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Short communication

Immobilization of glucose oxidase on carbon paper electrodes modified with conducting polymer and its application to a glucose fuel cell

Takashi Kuwahara, Hokuto Ohta, Mizuki Kondo, Masato Shimomura *

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

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ABSTRACT

A carbon paper electrode was modified with the conducting copolymer of 3-methylthiopene and thiophene-3-acetic acid prepared electrochemically on the electrode, and an enzyme electrode was fabricated by covalent immobilization of glucose oxidase on the modified electrode. The modification with the conducting copolymer increased the surface area of the electrode and the amount of the immobilized enzyme. As a result, the enzyme electrode showed a high catalytic activity. Moreover, it was found that the increased surface area led to a high rate of electron transfer reaction between the electrode and *p*-benzoquinone employed as an electron mediator. The enzyme electrode fabricated with the modified carbon paper gave a larger glucose oxidation current than that fabricated with the bare one. In addition, the glucose oxidation current was found to increase with increasing content of the conducting copolymer in the modified carbon paper. Corresponding to the large glucose oxidation current, high performance was confirmed for the glucose fuel cell constructed with the enzyme electrode based on the modified carbon paper.

1. Introduction

Conjugated polymers, which are synthesized from such monomers as aniline, pyrrole and thiophene, have electron conductivity in spite of being organic substances [1–5]. They have been applied to sensors [6–10], batteries [11,12] and display devices [13,14] because of their unique electronic and electrochemical characteristics. Among various kinds of applications, it is one of the very attractive subjects to use conducting polymers as a component of an enzyme electrode, for they can be used as both an electron-transferring medium and a supporting material for immobilization of enzyme. To give an example of such application, a polypyrrole film including glucose oxidase (GOx) has been prepared by the electrochemical polymerization of pyrrole in the presence of the enzyme, and used as an amperometric biosensor [15,16]. As for such conducting polymers as polypyrrole and polyaniline, it has been also reported that the conducting polymer film prepared electrochemically on an electrode plays an important role in electron transfer between the immobilized enzyme (GOx) and the electrode [17–22]. Recently, the authors investigated the properties of the enzyme electrode fabricated with GOx and the conducting film of a polythiophene derivative, and confirmed that the enzyme electrode well functioned as an anode of a biofuel cell [23]. On the other hand, it has been demonstrated that a high performance of the enzyme electrode can be achieved by covalent immobilization of enzyme on the surface of the conducting polymer film with high conductivity [24].

Carbon paper (CP) has drawn much attention in a variety of electrochemical applications because of its high conductivity, chemical stability, large surface area due to gathered structure of fine carbon fibers. For example, it has been used as an electrode in chemical fuel cells, which are driven with such fuels as methanol and hydrogen, for supporting catalysts, oxidizing fuels and collecting electrons [25–27]. Thus CP is an excellent conducting material and has been also applied to biofuel cells [28–30]. If an enzyme electrode is used in a biofuel cell, CP seems preferable as a component of the electrode because its large surface area can support a large amount of enzyme. However, CP does not have any functional groups on its carbon fiber surfaces and, therefore, enzyme molecules cannot be immobilized on the surfaces by stable covalent linkages. In order to overcome this problem, appropriate modification should be carried out to introduce the functional groups available for enzyme immobilization.

In the present study, for the purpose of fabricating an enzyme electrode, the modification of CP was attempted with the conducting polymer having carboxyl groups used as covalent binding sites for enzyme immobilization. It has been demonstrated in a previous study [24,31] that the copolymer of 3-methylthiophene (3MT) and thiophene-3-acetic acid (T3A) shows higher performance than Au as a conducting component of an enzyme electrode. Therefore, the copolymer (3MT/T3A copolymer), whose structural formula is shown in Scheme 1, was employed for the modification of CP. After the electrochemical copolymerization of 3MT and T3A on a CP electrode, GOx was immobilized covalently through amide linkages on the layer of 3MT/T3A copolymer covering the CP electrode by the condensation reaction with the carboxyl groups of the copolymer. The modified CP electrode bearing GOx (CP/Copolymer/GOx electrode) was used as an

* Corresponding author. Tel./fax: +81 258 47 9404.
E-mail address: smasato@vos.nagaokaut.ac.jp (M. Shimomura).

