Highly Sensitive Determination of Acetylcholinesterase Activity Based on the Chemisorption/Reductive Desorption-Process of Thiol Compound on a Silver Electrode

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(Received March 19, 2002; CL-020254)

The chemisorption/reductive desorption-process of a thiol compound on a silver electrode was applied to the highly sensitive determination of a thiol-producing enzyme, acetylcholinesterase.

Numerous works have dealt with the chemisorption of thiol compounds on metal electrodes[1,2] and the reductive desorption of the thiolate chemisorbed through one-electron path.[3] 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.[4] Indeed, we have succeeded in determining the activity of 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:

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

$$(CH_3)_3N^+CH_2CH_2SH + Ag \rightarrow (CH_3)_3N^+CH_2CH_2S\text{–}Ag + 1/2H_2 \ (\text{or} + e^- + H^+) \quad (2)$$

$$(CH_3)_3N^+CH_2CH_2S\text{–}Ag + e^- \rightarrow (CH_3)_3N^+CH_2CH_2S^- + Ag \quad (3)$$

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.

A silver disk electrode of 1.6 mm diameter (Bioanalytical systems) was polished with aqueous slurries of successively finer diamond (down to 1 μm) on a polishing microcloth (Bioanalytical systems) and was sonicated for 6 min in water. Then the silver electrode was cleaned electrochemically in 0.1 M KOH by potential cycling in the range from -0.4 to -1.4 V (vs Ag/AgCl) at the potential scan rate of 50 mV s^{-1} for more than 15 min. When a bare silver electrode was immersed in a solution containing AChE, the protein molecules adsorbed onto the electrode surface, so as to avoid the adsorption of thiocholine. Therefore, the silver electrode thus cleaned was covered with a dialysis membrane. 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. The dialysis membrane-covered silver electrode was immersed in the solution for 10 min immediately after the addition of AChE (from electric eel, 261 U mg^{-1}, Sigma). The silver electrode was rinsed with water, then 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.[5]

Our preliminary examination showed that the thiocholine on the silver electrode surface desorbed cathodically with a peak at -1.15 V (vs Ag/AgCl) in 0.1 M KOH. The reductive desorption of the thiocholine on the silver electrode took place over a wide potential range from -0.95 to -1.25 V owing to differences in the structure of the chemisorbed thiocholine molecules. Figure 1 shows the linear sweep voltammograms for the reductive desorption of thiocholine obtained after soaking in the solutions

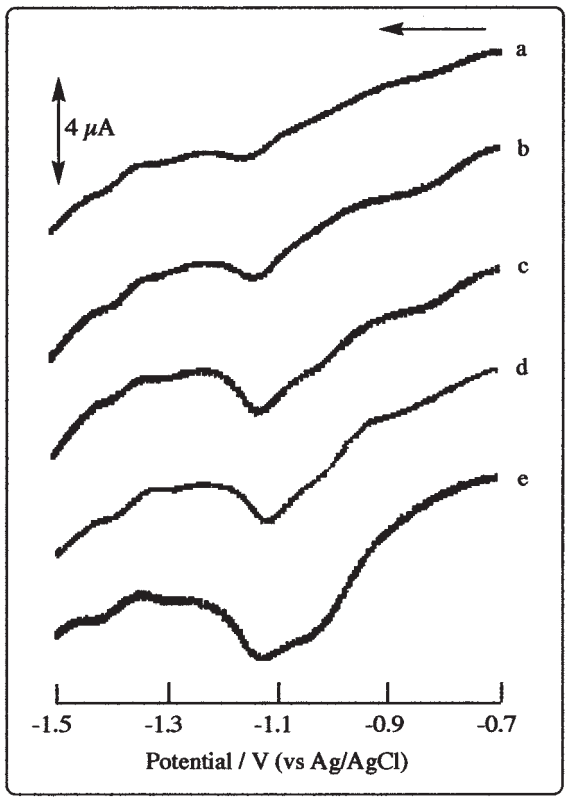

Figure 1. Linear sweep voltammograms for reductive desorption of thiocholine in 0.1 M KOH (sweep rate: 50 mV s^{-1}). AChE activities for producing thiocholine are 0 U l^{-1} (a), 0.08 U l^{-1} (b), 0.2 U l^{-1}(c), 0.3 U l^{-1}(d) and 0.5 U l^{-1}(e).

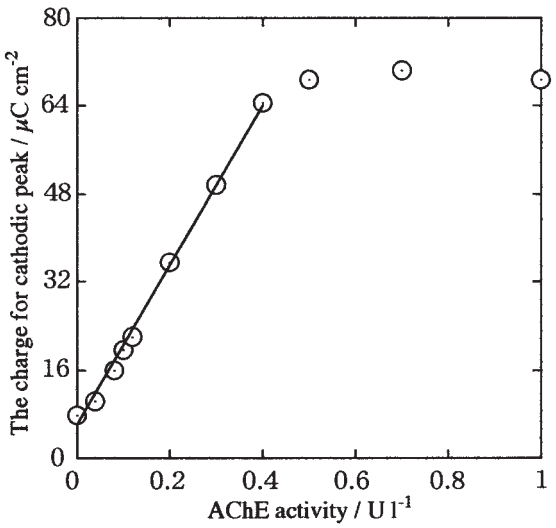

Figure 2. The relationship between the charge and the AChE activity.

containing different activities of AChE. 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} (Figure 2). 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.[6] The current response obtained in the enzyme-free solution was caused by the spontaneous hydrolysis of acetylthiocholine (see curve (a) in Figure 1). 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.[7]

In conclusion, we first applied the chemisorption/reductive desorption-process of thiocholine on the silver electrode to the determination of acetylcholinesterase activity. In the present method, the pretreatment of a sample solution will be required in order to avoid the adsorption of interferant. Although the pretreatment of the sample solution is somewhat troublesome, this would be circumvented by the high sensitivity.

References and Notes

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