Effect of the Electrochemical Pretreatments on Carbon Fiber Electrodes for Detection of Dopamine in the Presence of Ascorbic Acid and/or Metabolites

Hai-Tao XU, Fusao KITAMURA, Takeo OHSAKA and Koichi TOKUDA*

Received June 25, 1992 ; Accepted August 17, 1992

Mild and strong electrochemical pretreatments were applied to microcylinder carbon fiber electrodes and their differential pulse voltammetric responses for the oxidation of dopamine (DA) were investigated in the presence of ascorbic acid (AA), DOPAC and DOPA in phosphate buffer saline solution (PBS, pH 7.4). The "mild" pretreatment (potential was swept at 0.2 V/s four times between -0.3 and 2.0 V vs. SCE in PBS) was shown to be suitable for quantitative determination of DA in the concentration range higher than $2 \mu\text{mol}/\text{dm}^3$ in the presence of $30 \mu\text{mol}/\text{dm}^3$ DOPA and/or $50 \mu\text{mol}/\text{dm}^3$ DOPAC. In the presence of $200 \mu\text{mol}/\text{dm}^3$ AA detection of $2 \mu\text{mol}/\text{dm}^3$ is possible from the differential pulse voltammograms but the waves for DA and AA overlapped and resolution of these waves were not good enough to allow us the direct determination of DA without a calibration curve. Higher sensitivity and selectivity for dopamine are obtained by the "strong" pretreatment (the potential was swept up to 2.5 V) because the strong pretreatment causes exposure of edges of graphite planes and strong adsorption of dopamine on the electrode surface takes place. The presence of DOPAC less than $20 \mu\text{mol}/\text{dm}^3$, of DOPA less than $25 \mu\text{mol}/\text{dm}^3$ and of AA less than $200 \mu\text{mol}/\text{dm}^3$ is found not to interfere the differential pulse peak current for DA. Since there is no linearity between the current value and the DA concentration in the bulk solution, special care must be exercised in the interpretation of data obtained from *in vivo* voltammetry for the detection of DA.

1 INTRODUCTION

Carbon fiber electrodes are now widely used in *in vivo* voltammetry, especially for determination of dopamine (DA, 3,4-dihydroxyphenylethylamine) in extracellular fluid of the animal brain¹⁻³). Many recipes have been reported for the pretreatment procedures of carbon fiber electrodes and it has been shown that electrochemical responses of the resulting electrodes largely differ according to the procedures employed⁴⁻⁹). Carbon fiber electrodes in the forms of microdisks or elliptic microdisks have been pretreated by polishing or beveling with diamond paste or alumina powders^{7,9}), but electrochemical pretreatments seem to be used most commonly since they can be used even for microcylinder electrodes to which no mechanical pretreatment is applied. Other electroactive species

such as ascorbic acid (AA), 3,4-dihydroxyphenylacetic acid (DOPAC) and 3,4-dihydroxyphenyl alanine (DOPA) are also present in the extracellular fluid or tissues in brain and they are oxidized electrochemically at potentials similar to each other. For analytical determination of DA, enhancement of the sensitivity and selectivity for DA and discrimination against AA, DOPA and DOPAC are desirable. For this purpose, use of carbon fiber electrodes coated with perfluorinated ion exchange membrane has been reported⁹⁻¹²).

For convenience McCreery¹³) has classified electrochemical pretreatments of carbon fibers into two groups according to the potential limit employed and referred to as "mild"^{5,7-8,14}) and "strong"^{4,5,8,14}). In our previous work^{5,14}) we employed two types of electrochemical pretreatments for cylindrical carbon fiber electrodes to examine the effect on the voltammetric responses for DA and showed that the mild treatment is very effective to obtain the electrodes giving the current responses exactly predicted by theoretical equations for cyclic voltammetry and chronoampero-

Department of Electronic Chemistry, Interdisciplinary Graduate School of Science and Engineering, Tokyo Institute of Technology (Nagatsuta, Midori-ku, Yokohama 227, Japan)

Key Words: Carbon fiber, Dopamine, Electrochemical pretreatment, Differential pulse voltammetry

metry at microcylinder electrodes^{15,16}. We also confirmed¹⁴) that the strong treatment causes pronounced adsorption of DA on the electrode surface, which leads to higher sensitivity or lower detectable limit for DA.