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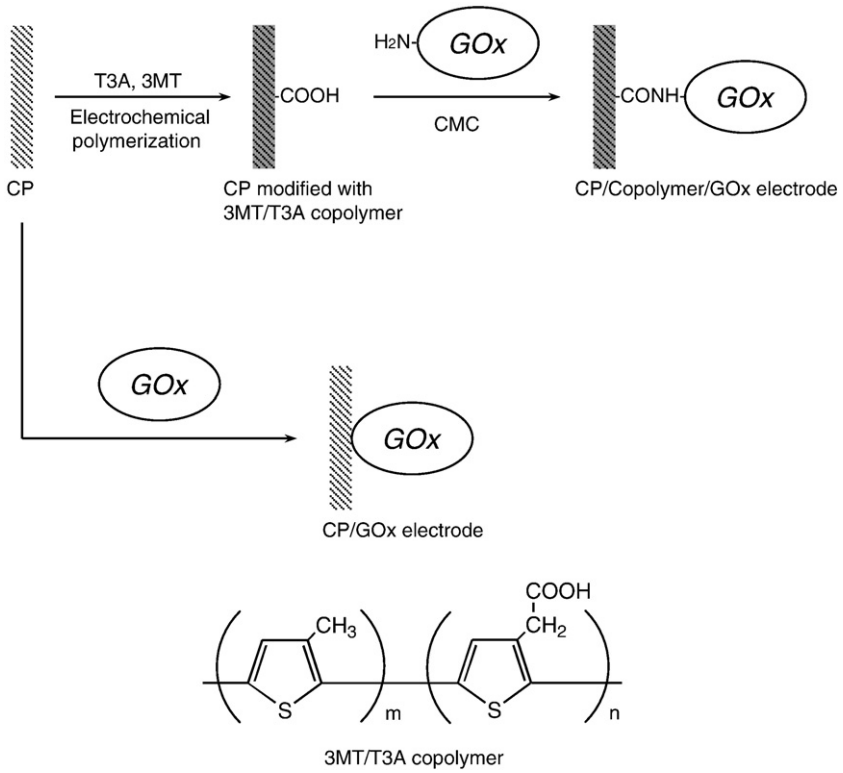


Scheme 1. Routes of preparing the CP/Copolymer/GOx electrode and the CP/GOx electrode.

anode of a glucose fuel cell, and the role of the modification with 3MT/T3A copolymer was investigated based on the performance of the fuel cell.

2. Experimental

2.1. Materials and apparatus

The CP used was TGPH-60 obtained from Toray Ind., which was cut in a size of 0.5 cm × 2.0 cm prior to the modification by electrochemical polymerization of 3MT and T3A. 3MT, tetraethylammonium perchlorate and *p*-benzoquinone (BQ) were purchased from Nacalai Tesque, Inc. T3A and D-glucose were purchased from Tokyo Kasei Kogyo Co. and Wako Pure Chemical Ind., respectively. All these chemicals were of guaranteed-reagent grade and used without further purification. GOx (EC 1.1.3.4, from *Aspergillus* species) was supplied by Toyobo Co., which had an activity of 154 units/mg. 1-Cyclohexyl-3-(2-morpholinoethyl)-carbodiimide metho-*p*-toluenesulfonate (CMC) from Aldrich Chemical Co. was used as a condensing agent. Nafion 112 (perfluorinated membrane with a thickness of 0.002 in.) was supplied by Aldrich Chemical Co.. Other chemicals and solvents were of guaranteed-reagent or analytical grade and used without further purification.

Electrochemical experiments were carried out in the three-electrode cell equipped with a potentiostat/galvanostat (Hokuto Denko Corp. HA-150G), a bipolar coulomb/ampere hour meter (Hokuto Denko Corp. HF-203D) and an arbitrary function generator (Hokuto Denko Corp. HB-105A). An outline of the apparatus is shown in Fig. 1. A platinum plate and a saturated calomel electrode (SCE) were used as a counter electrode and a reference electrode, respectively.

