Letters to the Editor

A Novel Amperometric Urea Sensor

Toshiyuki OSAKAI*, Tadaaki KAKUTANI** and Mitsugi SENDA*

*Department of Agricultural Chemistry, Faculty of Agriculture, Kyoto University, Kyoto 606

**Research Center for Cell and Tissue Culture, Faculty of Agriculture, Kyoto University, Kyoto 606

T.O.: Present address Department of Chemistry, College of Liberal Arts, Kobe University, Nada, Kobe 657

Keywords Amperometric sensor, urea sensor, biosensor

In a previous paper¹, an amperometric ammonia sensor based on an ammonium-ion selective PVC-nitrobenzene-dibenzo-18-crown-6 (DB18C6) gel electrode has been reported. The electrode was covered with a gas-permeable Teflon membrane, with an internal-solution layer being sandwiched between the electrode and the membrane. In this study, an amperometric urea sensor was fabricated by immobilizing urease on the gas-permeable membrane surface of the amperometric ammonia sensor.¹ Urea enzyme sensors based on potentiometric detection of ammonium ion^{2,3}, ammonia gas⁴⁻⁶, carbon dioxide⁷ and pH-change⁸ have been reported. An amperometric urea enzyme sensor based on the pH dependence of electrochemical oxidation of hydrazine has also been reported.^{9,10} To the authors' knowledge, however, no sensor based on direct, amperometric detection of ammonium ion, as described in this paper, has yet been reported.

Experimental

Urease was obtained from Toyobo Co. (Grade II, 119 U/mg). Bovine serum albumin (BSA) was obtained from Sigma Chemical Co. Glutaraldehyde, 20% solution in water, was obtained from Wako Pure Chemical Industries. Chemicals for constructing the ammonia sensor have been described previously.^{1,11} The other chemicals were of analytical grade. The water was triply distilled.

The urease was immobilized by the procedure of Mascini and Guilbault⁶ on a gas-permeable Teflon membrane (area ca. 0.8 cm²) of the ammonia sensor (see Fig. 1 in Ref. 1). In 100 μ l of 15% BSA solution (0.2 mol dm⁻³ Tris buffer; pH 8.5) 8.4 mg of urease was dissolved. Five μ l of this solution (50 units) and 3 μ l of 2% glutaraldehyde solution were placed on the flat surface of the Teflon membrane, and the mixture was stirred for a few seconds with a small Teflon rod to spread it over the membrane surface. After letting the

surface stand for 15 min, it was rinsed successively with distilled water for 15 min and with 0.1 mol dm⁻³ glycine solution for 15 min. When not in use, the urease-immobilized ammonia sensor was stored in Tris buffer solution (pH 8.5) in a refrigerator.

The urease-immobilized ammonia sensor, that is, the urea enzyme sensor was examined in test solutions which were prepared by injecting an aliquot of the urea standard solution into the base solution (Tris buffer; pH 9.0) using a water-jacketed vessel thermostated at 25 $\pm$ 0.1 $^{\circ}$ C. The test solution was stirred at a definite rate using a magnetic stirrer. In amperometric measurements, the same apparatus as that for ammonia sensor¹ was used. The applied potential was kept at 0.37 V.

Results and Discussion

This urea enzyme sensor is based on the amperometric detection of ammonia produced by hydrolytic decomposition of urea:

which is catalyzed by urease immobilized on the Teflon

Fig. 1 Principle of amperometric urea sensor.

Fig. 2 Calibration curves of urea obtained with amperometric urea sensor.

membrane of the amperometric ammonia sensor. As schematically illustrated in Fig. 1, the ammonia produced by the enzymatic reaction passes through the Teflon membrane to reach the internal-solution layer (pH 9.7) of the ammonia sensor. In the internal-solution layer, the ammonia partially changes to ammonium ion, and both are transferred to the PVC-nitrobenzene-DB18C6 (=L) gel/internal solution interface, where the facilitated transfer of ammonium ion occurs. As a result, the current flow corresponding to the urea concentration in the test solution is observed.

When an aliquot of the urea standard solution was injected into the base solution, the urea sensor showed a well-defined current response in a manner similar to the ammonia sensor;¹ the current increased until it reached a peak after about 1 min, then it decreased gradually. The peak current I_p , was proportional to the urea concentration, C_{urea}^S , in the test solution from $1 \mu\text{mol dm}^{-3}$ to $2000 \mu\text{mol dm}^{-3}$, as shown in Fig. 2.

The urea sensor was able to be used repeatedly by washing with distilled water.

Design of amperometric biosensors for other analytes such as amino acids and creatinine, based on the same principle as described here, appears to be feasible. Application of pulse voltammetric technique¹² will be useful.

The authors wish to thank Mr. Hiroshi Miki for his invaluable assistance in the experiment. This work was supported in part by a Grant-in-Aid from the Ministry of Education, Science and Culture.

References

1. T. Osakai, T. Kakutani and M. Senda, *Anal. Sci.*, **3**, 521 (1987).
2. G. G. Guilbault and J. G. Montalvo, Jr., *J. Am. Chem. Soc.*, **92**, 2533 (1970).
3. G. G. Guilbault and J. G. Montalvo, Jr., *Anal. Lett.*, **2**, 283 (1969).
4. T. Anfält, A. Graneli and D. Jagner, *Anal. Lett.*, **6**, 969 (1973).
5. E. H. Hansen and J. Růžicka, *Anal. Chim. Acta*, **72**, 353 (1974).
6. M. Mascini and G. G. Guilbault, *Anal. Chem.*, **49**, 795 (1977).
7. G. G. Guilbault and F. R. Shu, *Anal. Chem.*, **44**, 2161 (1972).
8. H. Nilsson A. C. Åkerlund and K. Mosbach, *Biochim. Biophys. Acta*, **320**, 529 (1973).
9. D. Kirstein, L. Kirstein and F. Scheller, *Biosensors*, **1**, 117 (1985).
10. D. Kirstein, F. Scheller, Bo Olsson and G. Johansson, *Anal. Chim. Acta*, **171**, 345 (1985).
11. M. Senda, T. Osakai, T. Kakutani and T. Kakiuchi, *Nippon Kagaku Kaishi*, **1986**, 956.
12. T. Osakai, T. Nuno, T. Kakutani and M. Senda, paper presented at the 33rd Annual Meeting on Polarography and Electroanalytical Chemistry, Nara, 1987; abstract: *Rev. Polarogr.* (Kyoto), **33**, 73 (1987).

(Received July 13, 1988)

(Accepted July 23, 1988)