Xanthine Biosensor Based on Didodecyldimethylammonium Bromide Modified Pyrolytic Graphite Electrode

TANG, Ji-Lin(唐纪琳)   HAN, Xiao-Jun(韩晓军)   HUANG, Wei-Min(黄卫民)
WANG, Er-Kang*(汪尔康)
State Key Laboratory of Electroanalytical Chemistry and National Analytical Research Center of Electrochemistry and Spectroscopy, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, Jilin 130022, China

The vesicle of didodecyldimethylammonium bromide (DDAB) which contained tetrathiafulvalene (TTF) was mixed with xanthine oxidase, and the mixture was cast on the pyrolytic graphite electrode. The lipid films were used to supply a biological environment resembling biomembrane on the surface of the electrode. TTF was used as a mediator because of its high electron-transfer efficiency. A novel xanthine biosensor based on cast DDAB film was developed. The effects of pH and operating potential were explored for optimum analytical performance by using the amperometric method. The response time of the biosensor was less than 10 s. The detection limit of the biosensor was 3.2×10^{-7} mol/L and the liner range was from 4×10^{-7} mol/L to 2.4×10^{-6} mol/L.

Keywords   didodecyldimethylammonium bromide (DDAB), biosensor, xanthine oxidase (XOD), tetrathiafulvalene (TTF).

Introduction

Ordered films of water-insoluble surfactants can be prepared by casting their solutions or dispersions onto a solid support.[1] Evaporation of the solvent after casting leaves thin film self-assembled into ordered stack of bilayer, which is similar to biological membrane formed by lipid in living organism.[2-4] The bilayer provides a natural environment for embedding a host of compounds such as ion carriers, peptides, proteins, pigments and receptor.[5]

In early 1990s, Rusling[6-8] and Nakashima[9-10] *et al.* began to explore the electrochemistry of small electroactive molecules incorporated in cast multi-bilayer surfac-

tant film. This film exhibited excellent behavior of charge transport. Stable, ordered surfactant films have many possible applications, including coatings for sensors.[11] Rusling *et al.* have studied the direct electron transfer of myoglobin and P_{450} in the lipid film on the electrode.[12-13] They reported that the electron transfer rate was greatly enhanced in the insoluble lipid film. They also reported that Myoglobin-lipid film was used to reduce organohalides by electrochemical catalysis with enhanced rate compared to homogeneous reaction.[14] Recently, Hu *et al.* investigated the electrochemistry and electrocatalysis of myoglobin in biomembrane-like surfactant-polymer composite films.[15] Now, the study of cast surfactant film has aroused interest of more and more scientists.

Xanthine oxidase is a flavoprotein, which can catalyze the oxidation of hypoxanthine and xanthine to uric acid. Xanthine oxidase is also very important for the purine metabolism in human. This enzyme is one of the most complex flavoproteins because the enzyme activity is due to a complicated interaction of flavin adenine dinucleotide (FAD), molybdenum, iron and labile sulfur moieties at or near the active site.[16-17] An excess of uric acid in human blood results in gout; uric acid deposits may also occur as calculi in the kidney, with resultant renal damage. It is of medical and biological importance to develop a sensor for xanthine and hypoxanthine.

In this paper, TTF and DDAB were dissolved in chloroform. After chloroform evaporating, the mixture of

TTF and DDAB was suspended in phosphate buffer solution and utrasonicated to produce clear vesicle dispersions. By using this technique the mediator was immobilized in the lipid film strongly. Xanthine oxidase was added to the TTF-DDAB vesicle, so that xanthine oxidase can be incorporated in the vesicle. Then the mixture of xanthine oxidase and TTF-DDAB was cast on the pyrolytic graphite electrode. The DDAB film could supply a biological environment on the surface of the electrode for the enzyme. TTF is a very efficient mediator with well-defined electron stoichiometry, low redox potential and relatively reversible heterogeneous and homogeneous electron transfer,[18] and is inexpensive, stable and practically applicable. It has been applied to bioelectrocatalysis.[18-20] After the DDAB, TTF and xanthine oxidase were immobilized on the pyrolytic graphite electrode, the effect of pH and potential was optimized; and the analytical performance of the biosensor with respect to response time, sensitivity, interference and stability was evaluated.