2.2. Fabrication of the CP/Copolymer/GOx electrode

The modification of CP was carried out by the electrochemical copolymerization of 3MT and T3A using the CP as a working electrode in the three-electrode cell shown in Fig. 1. 20 mL of acetonitrile solution containing 3MT (0.45 M), T3A (0.05 M) and tetraethylammonium perchlorate (0.10 M) was placed in the electrochemical cell, and nitrogen was passed through the solution for more than 20 min. A potential E (E=+2.2 V vs. SCE) was applied on the CP at room temperature until the passed charge Q reached a given amount. Then the CP modified with 3MT/T3A copolymer (CP/Copolymer) was rinsed

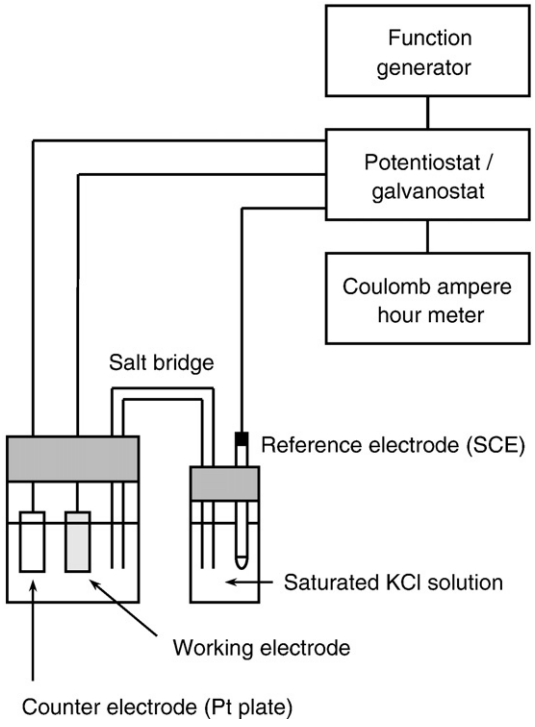


Fig. 1. Apparatus for electrochemical experiments.

with distilled water to remove residual monomers and weakly absorbed copolymer.

Subsequently, the CP/Copolymer was immersed in aqueous solution of GOx (33 μM) and CMC (57 mM) for 1.0 h at 4 °C to immobilize GOx covalently on the 3MT/T3A copolymer covering the CP. The CP/Copolymer/GOx electrode fabricated thus was rinsed several times with distilled water and stored in a phosphate buffer (0.10 M, pH 7.0) until its use in electrochemical measurements. For a comparison, a CP electrode adsorbing GOx (CP/GOx electrode) was prepared by immersion of CP in aqueous solution of GOx (33 μM) for 1.0 h at 4 °C.

2.3. Measurements of amount and activity of immobilized GOx

The amount of immobilized GOx was determined according to the method of Lowry et al. [32] by the analysis with Folin-Ciocalteu phenol reagent after alkaline copper treatment, in which colorimetry was carried out at 750 nm with a Shimadzu UV-3100 PC spectrometer.

The activity of immobilized GOx was measured at 30 °C by a colorimetric method based on the procedure of Trinder [33]. This method included the reaction of hydrogen peroxide, produced in oxidation of glucose by GOx, with phenol and 4-aminoantipyrine in the presence of peroxidase to yield a colored product. The activity was determined from a standard curve based on absorbance of the colored product at 505 nm.

2.4. Electrochemical measurements

The properties of CP, CP/Copolymer, CP/GOx and CP/Copolymer/GOx electrodes were examined electrochemically by cyclic voltammetry using the three-electrode cell shown in Fig. 1. Except for some specified cases, the measurements were carried out at a scan rate of 5 mV/s in a phosphate buffer (0.10 M, pH 7.0) in the presence of BQ (1.0 mM) and/or glucose (0.10 M) at 30 °C under air.

A two-compartment cell was constructed to investigate the performance of the CP/Copolymer/GOx electrode as an anode of a glucose fuel cell. The two compartments were separated by Nafion 112. Pt mesh was used as a cathode, which had been soaked in 50% H_2SO_4 for 10 min and then rinsed thoroughly with distilled water in advance. After a phosphate buffer (0.10 M, pH 7.0) was placed in each compartment, BQ and glucose were added to the buffer in the anodic compartment. The power output of the cell was determined by the measurement of current values at arbitrary potentials. Both the compartments were kept under atmospheric pressure during the measurement.

3. Results and discussion

3.1. Preparation of the CP/Copolymer/GOx electrode

The CP/Copolymer/GOx electrode and the CP/GOx electrode were prepared along the routes illustrated in Scheme 1. A 0.25 cm^2 portion of CP was subjected to the fabrication of these electrodes. The color of CP was found to change from gray to black in the course of the electrochemical copolymerization of 3MT and T3A on the CP. It was reported elsewhere [24] that the content of T3A unit in 3MT/T3A copolymer, which was obtained under the same condition as in the present study, was determined to be 10%. The CP/Copolymer/GOx electrode was fabricated by the covalent immobilization of GOx through amide linkages, which were formed by the condensation reaction between the amino groups of GOx and the carboxyl groups of the 3MT/T3A copolymer deposited. On the other hand, the CP/GOx electrode was prepared by adsorption of GOx on the carbon fibers of CP.

