Surface-Electrochemical Sensor for the Measurement of Anti-Cholinesterase Activity

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An organophosphorus pesticide, ethylthiometon (0.01–0.2 ppm) was determined by using a surface-electrochemical sensor system: the monolayer formation (chemisorption)-reductive desorption of thiocholine was applied to monitor the activity change of cholinesterase caused by the pesticide.

Keywords: Organophosphorus pesticide, Inhibition of cholinesterase activity, Thiolate monolayer

1. Introduction

The accurate and rapid determination of cholinesterase is required for environmental monitoring; the anti-cholinesterase activity measurement is used as a screening test for evaluating the pollution caused by organophosphorus pesticides (OPPs). Electrochemical sensors can accomplish this function⁽¹⁻³⁾. However, the development of high-sensitive sensor systems is still desired. Such systems can provide inexpensive measurements of OPPs, since the increase in the sensitivity resulted in the reduction of the amount of expensive indicator enzyme, cholinesterase, required for the measurement.

For the construction of high-sensitive electrochemical sensors, the surface coulometry⁽⁴⁾ is a useful technique. Here, the analyte is first chemisorbed on a metal (M) electrode surface to form a monolayer, and the adsorbate is then electrochemically desorbed. The chemisorption, i.e., accumulation of the analyte on the surface brings about a relatively large electrode response. We^(4,5) have successfully applied the chemisorption-desorption procedure for the determination of cholinesterase activity as low as 0.01 U l⁻¹, by employing the sequence of enzymatic reaction (1), chemisorption (2) and electrochemical desorption (3):

This paper describes preliminary results for measuring ethylthiometon, based on the above reaction sequence.

2. Experimental

Cholinesterase (acetyl, EC 3.1.1.7; 4 mU) was dissolved into 0.1 M phosphate buffer solution (pH 8, 20 ml) containing 0.5% (v/v) methanol and ethylthiometon, and the enzyme/ethylthiometon-solution was incubated for 30 min at room temperature. A silver disc electrode (diameter 1.6 mm, Bioanalytical Systems, West Lafayette, IN), which was covered with dialysis membrane to avoid the adsorption of cholinesterase, was immersed into the solution. Immediately after the addition of the acetylthiocholine (5 mM), a negative potential (-0.7 V vs. Ag/AgCl) was applied for 5 min on the silver electrode. The adsorption rate for the positively-charged reaction product, thiocholine, on the electrode surface remarkably increased by applying the negative potential⁽⁵⁾. Then the electrode was transferred into 0.1 M KOH and the thiolate on the electrode surface was cathodically desorbed.

3. Results and discussion

The decrease in the cholinesterase activity through the inhibition by ethylthiometon would result in the decrease of the number of thiocholine molecules produced, which brings about the decrease of the number of adsorbed thiolate. This can directly be monitored by the decrease of the charge for the electrochemical desorption. Figure 1 shows linear sweep voltammograms for the desorption of thiolate after soaking the enzyme/substrate/OPP-solution. The reductive desorption took place in a potential range from -0.95 V to -1.25 V vs. Ag/AgCl with a dominant peak at -1.15 V vs. Ag/AgCl. In association with the increase in ethylthiometon concentration, the peak height decreased as expected.

Figure 2 shows the relationship between the inhibition percentage (% I) by ethylthiometon and the OPP concentration. The inhibition was calculated according to the formula:

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Fig. 1. Linear sweep voltammograms for reductive desorption of thiolate on the silver electrode in 0.1 M KOH (potential sweep rate: 50 mV s^{-1}). Concentration of ethylthiometon: a) 0 ppm; b) 0.01 ppm; c) 0.05 ppm; d) 0.1 ppm; e) 0.5 ppm; f) 1 ppm. Other experimental conditions, see the text.

Fig. 2. Relationship between the inhibition percentage and the concentration of ethylthiometon.

$$\% I = \{(C_0 - C) / C_0\} \times 100 \quad (4)$$

Where C_0 denotes the charge for the reductive desorption of thiolate obtained after soaking in the ethylthiometon-free solution (blank) and C , that obtained after soaking in the ethylthiometon-containing solution. The charge was obtained from the cathodic current increase from -0.95 V to -1.25 V (vs. Ag/AgCl).

As shown in Fig. 2, the concentration of ethylthiometon could be determined from 0.01 to 0.2 ppm. The relative standard deviation for six successive measurements of 0.05 ppm ethylthiometon was 7.8%. The concentration of ethylthiometon in vegetables is regulated to be less than 0.1 ppm in Japan. Hence the present sensor system is useful for the screening test of the vegetable samples.

4. Conclusion

Electrochemical monitoring of electrode/monolayer (adlayer)/solution-systems, which proved to be useful for measuring the species adsorbed with high sensitivity, was successively applied to the measurement of anticholinesterase activity caused by organophosphorus pesticides. The concentration of ethylthiometon, could be measured in the concentration range from 0.01 to 0.2 ppm.

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