Consturuction of a Complete Bioelectrocatalytic Electrode Composed of Alcohol Dehydrogenase- and All Electron-transfer Components-modified Graphite Felt for Diol Oxidation

Yoshitomo Kashiwagi,* Qinghai Pan, Futoshi Kurashima, Chikara Kikuchi, Jun-ichi Anzai, and Tetsuo Osa
Faculty of Pharmaceutical Sciences, Tohoku University, Aramaki, Aoba-ku, Sendai 980-77

(Received October 6, 1997; CL-970775)

A poly(acrylic acid)-coated graphite felt electrode immobilizing all bioelectrochemical reaction components of ferrocene, diaphorase, NADH and alcohol dehydrogenase in the domain of the poly(acrylic acid) layer was applied to oxidize electrocatalytically cis-1,2-cyclohexanedimethanol and 3-methyl-1,5-pentanediol to (+)-(1*R*,6*S*)-cis-8-oxabicyclo[4.3.0]nonan-7-one and (3*S*)-3-methyl-δ-valerolactone, respectively, in a 0.15 M phosphate buffer (pH 8.0) at the constant potential of 0.45 V *vs.* SCE with high current efficiency (>92%) and high optical purity (>92%).

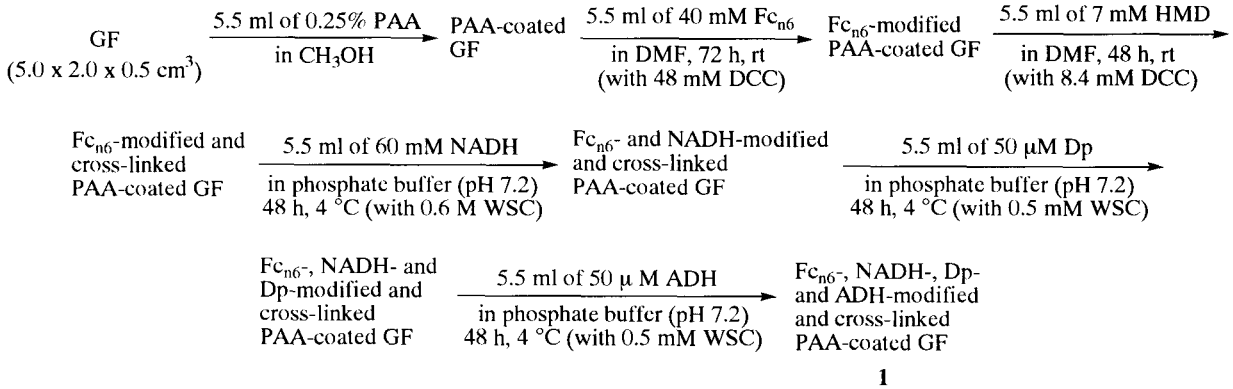
Recently, much effort has been focused on preparative electroenzymatic syntheses in high stereo- and species-selectivity. The efficient enzymatic reactions can be connected with electrochemical reactions by the electron transfer between electrodes and substrates via enzymes, whereby electrochemistry becomes a powerful tool for control of biocatalytic reactions. In order to achieve this purpose, the use of external electron-transfer redox mediators being artificial or semi-artificial is generally effective,[1] though some studies of direct bioelectrocatalysis providing a simple system have been reported,[2] and has been applied to many enzymatic reations.[3-4] The advantage of electroenzymatic reactions is characterized by the use of modified electrode: decrease in the amounts of expensive mediators, attainment of clean and simple process, high current density, high current efficiency, high conversion and yield, new type of reaction, etc. In a series of our studies on mediator-modified graphite felt (GF) electrode for bioelectrocatalysis,[5-9] we have reported that poly(acrylic acid) (PAA)-coated GF immobilizing ferrocene (Fc) of mediator, diaphorase (Dp) of flavoprotein and alcohol dehydrogenase (ADH) of enzyme, but not immobilizing NADH of coenzyme (present in a phosphate buffer) is efficient to oxidize alcohols in a preparative electrocatalytic process.[6] This paper deals with preparative, electrocatalytic oxidation of two diols on a PAA-coated GF electrode immobilizing the all components of Fc, Dp, NADH and ADH to the PAA layer (**1**).

The detailed preparation procedure of **1** is demonstrated in Scheme 1. The carboxyl groups of PAA-coated GF were immobilized with Fc-aminohexylamine (Fc_{n6}), hexamethylenediamine (HMD), NADH, Dp and ADH successively. The immobilized percentages of each component to the carboxyl groups of PAA measured by increased weight of the electrode, titration and UV are shown in Table 1. The immobilized values of Fc, HMD and NADH measured by these methods were considerably well coincident in each other, but those of Dp and ADH differed by weight and titration methods. The latter values measured by weight method will be more close to true ones than those by titration method, because the values by titration include both reaction amounts of carboxyl groups of PAA and enzymes.