Fig. 2 shows the SEM images of bare CP (A) and the CP/Copolymer samples prepared with Q=0.5 C (B) and Q=8.0 C (C). It can be clearly seen that 3MT/T3A copolymer was deposited on the carbon fibers of CP by the electrochemical polymerization. It should be noted that the

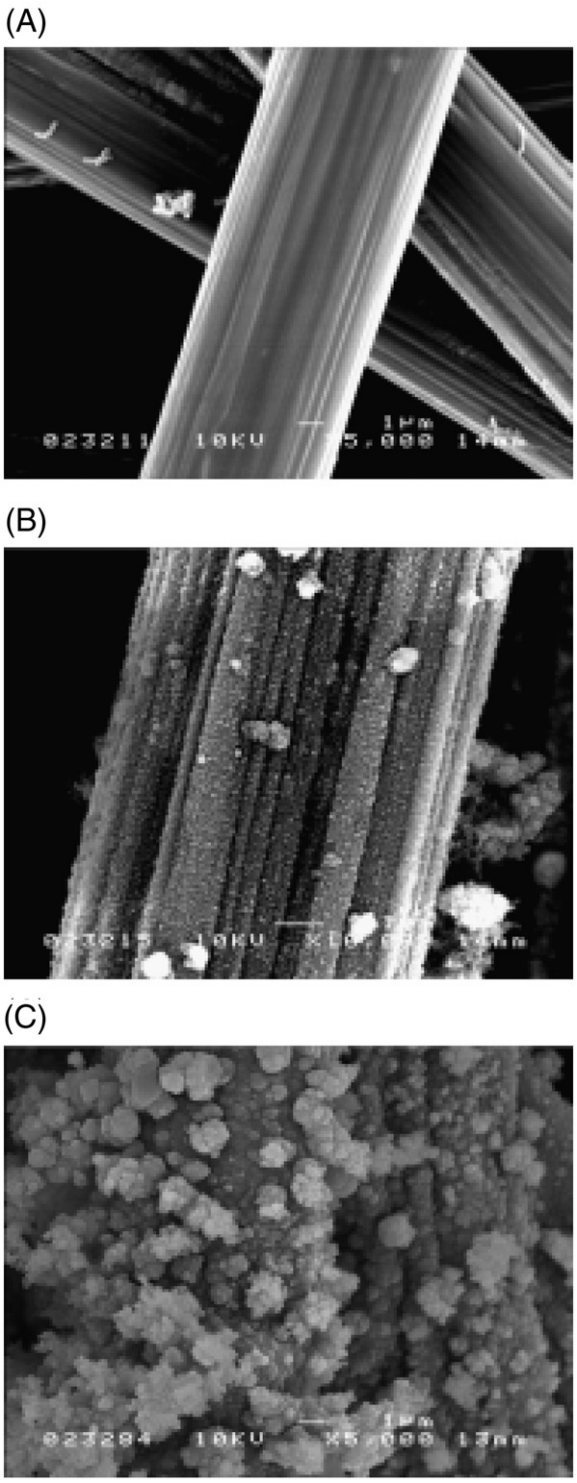


Fig. 2. SEM images of bare CP (A) and the CP/Copolymer samples prepared with Q=0.5 C (B) and Q=8.0 C (C).

3MT/T3A copolymer grew up in a granulated form on the carbon fibers. In addition, the deposition of the copolymer seems to occur on the fibers even inside CP. As seen from a comparison between the SEM images (B) and (C) in Fig. 2, the granular structure of the copolymer was developed by increasing Q. Thus, the surface area of the CP/Copolymer increased corresponding to the growth of the copolymer.