Experimental

Reagents

DDAB was obtained from Acros (Beligum). Xanthine oxidase purified from buttermilk), TTF and Xanthine were purchased from Sigma (USA). All other chemicals employed were of analytical grade and used as obtained. The phosphate buffer contained 5×10^{-3} mol/L NaH_2PO_4 and 5×10^{-3} mol/L Na_2HPO_4 + 0.1 mol/L NaCl. The solution of xanthine was prepared daily. Pure water (18.2 MΩ) was used throughout and obtained by means of Millipore Q water purification set.

Preparation of enzyme modified electrode

A pyrolytic graphite electrode (diameter 3.5 mm) was first polished with sand paper followed by 1.0, 0.3 and 0.05 μm alumina slurry, respectively, sonicated in a deionized water bath for 1 min and dried at room temperature.

5 mg of DDAB and 0.1 mg of TTF were dissolved in 1 μL of chloroform. After evaporating the solvent under purified nitrogen the mixture of DDAB and TTF was suspended in 1 mL of phosphate buffer (pH = 6.8) and utrasonicated in an ice bath for 2 h to produce clear

vesicle dispersions.

XOD and the vesicle were mixed. The enzyme modified electrode was made by spreading 10 μL of this mixture evenly with a microsyringe onto the surface of the pyrolytic graphite electrode. Films were dried gradually overnight with a small bottle covered over the electrode to serve as a closed evaporation chamber, followed by a period of standing in air.

Measurements

A computer-controlled electroanalytical system (model CS-1087, Cypress Systems, Inc., Lawrence, KS, USA) was used to perform cyclic voltammetric measurement. Amperometric experiments were performed with bioanalytical systems BAS100B/W (West Lafayette, IN) including both hardware and software. All experiments were carried out with a three-electrode system with an Ag/AgCl (saturated KCl) as a reference electrode, platinum coils as an auxiliary electrode and the enzyme electrode as a working electrode.

All experiments were carried out at room temperature. The buffer solutions were stirred by a magnetic stirring bar during the amperometric experiment.

Results and discussion

Voltammetric behavior of the enzyme electrode and response of the electrode to xanthine

After the enzyme electrode immersed into the phosphate buffer solution, the cyclic voltammetry response was recorded from 100 mV to 450 mV. Fig. 1a shows the electrochemical behavior of the enzyme electrode at scan rate of 50 mV/s. In the range of voltage from 100 mV to 350 mV the electrochemical reaction of TTF can be described as:[21-22]

$$TTF \leftrightarrow TTF^+ + e$$
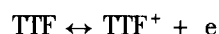
When xanthine (4×10^{-7} mol/L) was added into the solution, the oxidation current increased dramatically with a concomitant decrease in the reductive current (Fig. 1b). The result illustrates that TTF can effectively enhance electron communications between the enzyme in the DDAB film and the pyrolytic graphite electrode.
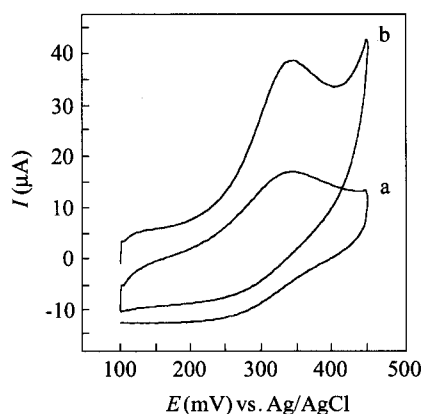
Fig. 1 Cyclic voltammograms of the xathine sensor in phosphate buffer (0.005 mol/L, pH 6.8). (a) Without xanthine; (b) with 4×10^{-7} mol/L xanthine. Scan rate, 50 mV/s.

