocathodes

The composition of the copolymer films employed for biocathode fabrication and the conductivity of them were determined in the same manner as reported elsewhere [13,14]. The composition was evaluated by means of IR spectroscopy based on the absorbance at 1650 cm^{-1} due to C=O stretching of carboxyl groups. The T3A content was calculated from a standard curve obtained with the mixtures of 3MT and T3A homopolymers. The conductivity was measured in the thickness direction by a two-probe direct-current method. Prior to the conductivity measurement, Au was deposited on the surface of each film, and ohmic contact of the film with Au was confirmed to verify no rectifying property.

The activity of the laccase on the biocathodes was measured with the aid of the reaction of 2.2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) with laccase in acetate buffer solution (0.05 M, pH 4.5) including O_2 at room temperature. The activity was determined from a standard curve based on absorbance at of oxidized ABTS at 420 nm resulting from laccase-catalyzed O_2 reduction.

2.4. Electrochemical measurement

The measurements were conducted with a potentiostat/galvanostat (μAutolab Type III, Metrohm Autolab B. V.) in a conventional three-electrode cell equipped with a Pt plate counter electrode and an Ag/AgCl reference electrode. Electrochemical properties of biocathodes were examined by the measurement of steady-state currents at an arbitrary potential in acetate buffer solution (0.05 M, pH 4.5) saturated with O_2 or N_2 at room temperature (around 25 °C). Chronoamperometry was carried out

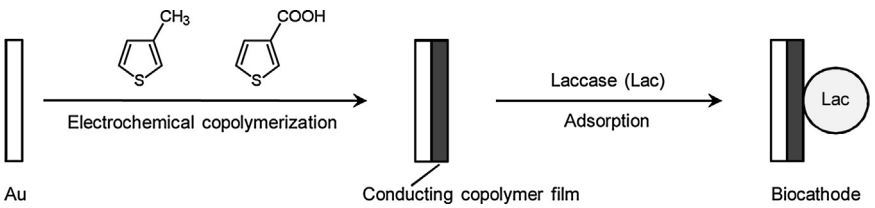

Fig. 1. Fabrication of the biocathode with the conducting copolymer film by adsorption of laccase.