Table 1 shows the amounts and activities of GOx immobilized on bare CP by adsorption and that immobilized on CP/Copolymer covalently, which are important factors determining the catalytic properties

Table 1
Amounts and activities of GOx immobilized on bare CP and CP/Copolymer

Support electrode	Immobilized GOx/mol	Apparent activity/unit	Specific activity/unit mol^{-1}
CP	1.4×10^{-11}	2.9×10^{-2}	2.0×10^{9}
CP/Copolymer (0.5 C)[a]	2.3×10^{-11}	5.9×10^{-2}	2.6×10^{9}
CP/Copolymer (8.0 C)[a]	2.4×10^{-11}	6.8×10^{-2}	2.8×10^{9}

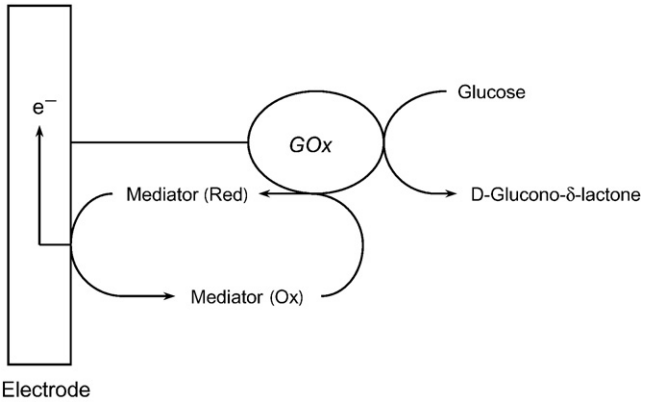
[a] The values of Q passed through the electrochemical copolymerization of 3MT and T3A are given in the parentheses.

of the enzyme electrodes. It is a matter for argument whether there is a significant difference between the amounts of GOx immobilized on CP and CP/Copolymer. However, a larger amount of GOx seemed to be immobilized on CP/Copolymer than on bare CP and, accordingly, apparent activity (activity per each electrode) of GOx on CP/Copolymer became higher. Thus, the modification with 3MT/T3A copolymer resulted in efficient immobilization of GOx. Taking the SEM images in Fig. 2 into account, the large amount of GOx immobilized on CP/Copolymer can be attributed to the increased surface area due to the granular structure of deposited 3MT/T3A copolymer. Although a larger amount of the copolymer was deposited on CP with Q=8.0 C than with Q=0.5 C, almost the same amount of GOx was immobilized on both the CP/Copolymer electrodes. The reason for this result is uncertain and still under investigation. On the other hand, almost the same specific activity was observed for GOx immobilized on CP/Copolymer and that immobilized on bare CP. This result suggests that the activity of GOx was influenced neither by the covalent immobilization on the deposited 3MT/T3A copolymer layer nor by the accompanying microenvironmental change around GOx molecules.

3.2. Electrochemical properties of the CP/Copolymer/GOx electrode

Scheme 2 illustrates the anodic reaction on a GOx-immobilized electrode in the presence of an electron mediator, where the GOx oxidizes glucose to turn into a reduced state and then reduces the electron mediator to return to an oxidized state. The electron mediator of reduced form is oxidized on the electrode, and electrons transfer to the electrode to generate the glucose oxidation current.

In the present study, the glucose oxidation current was measured with each of the CP/Copolymer/GOx electrode and the CP/GOx electrode by use of BQ as an electron mediator. Fig. 3 shows the relation between the glucose oxidation current density j and the applied potential E for each electrode. Although it was found for both electrodes that j increased when the potential E was over 0.1 V vs. SCE, the CP/Copolymer/GOx electrode gave a much larger value of j than the CP/GOx electrode. The large current density observed with the CP/Copolymer/GOx electrode seemed to reflect the effect that the modification with 3MT/T3A copolymer increased the amount of



Scheme 2. Anodic reaction on a GOx-immobilized electrode in the presence of an electron mediator.

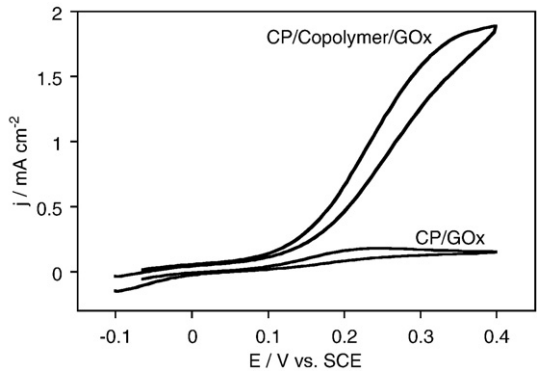


Fig. 3. Glucose oxidation current density j as a function of applied potential E measured for the CP/Copolymer/GOx electrode and the CP/GOx electrode. The CP/Copolymer/GOx electrode was fabricated by use of the CP/Copolymer prepared with Q=0.5 C.

immobilized GOx. However, it was considered to be a matter of argument whether the larger current density was attributed only to the larger amount of GOx, for the current density given by the CP/Copolymer/GOx electrode was eight times as large as that given by the CP/GOx electrode though the apparent enzyme activity of the former was only twice as high as that of the latter.