Table 1. Reacted percentage of carboxyl groups of PAA layer on PAA-coated GF (5.0 x 2.0 x 0.5 cm^3, 462mg) measured by weight, titration and UV methods

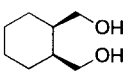
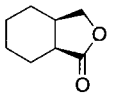
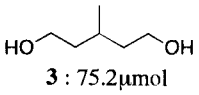
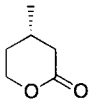
measurement method	Reacted percent of COOH groups of PAA				
	Fc_{n6}-	Cross-linked	NADH-	Dp-	ADH-
weight	41 (25.7 mg)	11 (2.5 mg)	11.7 (17.4 mg)	0.14 (12.3 mg)	0.39 (17.4 mg)
titration	43	14	11.5	1.39	3.74
UV			12.7		

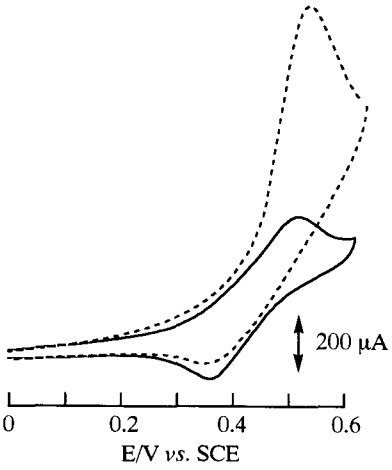
Cyclic voltammogram of alcohols on **1** (size: 1.0 x 1.0 x 0.5 cm^3) showed a catalytic current in a phosphate buffer (pH 8.0). An example of cis-1,2-cyclohexanedimethanol (**2**) is shown in Figure 1 in which the catalytic current observed at 0.49 V *vs.*



Scheme 1. Preparation procedure of Fc-, NADH-, Dp- and ADH-modified GF electrode (**1**).

Table 2. The results of electroenzymatic oxidation of diols on **1**

Substrate	Product	Charge passed (C)	Current efficiency (%)	Conversion (%)	Optical purity of product (%ee)	Turnover number based on Fc
 **2** : 54.9μmol	 18.4μmol	7.6	96.7	33.6	96	9.1
 **3** : 75.2μmol	 31.4μmol	13.5	92.3	41.7	92	15.8



Figuer 1. Cyclic voltammograms on **1** in the presence (- - - -) and absence (——) of 5.49 mM **2**. Phosphate buffer: pH 8.0. Electrode size: 1.0 x 1.0 x 0.5 cm^3. Scan rate: 2 $mV \cdot s^{-1}$.

SCE was 2.5 times larger than the blank one (in the absence of **2**). The modified PAA-coated GF electrodes in any absence of Fc_{n6}, NADH, Dp or ADH, however, did not give a large catalytic current for the oxidation of diols in cyclic voltammetry.

The electrolysis of 55-75 μmol level of saturated **2** and 3-methyl-1,5-pentanediol (**3**) was carried out in a bioelectrochemical reactor which is equipped with anode of **1** (1.0 x 1.0 x 0.5 cm^3), cathode of Pt wire and reference electrode of SCE without divided membrane in 10 ml of 0.15 M phosphate buffer (pH 8.0), at constant potential of 0.45 V *vs.* SCE for 8 h. After electrolysis, the reacted solutions were extracted with chloroform or diethyl ether, distilled, and analyzed by gas chromatography (column: CP-cyclodextrin-B-2,3,6-M-19). Substrates **2** and **3** were selectively oxidized to (+)-(1*R*,6*S*)-cis-8-oxabicyclo[4.3.0]-nonan-7-one and (3*S*)-3-methyl-δ-valerolactone, in high current efficiency and with optical purity of 96 and 92%, respectively, though conversion was low (Table 2). The electrode **1** can be easily applied to oxidze a various kind of monoalcohols such as cyclohexanol. In order to attain a smooth electrical communication between ADH and GF electrode, Fc derivatives possessing different alkyl chain length ($Fc\text{-}CH_2NH(CH_2)_nNH_2$, n=4-12) have been immobilized to PAA layer and the best electrical communication was obtained in the case of flexible Fc derivatives with spacer length of 12-15 Å (n=6-8).[7] According to the results, flexible Fc_{n6} (n=6) was used in this study. However, electrocatalytic current of alcohols on **1** was smaller than that on PAA-coated GF electrode immobilizing Fc, Dp and ADH in the presence of NADH in solution due to low electrical communication with immobilization of NADH. Therefore, more detailed immobilization study, especially more immobilization of Dp and ADH, to PAA-coated GF electrode is in progress.

This paper is dedicated to professor Hans-J. Schäfer, University of Münster, Germany, on the occasion of his 60th birthday. This research was partly supported by Grants-in-Aid for Scientific Research on Priority Areas (No. 05235102 and No. 283: "Innovative Synthetic Reactions") from the Ministry of Education, Science, Sports and Culture, Government of Japan.

References

1 A. E. O. Cass, G. Davis, G. D. Francis, H. A. O. Hill, W. J. Aston,, J. Higgins, E. V. Plotkin, L. D. L. Scott, and A. P. F. Turner, *Anal. Chem.*, **56**, 667 (1984).
2 T. Ikeda, H. Hamada, K. Miki, and M. Senda, *Agric. Biol. Chem.*, **49**, 541 (1985).
3 E. Steckhan, *Top. Curr. Chem.*, **179**, 84 (1994) and references therein.
4 W. A. C. Somers, W. van Hartingsveldt, E. C. A. Stigter, and J. P. van Derlug, *Agro-food-Ind. Hi-Tech* , March/April, 32 (1997).
5 Y. Kashiwagi and T. Osa, *Chem. Lett.*, **1993**, 677.
6 T. Osa, Y. Kashiwagi, and Y. Yanagisawa, *Chem. Lett.*, **1994**, 367.
7 Y. Kashiwagi, Q.-h. Pan, Y. Yanagisawa, N. Shibayama, and T. Osa, *Denki Kagaku*, **62**, 1240 (1994).
8 Y. Kashiwagi, Y. Yanagisawa, N. Shibayama, K. Nakahara, F. Kurashima, J. Anzai, and T. Osa, *Chem. Lett.*, **1996**, 1093.
9 Y. Kashiwagi, Y. Yanagisawa, N. Shibayama, K. Nakahara, F. Kurashima, J. Anzai and T. Osa, *Electrochim. Acta*, **42**, 2267 (1997).