In this paper we have examined the effect of the mild and strong electrochemical pretreatments on current responses of cylindrical carbon fiber electrodes for DA in the presence of AA, DOPA and DOPAC. We have found both the treatments yield current responses to DA in high reproducibility in differential pulse voltammetry. It has been found that determination of DA with the electrodes mildly treated is possible in the presence of DOPA or DOPAC but the presence of AA in 0.2 mmol/dm³ makes difficult the direct determination of DA in $\mu\text{mol/dm}^3$ order. The electrodes strongly treated causes pronounced and preferential adsorption of DA and thus higher sensitivity and selectivity for DA are achieved. However, no linear relationship is observed between the peak current value for DA and the DA concentration in solution. The results obtained in the present work indicate that special care must be taken in determining DA from the interpretation of *in vivo* voltammetric data.

2 EXPERIMENTAL

2.1 Chemicals

Dopamine hydrochloride (Kanto Chemical), sodium *L*-ascorbate (Kanto Chemical), DOPAC (Tokyo Chemical Industry) and DOPA (Tokyo Chemical Industry) were used as received. Phosphate buffer saline (PBS) solution (aqueous solution containing 0.2 g/dm³ of KCl, 0.2 g/dm³ KH₂PO₄, 0.8 g/dm³ NaCl and 1.15 g/dm³ Na₂HPO₄, pH was adjusted to 7.4 with addition of NaOH or HCl) was used as the base solution throughout the present work. Milli-Q water was used for preparing solutions. Sample solutions were deaerated before the measurement by bubbling high purity nitrogen through the solutions.

2.2 Apparatus

Electrochemical pretreatments as well as cyclic voltammetry studies were performed with a HECS 318 potentiostat and a HECS 321B potential sweeper (Huso Electrochemical System) and voltammograms were recorded on an X-Y recorder (D-80G, Riken Electronics). A Polarographic Analyzer P-1100 (Yanaco) and a computer-controlled electrochemical system (Cypress System CS-1090) were used for normal and differential pulse voltammetry. Conven-

tional electrolysis cells of Pyrex glass with water jacket for circulating water at a controlled temperature (25 °C) were used. A spiral platinum wire and a saturated calomel electrode (SCE) were used as the auxiliary and the reference electrodes. In this paper all the values of electrode potential are referred to the SCE.

2.3 Fabrication and pretreatment of carbon fiber electrodes

Carbon fibers (FORCA FT-700) used in this study were a gift from Tonen Industries. They are made from pitch and their surface has no external coating. Fabrication of carbon fiber cylindrical electrodes has been described elsewhere¹⁴). An individual fiber connected to a copper wire with a silver paint under a microscope was placed in a glass capillary (100 μL micropipette, Drummond Scientific), which was then pulled vertically to a fine taper on a home-made capillary puller. A proper choice of a combination of the heating time and the weight of the capillary puller made it possible to seal the carbon fiber with the glass sheath without using any adhesive materials such as epoxy. The exposed fiber was carefully cut using a cutter made of a razor blade under an optical microscope so that a length of 2.50 mm of the carbon fiber extended beyond the end of the glass. The diameter of carbon fibers was measured to be $9.5 \pm 0.4 \mu\text{m}$ from the SEM photographs.

Two types of electrochemical pretreatments were carried out in connection with our earlier work^{5,14}). The "mild" treatment consists of four cyclic potential sweeps at a sweep rate of 200 mV/s between -0.3 V and 2.0 V followed by potential sweeps at 20 mV/s between -0.1 V and 0.8 V for 15 min in PBS of pH 7.4, whereas 2.5 V instead of 2.0 V was used for the "strong" treatment as the upper limit of the potential sweep.

3 RESULTS AND DISCUSSION

3.1 Overview of the individual voltammetric behavior of DA, DOPA, DOPAC and AA

The mild and strong treatment procedures give rise to large difference in the surface states of carbon fibers and thus cause large difference in the cyclic voltammograms (CV) for DA, DOPA, DOPAC and AA as can be seen from Figs. 1 and 2. It has been clarified¹⁴) that the electrochemical pretreatment forms some anionic functional groups, possibly carboxyl group, on the surface. The cylindrical surface of the fiber is believed to consist mostly of microstructures of basal

planes of graphite like onion skin of 20–30 nm thick and the inside is composed of graphite planes in a radial structure according to TEM photographs of the cross section of the carbon fibers taken by Tonen Industries. We came to the same conclusion as McCreery¹³) that the mild treatment forms some damages on the surface of basal planes to expose edge structure to some extent, which enhances the rate of electron transfer between the carbon and the electroactive species in the solution. DA shows a reversible wave at the mildly treated electrode and the peak or maximum currents have been confirmed to be equal to those predicted from the theory¹⁴). Electrochemical reversibility decreases in the order: DA > DOPA > DOPAC > AA. This order may also reflect the facts that the surface of the mildly treated electrode has some weak anionic nature at pH 7.4¹⁴) and thus the access of anions to the electrode surface is hindered to some extent. Note that DA (pK_a : 10.6) is a protonated cation, DOPA (pK_a : 2.32 and 8.72) is a neutral zwitter ion whereas DOPAC (pK_a : 4.22) and AA (pK_a : 4.19¹⁷) are present as deprotonated anions at this pH.