For the purpose of clarifying the reason why such a large current density was observed with the CP/Copolymer/GOx electrode, the redox behavior of BQ on each of the CP/Copolymer electrode and the CP electrode was analyzed by cyclic voltammetry. As shown in Fig. 4, higher redox peaks were observed with the CP/Copolymer electrode than with the CP electrode. Therefore, the large current density accompanying the glucose oxidation with the CP/Copolymer/GOx electrode shown in Fig. 3 can be attributed to facilitated electron transfer from BQ to the electrode rather than the large amount of immobilized GOx. It goes without saying that the 3MT/T3A copolymer of the CP/Copolymer/GOx electrode is relevant to the electron transfer reaction between BQ and the electrode. As for the performance of enzyme electrodes, various factors should be taken into account. For example, if an enzyme electrode is fabricated with an oxidoreductase, such as GOx, which catalyzes the oxidation of its substrate, the anodic performance of the enzyme electrode will be affected directly by the amount and activity of the immobilized enzyme. On the other hand, the performance is also dependent on the rate of electron transfer from the enzyme reaction system to the electrode, in which an electron mediator, such as BQ, plays an important role. With respect to the CP/Copolymer/GOx electrode, its large surface area due to the granular structure of deposited 3MT/T3A copolymer can be considered to afford a high rate of electron transfer from BQ to the electrode. This is a possible major reason for the large current density resulting from the glucose oxidation with the CP/Copolymer/GOx electrode.

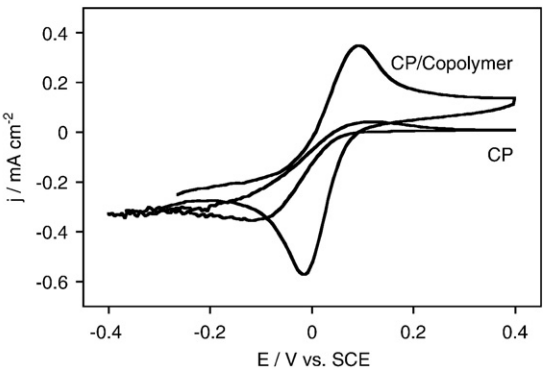


Fig. 4. Cyclic voltammogram of BQ measured with each of the CP/Copolymer electrode and the CP electrode. The CP/Copolymer was prepared with Q=0.5 C.

Since the yield of 3MT/T3A copolymer can be controlled by changing Q in the electrochemical polymerization, the CP/Copolymer samples having various contents of 3MT/T3A copolymer are prepared without difficulty by the polymerization with different amounts of Q. The CP/Copolymer/GOx electrodes, then, were fabricated with several kinds of CP/Copolymer prepared with various amounts of Q. With these electrodes, the glucose oxidation current density j was determined by cyclic voltammetry (scan rate 50 mV/s) at an applied potential of 0.40 V vs. SCE in the presence of 0.50 M of glucose. Fig. 5 (A) shows the relation between j and the passed charge Q in the electrochemical polymerization, where Q=0 C corresponds to the CP/GOx electrode. When Q=8.0 C, the current density j reached 12 mA/cm^2 which was six times as large as the case of Q=0.5 C. Thus, the current density j increased markedly with increasing Q, suggesting that a large content of 3MT/T3A copolymer contributed to the enhanced performance of the CP/Copolymer/GOx electrode. However, the value of Q should be limited to 8.0 C because further increase in Q results in the formation of a brittle layer of 3MT/T3A copolymer. It is obvious from the SEM images in Fig. 2 (B) and (C) that a larger amount of Q gave a higher yield of 3MT/T3A copolymer with a large surface area on the fibers of CP. Nevertheless, as shown in Table 1, almost the same amount and activity were determined for the immobilized GOx on the two kinds of CP/Copolymer prepared with Q=0.5 C and 8.0 C. Therefore, the increased glucose oxidation current density with increasing Q, which was observed for the CP/Copolymer/GOx electrodes, cannot be related to the amount and activity of immobilized GOx.

