CHEMISTRY LETTERS, pp. 1569-1572, 1994.

Highly-Sensitive Measurement of Dihydroxyphenols Using Carbon Felt Electrode Impregnated with Fructose Dehydrogenase-Containing Solution

Fumio MIZUTANI* and Soichi YABUKI
National Institute of Bioscience and Human-Technology, 1-1 Higashi, Tsukuba, Ibaraki 305

A carbon felt electrode impregnated with a fructose dehydrogenase/fructose-containing solution was used for measuring dihydroxyphenols (e.g., catechol and dopamine). Dihydroxyphenol molecules are oxidized on the electrode but regenerated by the enzyme reaction. The consumption/regeneration cycle for dihydroxyphenols resulted in an amplified electrode response. Further, the measuring method could be free from the electrochemical interference by oxidizable species such as L-ascorbic acid.

Chemical amplification in enzymatic substrate determination is an excellent way of increasing sensitivity. Several groups have reported enzyme electrodes with chemically-amplified responses.[1] Recently we have reported an amperometric method for the highly-sensitive measurement of dihydroxyphenols using a glucose oxidase (GOD)-immobilized electrode.[2] Dihydroxyphenol molecules are oxidized on the electrode surface but regenerated by the GOD reaction in the presence of glucose. The consumption-regeneration cycle for dihydroxyphenol resulted in an increased sensitivity. For example, the detection limit of catechol was as low as 1 nM. However, there exist problems with interference from oxygen and oxidizable species such as L-ascorbic acid and uric acid. In the presence of oxygen, GOD in the immobilized layer catalyzes the natural reaction, i.e., the oxidation of glucose by oxygen, which results in the decrease of the regeneration rate for dihydroxyphenol and also of the electrode response to the analyte. L-Ascorbic acid and uric acid are known as typical electrochemical interferents in biological samples, which give an extra current response for measuring dihydroxyphenols in such samples.

In order to circumvent the interferential problem, we have developed an alternative electrode/enzyme-based method, i.e., the use of a carbon felt (CF) electrode impregnated with a fructose dehydrogenase (FDH)/fructose solution. FDH catalyzes the reaction to regenerate dihydroxyphenol:

fructose + quinone $\longrightarrow$ 5-keto-D-fructose + dihydroxyphenol

Since FDH does not use oxygen as the electron acceptor for fructose, the response to dihydroxyphenol from the FDH-based system would be independent of the oxygen concentration in the solution. As indicated by Uchiyama et al.,[3-5] the electrochemical cell consisting of a CF impregnated with an electrolytic solution is useful for the rapid coulometric determination of electroactive species: a small volume of sample diffuses quickly into the porous CF electrode so as to undergo the electrode reaction. Therefore the current for oxidizing the interferents coexisted in a sample (e.g., L-ascorbic acid) are expected to diminish rapidly, whereas the current for oxidizing dihydroxyphenol in the same sample remains unchanged owing to the regeneration: the concentration of

dihydroxyphenol would exactly be measured from the steady-state current obtained after the consumption of the interferents. Some of the preliminary results for measuring catechol and dopamine are reported here.

The electrochemical cell used is shown in Fig. 1. A CF electrode (diameter, 20 mm; thickness, 2 mm; TOA Electronics, Tokyo) and an Ag/AgCl electrode (Model 11-2020, Bioanalytical Systems, West Lafyette, IN) were used as the working and counter electrodes, respectively. The CF electrode was impregnated with 0.1 M potassium acetate buffer solution (0.6 ml, pH 5) containing FDH (EC 1.11. 99.11, from *Gliconobacter* sp., 30 U mg^{-1}, containing ca. 70% of stabilizers; Toyobo, Osaka) and fructose. The pH of the solution was close to the optimal pH for FDH. The concentrations of FDH and fructose in the solution were usually set at 0.1 mg ml^{-1} (which corresponds to ca. 0.2 μM) and 20 mM (which is a few times higher than the Michaelis constant of FDH for fructose[6]), respectively. The temperature of the solution was kept at 25.0±0.2 °C. The potential of the CF electrode was set at 0.6 V vs. Ag/AgCl. This potential was positive enough to oxidize catechol and dopamine.[2] The volume of the sample added to the cell was 3 μl. The concentration of analytes given in the present paper is the final concentration.