Fig. 1. Cyclic voltammograms at the carbon fiber electrodes mildly pretreated for DA, DOPA, DOPAC and AA in PBS, pH 7.4. Concentration of each species is 0.1 mmol/dm³. Potential sweep rate is 100 mV/s.

Fig. 2. Cyclic voltammograms at the carbon fiber electrodes strongly pretreated for DA, DOPA, DOPAC and AA in PBS, pH 7.4. Concentration of each species is 0.1 mmol/dm³. Potential sweep rate is 100 mV/s.

The current values observed at the strongly treated electrodes for DA are much larger than those observed at the mildly treated (Figs. 1 and 2). This is a clear indication of the pronounced adsorption of DA on the strongly pretreated surface. We showed¹⁴) that the strong electrochemical pretreatment removes the basal plane graphite skins from the fiber surface to expose the radial edge structures, causing a markedly large increase in the differential capacitance (about 1800 $\mu\text{F}/\text{cm}^2$ based on the geometric surface area) and an increase in the real surface area. Formation of anionic species has also been evidenced (the detail will be given in subsequent papers). Figure 2 also indicates that adsorption of DOPAC occurs at the strongly treated electrodes, though DOPAC is present as an anion. However, the CV for AA is almost the same as the background CV (not shown). The fact that DOPAC is more hydrophobic than AA may explain the difference in CV for DOPAC and AA.

3.2 Normal and differential pulse voltammetry at carbon fiber electrodes mildly pretreated

Differential pulse voltammograms (DPV) and normal pulse voltammograms (NPV) at the electrodes mildly pretreated are shown in Figs. 3 and 4 for DA, DOPA and DOPAC alone or in the presence of the other. Though DOPA exhibits well-defined NPV in the same potential region as DA, in the presence of DA the wave height of NPV is almost the same as that for DA alone. Though we have no explanation for this feature, it is advantageous for the determination of DA in the presence of DOPA. It is

Fig. 3. Differential pulse (A) and normal pulse voltammograms (B) for DA and DOPA at the electrodes mildly pretreated. (A-a): 20 $\mu\text{mol}/\text{dm}^3$ DA, (A-b): 20 $\mu\text{mol}/\text{dm}^3$ DA + 10 $\mu\text{mol}/\text{dm}^3$ DOPA, (B-a): 20 $\mu\text{mol}/\text{dm}^3$ DA, (B-b): 10 $\mu\text{mol}/\text{dm}^3$ DOPA and (B-c): 20 $\mu\text{mol}/\text{dm}^3$ DA + 10 $\mu\text{mol}/\text{dm}^3$ DOPA. Pulse height: 50 mV, potential sweep rate: 10 mV/s, pulse width: 50 ms and pulse interval: 1 s.

more apparent in DPV as seen from Fig. 5, which shows the peak height, Δi_p , of DPV for DA+DOPA does not depend on the concentration of DOPA if it is less than $30 \mu\text{mol}/\text{dm}^3$. The contribution from DOPA to the DPV peak values becomes no more negligible at the concentration higher than $30 \mu\text{mol}/\text{dm}^3$. This finding indicates that quantitative determination of DA with mildly treated electrodes is possible without interference from DOPA. Note that the top range of DOPA found in the rat brain is $1 \mu\text{mol}/\text{dm}^3$ ¹⁸.

The waves for DOPAC indicate that the electrode reaction is less reversible than DOPA. However, the contributions of DOPAC to the current responses for

Fig. 4. Differential pulse (A) and normal pulse voltammograms (B) for DA and DOPAC at the electrodes mildly pretreated. (A-a): $20 \mu\text{mol}/\text{dm}^3$ DA, (A-b): $20 \mu\text{mol}/\text{dm}^3$ DA + $10 \mu\text{mol}/\text{dm}^3$ DOPAC, (A-c): $20 \mu\text{mol}/\text{dm}^3$ DA + $20 \mu\text{mol}/\text{dm}^3$ DOPAC, (B-a): $20 \mu\text{mol}/\text{dm}^3$ DA, (B-b): $20 \mu\text{mol}/\text{dm}^3$ DOPAC and (B-c): $20 \mu\text{mol}/\text{dm}^3$ DA + $20 \mu\text{mol}/\text{dm}^3$ DOPAC. Differential pulse conditions are the same as in Fig. 3.