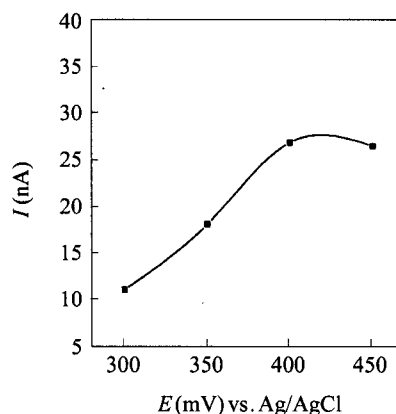*Effect of* pH *and potential on the current response of the biosensor*

The environmental pH is well known to affect the substrate specificity of enzymes. The effect of pH on the current response of the enzyme electrode was studied from pH 5.5 to pH 8.0. A series of phosphate buffers was employed for this purpose. In Fig. 2, the pH effect on the enzyme electrode for 4×10^{-7} mol/L xanthine is shown. As shown in Fig. 2, the steady-state response current increased with increasing pH up to 7.0.
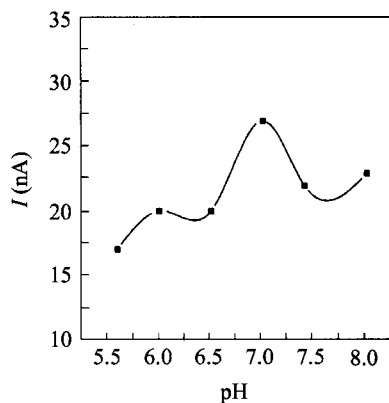
Fig. 2 Effect of pH on the sensor response for xanthine $(4 \times 10^{-7}$ mol/L) in phosphate buffer at the applied potential of 400 mV.

The effect of potential on the steady-state current of the sensor for xanthine is shown in Fig. 3. The steady-state current increased with the applied potential increas-

ing from 300 mV to 450 mV. We select 400 mV as the working potential.

Fig. 3 Effect of potential on the sensor response for xanthine $(4 \times 10^{-7}$ mol/L) in phosphate buffer (0.005 mol/L, pH 6.8).

Current response of biosensor

The enzyme electrode was tested by recording the amperometric response resulting from consecutive increments in the concentration of xanthine at an applied potential of 400 mV. The typical time dependence of the response current of the enzyme electrode on successive step changes of xanthine is shown in Fig. 4. When an aliquot of xanthine was added into the buffer solution, the oxidiation current rose steeply to reach a stable value. The enzyme electrode achieved 90% of steady-state current in 10 s, which indicated a fast diffusional process.
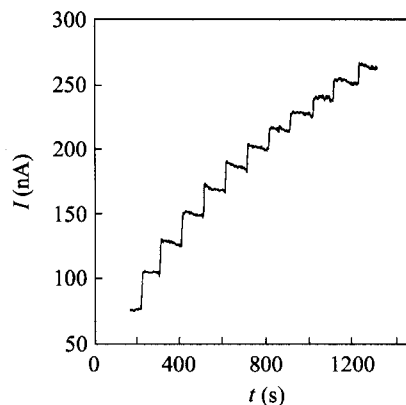
Fig. 4 Typical current-time response curve for successive addtion of xanthine $(4 \times 10^{-7}$ mol/L) for the sensor in phosphate buffer (0.005 mol/L, pH 6.8). Potential, 400 mV.

The calibration curve of the xanthine biosensor is shown in Fig. 5. The liner range of xanthine concentration is between 4.0×10^{-7} mol/L and 2.4×10^{-6} mol/L with a correlation coefficient of 0.993. The xanthine sensor has a detection limit of 3.2×10^{-7} mol/L at a signal-to-noise ratio of 3.
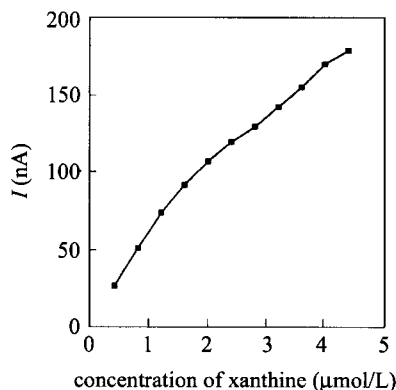
Fig. 5 Calibration curve for successive addition of xanthine (4×10^{-7} mol/L) in phosphate buffer (0.005 mol/L, pH 6.8). Potential, 400 mV.