Fig. 5 (B) shows the current density j determined by cyclic voltammetry (scan rate 50 mV/s) for the maximum oxidation of BQ on the CP/Copolymer electrodes prepared with various amounts of Q. It was seen that j increased with increasing Q. This result is consistent with the consideration that the increase in the glucose oxidation current density with increasing Q, observed for the CP/Copolymer/GOx electrode, is attributed to facilitated electron transfer from BQ to the electrode due to the increased surface area of the deposited 3MT/T3A copolymer layer. Thus, the surface morphology of the enzyme electrode had a significant effect on the rate of electron transfer from the electron mediator to the electrode. In addition, according to a previous study of the enzyme electrodes based on 3MT/T3A copolymer films [34], the conductivity of the copolymer also affects the performance of the enzyme electrode. Needless to say, the activity of immobilized enzyme is an essentially important factor to determine the function of the enzyme electrode.

3.3. Application the CP/Copolymer/GOx electrode to a glucose fuel cell

A glucose fuel cell was constructed by use of the CP/Copolymer/GOx electrode and a Pt mesh electrode as an anode and a cathode, respectively. The cell current density j_{cell} (the cell current per area of the anode) was measured as a function of the cell voltage U_{cell}, and the power output density P given by $j_{cell} \times U_{cell}$ was determined. These data for the glucose fuel cell using the CP/Copolymer/GOx electrode as an anode are shown in Fig. 6, together with the data for the fuel cell using the CP/GOx electrode instead. The fuel cell using the CP/Copolymer/GOx electrode gave a more sloped current–voltage line than that using the CP/GOx electrode. The short-circuit current density j_{sc}, i. e. j_{cell} at U_{cell}=0 V, of the former fuel cell was larger than that of the latter by 0.15 mA/cm^2-anode. The open-circuit voltage U_{oc}, i. e. U_{cell} at j_{cell}=0 A, of the former was larger than that of the latter by 0.15 V. The values of maximum power output density P_{max} of the former and the latter fuel cells were 22 and 13 μW/cm^2-anode, respectively. It is apparent that the difference of performance between these fuel cells is relevant to the characteristics of the enzyme electrodes used. The values of j_{cell} and j_{sc} seem to be related to the glucose oxidation current shown in Fig. 3. The deposited 3MT/T3A copolymer layer of the CP/Copolymer/GOx electrode, on the other hand, may lower the over potential of total electrochemical reactions to increase U_{oc}. Thus, a higher performance of the fuel cell would be achieved by use of the CP/Copolymer/GOx electrode than the CP/GOx electrode.

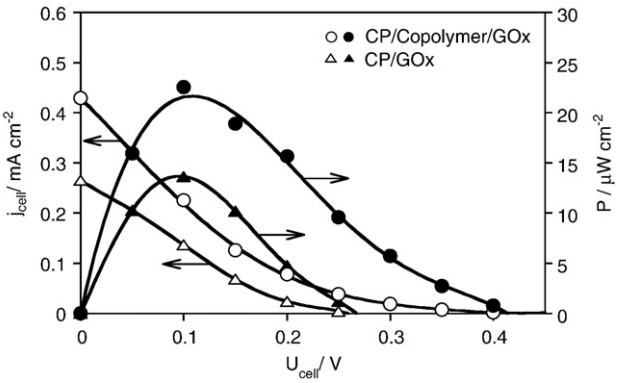


Fig. 6. Cell current density j_{cell} and power output density P as functions of the cell voltage U_{cell} measured for the glucose fuel cells using the CP/Copolymer/GOx electrode and the CP/GOx electrode as anodes. The CP/Copolymer/GOx electrode was fabricated by use of the CP/Copolymer prepared with Q=0.5 C.

Fig. 7 shows the values of P_{max} measured for the glucose fuel cells using the CP/Copolymer/GOx electrodes based on several kinds of CP/Copolymer prepared with various amounts of Q. P_{max} increased with increasing Q, and reached 46 μW/cm^2-anode when Q=8.0 C. This relation between P_{max} and Q is consistent with the fact that the glucose oxidation current observed with the CP/Copolymer/GOx electrode became larger when the CP/Copolymer was prepared with