Fig. 5. Dependence of the differential pulse voltammetric current for DA at the electrodes mildly pretreated on the DOPA concentration c_{DOPA} . The concentration of DA is $20 \mu\text{mol}/\text{dm}^3$. The error bars indicate the maximum and the minimum values obtained with five or three different electrodes.

Fig. 6. Dependence of the differential pulse voltammetric currents for DA and DOPAC at the mildly treated electrodes on the DOPAC concentration c_{DOPAC} . The concentration of DA is $20 \mu\text{mol}/\text{dm}^3$. The error bars have the same meaning as in Fig. 5.

DA+DOPAC are seen as shoulders in the DPV and NPV (Fig. 4). It was found that peak height of DA in NPV is constant irrespective of the change in DOPAC concentration up to $50 \mu\text{mol}/\text{dm}^3$, and that simultaneous determination of DA and DOPAC is possible as is seen from in Fig. 6.

The reproducibility of the DPV current responses was found to be very good and the standard deviation was usually the same order as that for the diameter of the carbon fibers.

Though the electrochemical responses of AA are the most irreversible among the four species studied in this work, AA is usually present in the extracellular fluid in the top concentration range of about $0.2 \text{ mmol}/\text{dm}^3$ that is much higher than other species. Figure 7A shows DPV for $0.2 \text{ mmol}/\text{dm}^3$ AA in the presence of DA in different concentrations. The waves for DA and for AA overlap but the peak for DA appears as a shoulder on the wave at about 0.1 V vs. SCE. Since the resolution of the wave is not good enough for us to determine the peak height unequivocally, the peak height was measured from the tangent of the DPV and plotted against DA concentration in the test solutions in Fig. 8. Figure 8 also shows the similar plot in the absence of AA, which indicates that the current values are very close to those calculated from the theoretical equation for reversible DPV peak current, Δi_p , at microcylinder electrodes^{5,19}:

$$\Delta i_p = 4\pi F c D l f(D\Delta t/a^2) \tanh(F\Delta E/2RT) \quad (1)$$

with

$$f(\theta) = (\pi\theta)^{-1/2} + 0.422 - 0.0675 \log(\theta) + 0.0058 [\log(\theta) - 1.47] \{\log(\theta) - 1.47\}$$

where ΔE is the magnitude of differential pulse, Δt is

the pulse width, a and l are the radius and length of the electrode, respectively, and c and D are the concentration and the diffusion coefficient of DA, respectively. A value of $D = 6.0 \times 10^{-6} \text{ cm}^2/\text{s}^{14}$ was used (see line c). It is concluded from Fig. 8 that DA concentration higher than $90 \mu\text{mol}/\text{dm}^3$ can be determined from the peak current thus measured in the presence of AA in $0.2 \text{ mmol}/\text{dm}^3$ and that any method for resolving DPV or a calibration curve may be necessary for the DA determination less than $90 \mu\text{mol}/\text{dm}^3$. It should be mentioned that the resolution of the DPV is more apparent for lower concentration of AA.

Fig. 7. DPV for $0.2 \text{ mmol}/\text{dm}^3$ AA in the absence and the presence of DA at the electrodes mildly (A) and strongly (B) pretreated. (A-a): $200 \mu\text{mol}/\text{dm}^3$ AA, (A-b): (A-a) + $2 \mu\text{mol}/\text{dm}^3$ DA, (A-c): (A-a) + $5 \mu\text{mol}/\text{dm}^3$ DA, (A-d): (A-a) + $10 \mu\text{mol}/\text{dm}^3$ DA, (B-a): $200 \mu\text{mol}/\text{dm}^3$ AA, (B-b): (B-a) + $2 \mu\text{mol}/\text{dm}^3$ DA, (B-c): (B-a) + $5 \mu\text{mol}/\text{dm}^3$ DA, and (B-d): (B-a) + $10 \mu\text{mol}/\text{dm}^3$ DA. Pulse height: 50 mV , potential sweep rate: $20 \text{ mV}/\text{s}$, pulse width: 50 ms , pulse interval: 0.2 s .