Figure 2 shows current-time curves obtained after the addition of (a) 1 μM L-ascorbic acid, (b) 50 nM catechol, and (c) 1 μM L-ascorbic acid plus 50 nM catechol. After the addition of L-ascorbic acid, the electrode current increased but returned to the base line within 1.5 min. The charge passed for oxidizing L-ascorbic acid was 0.115 mC, which coincided well with the value calculated for the two-electron reaction to form dehydroascorbic acid. On the other hand, a steady-state current response was obtained after the addition of catechol. This shows that the cyclic reactions of electrooxidation/enzymatic regeneration for catechol proceeds

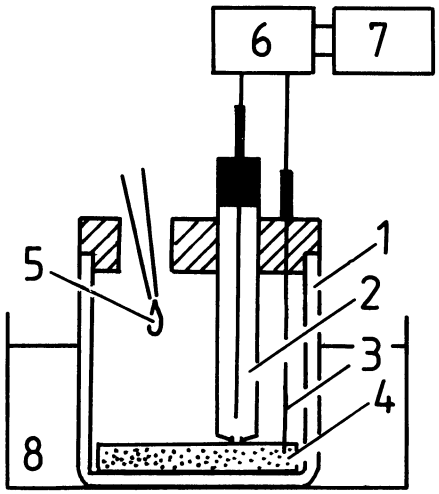

Fig. 1. Schematic illustration of measuring cell: (1) cylindricall glass cell; (2) Ag/AgCl electrode; (3) platinum wire; (4) carbon felt electrode impregnated with FDH/fructose solution; (5) sample; (6) potentiostat; (7) recorder; (8) thermostat.

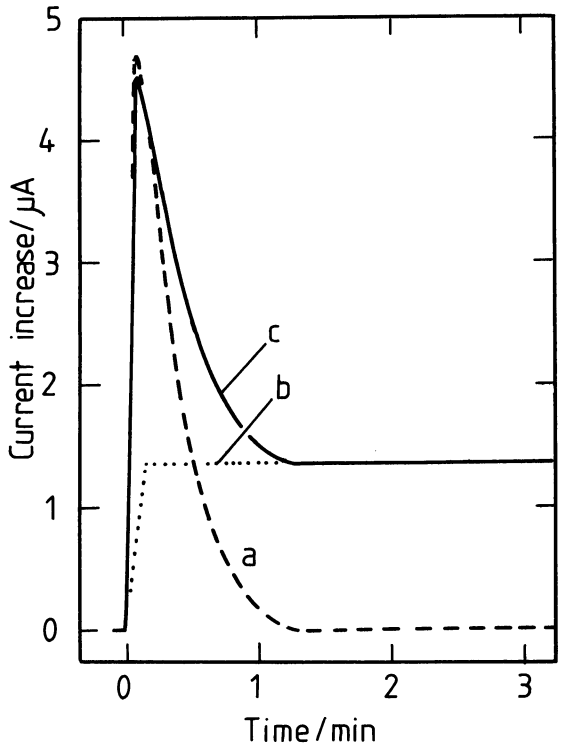

Fig. 2. Current response to (a) 1 μM L-ascorbic acid, (b) 50 nM catechol and (c) 1 μM L-ascorbic acid plus 50 nM catechol.

continuously in the measuring cell, as expected. The charge passed after the continuous electrolysis for 3 min was 0.243 mC, which was more than 40 times larger than the theoretical value corresponding to the simple electrooxidation of 50 nM catechol. The steady-state current increase obtained 1.5 min after the addition of the mixture of L-ascorbic acid and catechol (Curve c in Fig. 2) was coincide with that obtained by the addition of the L-ascorbate-free catechol solution (Curve b in Fig. 2). Thus the concentration of catechol could exactly be determined from the steady-state current response obtained after the consumption of L-ascorbic acid. Uric acid also did not influence the steady-state current response obtained 1.5 min after the addition of a catechol/uricate-mixture.

Figure 3 shows the effects of the FDH concentration upon the steady-state current response to 50 nM catechol and that to 50 nM dopamine. The current response of each analyte was independent of the FDH concentration in the range higher than 10 μg ml^{-1} (ca. 20 nM). In such a high FDH concentration region, the rate of the regeneration of the analyte is considered to be determined by the molecular activity (or turnover number, k_{cat}) of FDH and to be independent of the concentration of the enzyme.[7] Hence the electrode response has been independent of the FDH concentration as shown in Fig. 3. The smaller k_{cat}-value for dopamine would result in the smaller electrode response for the analyte, as compared to the case of catechol. Further investigations for the kinetics of the FDH-catalyzed reactions are in progress. Other enzymes which give larger k_{cat}-values are required in order to increase the electrode response to the analytes. The current response to catechol and that to dopamine were independent of the concentration of fructose at least in the range from 20 mM to 0.2 M.