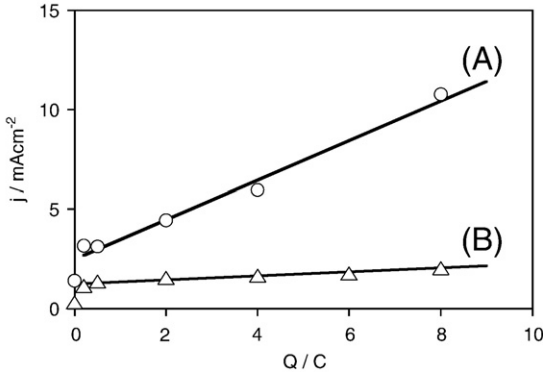


Fig. 5. Current density j measured for the glucose oxidation with the CP/Copolymer/GOx electrodes based on the CP/Copolymer supports prepared with various amounts of passed charge Q (A) and measured for the BQ oxidation measured with the CP/Copolymer electrodes prepared with various amounts of passed charge Q (B).

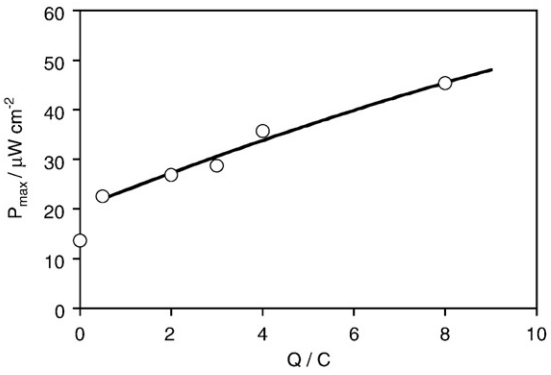


Fig. 7. Maximum power output density P_{max} measured for the glucose fuel cells using the CP/Copolymer/GOx electrodes based on the CP/Copolymer supports prepared with various amounts of passed charge Q.

a larger amount of *Q*. The performance of the fuel cell thus reflects the properties of the enzyme electrode used. Although the value of *Q* was limited to 8.0 C in the present study by the reason mentioned earlier, there is still a possibility to raise the performance of the fuel cell by preparing the CP/Copolymer with a larger amount of *Q* unless it makes the deposited 3MT/T3A copolymer layer brittle. By choosing a more appropriate support electrode than the CP used in the present study, a more stable layer of the granulated copolymer may be obtained on the electrode even with a large amount of *Q*. If this comes true, the power output of the fuel cell will be increased. On the other hand, the modification with the conducting polymer is available for the fabrication of an enzyme electrode used as a cathode of the fuel cell. The application of such a cathode, as well as the improvement in physical properties of the 3MT/T3A copolymer layer, is under investigation.

4. Conclusions

The CP was modified by the electrochemical copolymerization of 3MT and T3A, and applied to the preparation of the GOx-immobilized electrode used as an anode of a glucose fuel cell. The modified CP, i. e. the CP/Copolymer, had a large surface area due to the granular structure of deposited 3MT/T3A copolymer. By the condensation reaction with the carboxyl groups of the copolymer, GOx was immobilized covalently on the CP/Copolymer through amide linkages. The CP/Copolymer/GOx electrode thus prepared bore a larger amount of GOx and showed a higher activity than the CP/GOx electrode fabricated by the adsorption of GOx on bare CP. It was a point of interest, in addition, that the large surface area of the CP/Copolymer/GOx electrode led to a high rate of electron transfer reaction between the electrode and *p*-benzoquinone employed as an electron mediator. Thus, a large glucose oxidation current was observed with the CP/Copolymer/GOx electrode, which was reflected in the performance of the glucose fuel cell using the enzyme electrode as an anode.

The yield of 3MT/T3A copolymer was dependent on the charge *Q* passed through the electrochemical polymerization. Increased current density resulted from the glucose oxidation with the CP/Copolymer/GOx electrode based on the CP/Copolymer prepared with increasing *Q*, which suggested that a large content of 3MT/T3A copolymer contributed to the enhanced performance of the electrode. Similarly, the charge *Q* passed through the preparation of CP/Copolymer affected the glucose fuel cell using the CP/Copolymer/GOx electrode as an anode, and P_{max} of the cell was increased by use of the CP/Copolymer prepared with an increasing amount of *Q*. However, the value of *Q* was limited to 8.0 C because further increase in *Q* resulted in the formation of a brittle layer of 3MT/T3A copolymer. A more stable layer of the granulated copolymer should be obtained even with a large amount of *Q* by choosing a more appropriate support electrode than the CP used in the present study. The fabrication of the GOx-immobilized electrode with such a copolymer layer, though it is still under investigation, will be a promising approach to improve the performance of the glucose fuel cell.

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