Fig. 8. Dependence of DPV peak currents for DA measured from the tangent of the DVP on the DA concentration in the absence (a) and the presence (b) of $0.2 \text{ mmol}/\text{dm}^3$ AA at the electrode mildly treated. Line (c) is calculated from eq. (1).

3.3 Differential pulse voltammetry at carbon fiber electrodes strongly treated

Figure 9 shows DPV obtained for $20 \mu\text{mol}/\text{dm}^3$ DA in the presence of DOPAC and DOPA at the strongly treated electrodes. The current values are much higher than those observed at the mildly treated ones for the same concentrations of DA. DPV peak current values for $20 \mu\text{mol}/\text{dm}^3$ DA was found to remain almost constant even if DOPAC is present in the concentration range up to $25 \mu\text{mol}/\text{dm}^3$ or in the presence of DOPA up to $20 \mu\text{mol}/\text{dm}^3$ (Fig. 10). Since $10 \mu\text{mol}/\text{dm}^3$ DOPA shows a clear peak in DPV (curve B-a in Fig. 9) when it is the only electroactive species in the solution, it is obvious that DOPA is adsorbed to the electrodes strongly treated as is expected from CV

Fig. 9. DPV for DA with DOPAC (A) and DA with DOPA (B) at the electrodes strongly pretreated. (A-a): $25 \mu\text{mol}/\text{dm}^3$ DOPAC, (A-b) and (B-b): $20 \mu\text{mol}/\text{dm}^3$ DA, (A-c): $20 \mu\text{mol}/\text{dm}^3$ DA + $25 \mu\text{mol}/\text{dm}^3$ DOPAC, (B-a): $10 \mu\text{mol}/\text{dm}^3$ DOPA, (B-c): $20 \mu\text{mol}/\text{dm}^3$ DA + $10 \mu\text{mol}/\text{dm}^3$ DOPA. Differential pulse conditions are the same as in Fig. 3.

Fig. 10. Effect of DOPAC and DOPA concentrations on the DPV peak current for DA at the electrodes strongly pretreated. Concentration of DA is $20 \mu\text{mol}/\text{dm}^3$. Differential pulse conditions are the same as in Fig. 3.

in Fig. 2. However, the indifference of the DPV peak current to the DOPA concentration shown in Fig. 10 indicates that DA is adsorbed to the electrode surface in place of DOPA, since the former is a cation while the latter is neutral.

It should be noted that differential pulse voltammetry is in general much less sensitive than other techniques to the electrode reaction of adsorbed species because the current due to the reaction of the adsorbed species decays rapidly after the application of the pulse and thus do not contribute to the sampled current at 40 or 50 ms. However, the electrodes strongly treated have a very large differential capacitance value as stated above and exhibit very slow response time, which makes it possible to detect the reaction of the adsorbed species.

As shown in Figs. 7B and 11A, the differential pulse current responses for AA are very small. This is ascribed to the fact that the electrode surface following strong ECP is highly anionic and AA is also anionic so that AA is re-pelled from the electrode. Figure 11B shows that the pre-sence of AA in the concentration range up to 0.2 mmol/dm³ does not affect the measured peak current, Δi_p , for 2 $\mu\text{mol/dm}^3$ DA. This characteristic also holds for other DA concentrations.

It should be mentioned that both the sensitivity and selectivity of the carbon fiber electrodes for DA are highly enhanced by strong ECP. Detection of DA less than 10 nmol/dm³ is readily performed. However, this enhancement is due to both the preferential adsorption of DA to the electrodes strongly treated and the high capacitance value. This is apparent from Fig. 12,

Fig. 11. DPV for 0.2 mmol/dm³ AA and for DA (A) and the effect of AA concentration on the DPV peak current for 2 $\mu\text{mol/dm}^3$ DA (B) at the electrodes strongly pretreated. (A-a): 0.2 mmol/dm³ AA, (A-b): 2 $\mu\text{mol/dm}^3$ DA, (A-c): 2 $\mu\text{mol/dm}^3$ DA + 0.2 mmol/dm³ AA.