Figure 4 shows calibration graphs for catechol and dopamine. A linear relationship was obtained up to

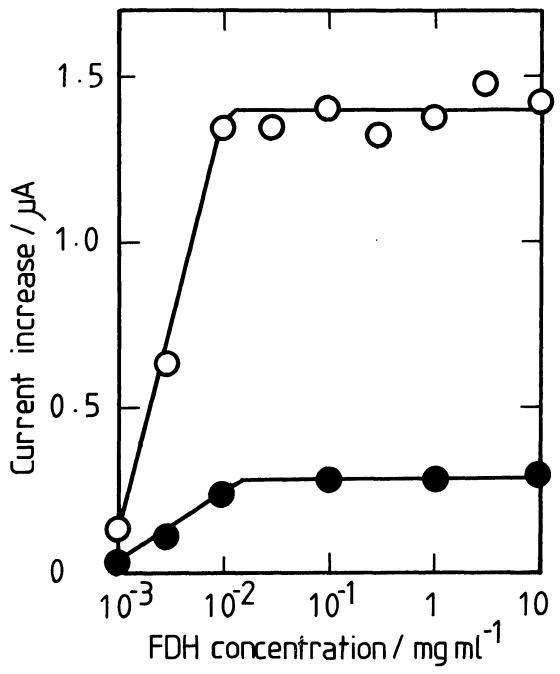

Fig. 3. Relationships between FDH concentration and electrode responses: for catechol(O); for dopamine (●).

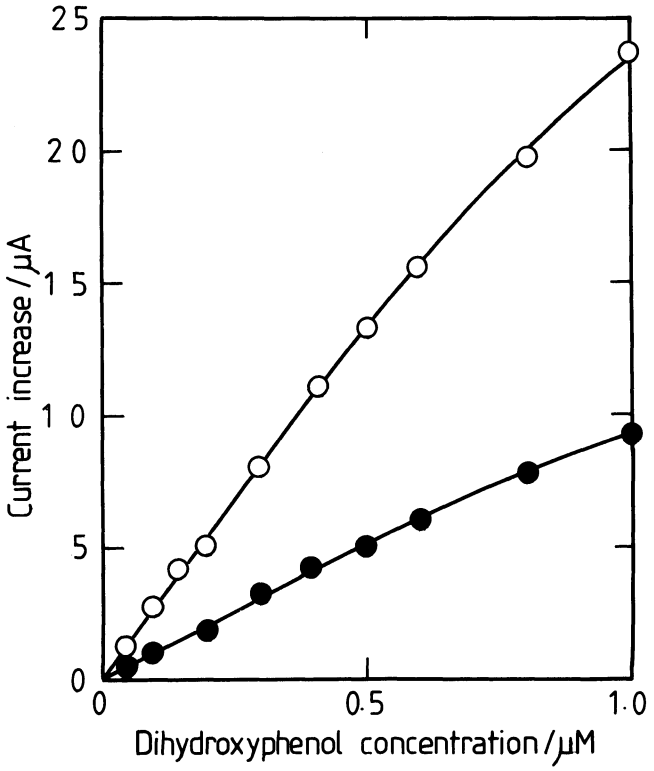

Fig. 4. Calibration graphs for catechol (O) and dopamine (●).

0.5 μM for each analyte. The detection limits were 1 nM for catechol and 5 nM for dopamine (signal-to-noise ratio, 5). The relative standard deviations (RSDs) for 10 measurements were ca. 5% for both analytes. The possible inhomogeneity in the unstirred test solution after the addition of the analyte may be responsible for the rather large RSD-values.

In the present method, the replacement of the FDH/fructose-containing test solution was required in order to restart the measurement. Although the replacement of the solution is somewhat troublesome, this would be circumvented by the high sensitivity and low interferential level.

References

1) e.g., F. Mizutani, Y. Shimura, and K. Tsuda, *Chem. Lett.*, **1984**, 199; F. Schubert, D. Kirstein, K. L. Schroeder, and F. W. Scheller, *Anal. Chim. Acta*, **169**, 391 (1985); T. Yao, H. Yamamoto, and T. Wasa, *ibid.*, **236**, 437 (1990); S. Uchiyama, Y. Hasebe, H. Shimizu, and H. Ishihara, *ibid.*, **276**, 341 (1993).
2) F. Mizutani, S. Yabuki ,and M. Asai, *Biosensors Bioelectron.*, **6**, 305 (1991).
3) S. Uchiyama, M. Ono, S. Suzuki, and O. Hamamoto, *Anal. Chem.*, **60**, 1835 (1988).
4) S. Uchiyama, Y. Kobayashi. S. Suzuki, and O. Hamamoto, *Anal. Chem.*, **63**, 2259 (1991).
5) S. Uchiyama, S. Maeda, Y. Hasebe, and S. Suzuki, *Anal. Chim. Acta*, **285**, 89 (1994).
6) M. Ameyama, E. Shinagawa, K. Matsushita, and O. Adachi, *J. Bacteriol.*, **145**, 814 (1981).
7) H. U. Bergmeyer, "Method of Enzymatic Analysis," ed by H. U. Bergmeyer, Verlag Chemie-Academic Press, New York and London (1974), p.131.

(Received June 9, 1994)