The effect of substance that might interfere with the response of the enzyme was studied at 400 mV. The interference was measured by current obtained for each interfering substance at a concentration of 4×10^{-7} mol/L and compared with the current obtained for the same concentration of xanthine, and this ratio was used as a criterion for the selectivity of the sensor. In the experiment, glucose, sucrose and lactic acid did not cause any observable interference.

The reproducibility of the sensor was examined at a xanthine concentration of 4.0×10^{-7} mol/L with the same enzyme electrode, and the relative standard deviation is 7.5% ($n = 7$). The lifetime of the xanthine sensor was 3 d.

Conclusions

In this paper, a new method to immobilize mediator was developed. The biosensor of xanthine was made by casting the vesicle of TTF and DDAB mixed with xanthine oxidase on the pyrolytic graphite electrode. The lipid film can provide a biological environment resembling biomembrane on the surface of electrode for xanthine oxidase. The xanthine sensor has a low detection limit of 3.2×10^{-7} mol/L and is easy to make.

Reference

1   Suga, K. S.; Rusling, J. F. *Langmuir* **1993**, *9*, 3649.
2   Nakasima, N.; Ando R.; Kunitake, T. *Chem. Lett.* **1983**, 1577.
3   Kunitake, T.; Tsuge, A.; Nakashima, N. *Chem. Lett.* **1984**, 1783.
4   Kunitake, T.; Shimomura, M. *Thin Solid Film* **1984**, *121*, L89.
5   Tien, H. T.; Ottaova, A. L. *Electrochim. Acta* **1998**, *43*, 3587.
6   Rusling, J. F.; Hu, N. *Anal. Chem.* **1991**, *63*, 2163.
7   Rusling, J. F.; Nassar, A. E. F. *J. Am. Chem. Soc.* **1993**, *115*, 11891.
8   Rusling, J. F.; Zhang, H. *Langmuir* **1991**, *7*, 1791.
9   Nakashima, N.; Masuyama, K.; Mochida, M.; Kunitake, M.; Manabe, O. *J. Electroanal. Chem.* **1991**, *319*, 355.
10 Nakashima, N.; Wake, S.; Nishino, T.; Kunitake, M.; Manabe, O. *J. Electroanal. Chem.* **1992**, *333*, 345.
11 Okahata, Y.; Ebato, H. *Anal. Chem.* **1991**, *63*, 1791.
12 Zhang, Z.; Nassar, A. E. F.; Lu, Z.; Schenkman, J. B.; Ruslings, J. F. *J. Chem. Soc., Farady Trans.* **1997**, *93*, 1769.
13 Nassar, A. E. F.; Bobbitt, J. M.; Stuart, J. D.; Rusling, J. F. *J. Am. Chem. Soc.* **1995**, *117*, 10986.
14 Hu, Y.; Hu, N.; Zeng, Y. *Talanta* **2000**, *50*, 1183.
15 Arai, G.; Takahashi, S.; Yasumori, I. *J. Electroanal. Chem.* **1996**, *410*, 173.
16 Massey, V.; Brumby, P. E.; Komal, H.; Palmer, F. *J. Biol. Chem.* **1969**, *244*, 1682.
17 Zhao, S.; Luong, J.; Tien, H. T. *Anal. Chim. Acta* **1993**, *287*, 319.
18 Wu, Z.; Wang, B.; Dong, S.; Wang, E. *Biosens. Bioelectron.* **2000**, *15*, 143.
19 Wang, B., Li, B.; Deng, Q.; Dong, S. *Anal. Chem.* **1998**, *70*, 3170.
20 Turner, A. P. F., Hendry, S. P.; Cardosi, M. F. *Biosensors: Instrumentation and Processing*, The World Biotechnology Report, Online Publications, London, **1987**, p. 125.
21 Gunasingham, H.; Tan, C. *Electroanalysis* **1989**, *1*, 423.