Fig. 12. Dependence of DPV peak current for DA on the bulk concentration of DA in the presence of 0.2 mmol/dm³ AA, 10 $\mu\text{mol/dm}^3$ DOPAC and 1 $\mu\text{mol/dm}^3$ DOPA at the electrodes strongly pretreated.

which shows the dependence of Δi_p on the DA concentration in the bulk of solution in the presence of 0.2 mmol/dm³ AA, 10 $\mu\text{mol/dm}^3$ DOPAC and 1 $\mu\text{mol/dm}^3$ DOPA. This figure also shows that no linear relation is observed between Δi_p and the DA concentration, indicating that some calibration curves are required for the reliable determination of DA, though it is not easy to have such curves for *in vivo* voltammetry.

We should mention that the DPV with the electrodes strongly treated shown in this paper was recorded after the adsorption equilibrium was attained. The longer time was required to reach the adsorption equilibrium, the lower the DA concentration was; with 2 $\mu\text{mol/dm}^3$ DA, 10 min or more were required. The electrodes strongly treated showed so-called memory effect. This indicates that the electrodes strongly pretreated require a considerable time to respond to changes in DA concentrations, which is a disadvantage for rapid *in situ* measurements.

4. CONCLUSIONS

The mild electrochemical pretreatment of carbon fiber electrodes proposed here was found to yield electrodes suitable for the reliable determination of DA without interference from DOPAC and DOPA. However, the resolution of DPV for DA and AA in the high concentration of the latter was found not to be good enough to allow us a direct determination of DA without calibration curves. Though much higher sensitivity and selectivity for DA are obtained by the strong electrochemical pretreatment, there is no linearity between the current value and the DA concentration in the bulk solution. Thus special care must be exercised

in the interpretation of data obtained from *in vivo* voltammetry for the determination of DA.

REFERENCES

- 1) F. Gonon, M. Buda, R. Cespuglio, M. Jouvet and J.-F. Pujol, *Nature (London)* **286**, 902 (1980).
- 2) R.M. Wightman, L.J. May and A.C. Michael, *Anal. Chem.*, **13**, 769A (1988).
- 3) J.A. Stamford, *Brain Res. Rev.*, **10**, 119 (1985).
- 4) F.G. Gonon, C.M. Fombarlet, M.J. Buda and J.F. Pujol, *Anal. Chem.*, **53**, 1386 (1981).
- 5) S. Sujaritvanichpong, K. Aoki, K. Tokuda and H. Matsuda, *J. Electroanal. Chem.*, **182**, 267 (1985) and references 1–16 cited herein.
- 6) P. Kovach, M. Deakin and R.M. Wightman, *J. Phys. Chem.*, **90**, 4612 (1986).
- 7) A.C. Michael and J.B. Justice, Jr., *Anal. Chem.*, **59**, 405 (1987).
- 8) J.-X. Feng, M. Brazell, K. Renner, R. Kasser and R.N. Adams, *Anal. Chem.*, **59**, 1863 (1987).
- 9) J. Baur, E. Kristensen, L. May, D. Wiedemann and R. M. Wightman, *Anal. Chem.*, **60**, 1268 (1988).
- 10) G.A. Gerhardt, A.F. Oke, G. Nagy, B. Moghaddam and Adams, *Brain Res.*, **290**, 390 (1984).
- 11) G. Nagy, G.A. Gerhardt, A.F. Oke, M.E. Rice, R. N. Adams, R.B. Moore, III, M.N. Szentirny and C.R. Martin, *J. Electroanal. Chem.*, **188**, 85 (1985).
- 12) E.W. Kristensen, W.G. Kuhr and R.M. Wightman, *Anal. Chem.*, **59**, 1725 (1987).
- 13) R.M. McCreery, *Electroanalytical Chemistry* (Ed. A.J. Bard), Vol. 17, Marcel Dekker, New York, p. 221 (1990).
- 14) H.-T. Xu, F. Kitamura, T. Ohsaka and K. Tokuda, submitted.
- 15) K. Aoki, K. Honda, K. Tokuda and H. Matsuda, *J. Electroanal. Chem.*, **182**, 267 (1985).
- 16) K. Aoki, K. Honda, K. Tokuda and H. Matsuda, *J. Electroanal. Chem.*, **186**, 79 (1985).
- 17) L.A. Coury, Jr., E.W. Huber and W.R. Heineman, *Biotechnology Series 12: Applied Biosensors* (Ed. D.L. Wise), Butterworths, Chap. 1 (1989).
- 18) S. Hafizi, Z.L. Kruk, and J.A. Stamford, *J. Electroanal. Chem.*, **283**, 125 (1990).
- 19) S. Sujaritvanichpong, K. Aoki, K. Tokuda and H. Matsuda, *J. Electroanal. Chem.*, **199**, 271 (1986).