Sensitive Determination of Acetylcholinesterase Based on Chemisorption/Electrochemical Desorption-Process of Thiol Compound on a Silver Electrode

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Numerous works have dealt with the chemisorption of thiol compounds on metal electrodes and the reductive desorption of the thiolate chemisorbed through one-electron path. The chemisorption/desorption-process seems to be useful for determining thiol compounds (and thiol-producing catalysts) with high sensitivity. The thiol molecules in a test solution can be accumulated on the electrode surface through the chemisorption. The accumulation of the analyte resulted in a relatively large electrochemical response, as in the case of stripping voltammetric determinations.[1]

We[2] have successfully determined acetylcholinesterase (AChE, EC 3.1.1.7) with a high sensitivity (detection limit, 10^{-2} U l^{-1}), based on the following enzymatic thiol-production (eq 1), chemisorption (accumulation) (eq 2), and desorption (current measurement) (eq 3) processes:

$$(CH_3)_3N^+CH_2CH_2SCOCH_3 + H_2O \xrightarrow{\text{AChE}} (CH_3)_3N^+CH_2CH_2SH + CH_3COOH \quad (1)$$

$$(CH_3)_3N^+CH_2CH_2SH + Ag \rightarrow (CH_3)_3N^+CH_2CH_2S—Ag + 1/2H_2 \quad (2)$$

$$(CH_3)_3N^+CH_2CH_2S—Ag + e^- \rightarrow (CH_3)_3N^+CH_2CH_2S^- + Ag \quad (3)$$

The test solution used was 0.1 M phosphate buffer (pH 7.6, 23±1 °C) containing 10 mM acetylthiocholine (Sigma). The solution was stirred with a magnetic bar. A silver disc electrode (diameter, 1.6 mm) covered with a dialysis membrane was immersed in the solution for 10 min, immediately after the addition of AChE (from electric eel, Sigma). Then the silver electrode was rinsed with water, and the dialysis membrane on the electrode surface was removed. The electrode was transferred into 0.1 M KOH and the thiocholine on the electrode surface was electrochemically desorbed. The relationship between the charge and the AChE activity was examined. The AChE activity was measured by the method of Ellman et al.[3]

Thiocholine molecules on the silver electrode surface desorbed cathodically with a peak at –1.15 V (vs Ag/AgCl) in 0.1 M KOH. In association with the increase in the AChE activity, the peak height at –1.15 V increased. A linear relationship was obtained between the charge for the cathodic peak and the AChE activity from 0.08 to 0.4 U l^{-1}. The charge for the cathodic peak was estimated from the current response obtained over the potential range from –0.95 to –1.25 V. When the AChE activity was larger than 0.5 U l^{-1}, the charge reached a plateau, 70 μC cm^{-2}. The value well agreed with that for the close-packed monolayers for alkanethiols.[4] The relative standard deviation for 6 measurements of 0.2 U l^{-1} AChE activity was 4.8%.

The dynamic range for the determination of AChE activity can be shifted by varying the soaking time for the dialysis membrane-covered electrode into the AChE/acetylthiocholine solution. When the soaking time was shorter (e.g. 5 min), the AChE with higher activity range (from 0.1 to 5 U l^{-1}) could be measured. In contrast, a longer soaking time (e.g. 30 min) resulted in the determination of AChE activity in the lower activity range (from 0.01 to 0.08 U l^{-1}). The lower detection limit obtained by soaking for 30 min, 0.01 U l^{-1}, was 100-fold higher than that was obtained by conventional amperometric method.[5]

Such a sensitive determination method is useful for the diagnosis of liver disease using a tiny amount of biological samples, and applicable to the construction of a novel enzyme immunoassay system.

References

1 E. Barendrecht, and A. J. Bard, “Electroanalytical Chemistry,” Marcel Dekker, Inc., New York (1967), Vol.2; K. Z. Brainina, *Talanta*, **18**, 513 (1971); H. Sawamoto, *J. Electroanal. Chem.*, **113**, 301 (1980).

2 H. Matsuura, Y. Sato, T. Sawaguchi, and F. Mizutani, *Chem. Lett.*, **2002**, 618; *Sensors Actuators B*, in press.

3 G. L. Ellman, K. D. Courtney, V. Andres, and R. M. Featherstone, *Biochem. Pharmacol.*, **7**, 88 (1961).

4 C. A. Widrig, C. A. Alves, and M. D. Porter, *J. Am. Chem. Soc.*, **113**, 2805 (1991).

5 F. Mizutani and K. Tsuda, *Anal. Chim. Acta.*, **139**, 359 (1982).