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Sensitivity of Quartz Crystal Microbalance Precipitation Sensor for Paraoxon-ethyl

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Abstract

A quartz crystal microbalance biosensor which measured the inhibition in enzyme activity by an organophosphorus pesticide, paraoxon-ethyl, was developed. When inhibitor concentration increased, sensor response gradually decreased owing to the inhibition of the immobilized biocatalyst. The relationship between inhibitor concentration and percent inhibition (%I) was Y (%I)=12.48X ($\log_{10}$ paraoxon-ethyl)+122.30 (r=0.8477) at the paraoxon-ethyl concentrations of 3.63×10^{-10}-3.63×10^{-6} M. The %I values found for newly prepared acetylcholinesterase-immobilized sensor chips in the presence of 3.63×10^{-7} M paraoxon-ethyl were determined and the coefficient of variability for six measurements was found as 5.46%.

Keywords: QCM-precipitation sensor, Pesticide detection, Enzyme inhibition

Introduction

Most of the insecticides used recently have been classified as cholinesterase (ChE) inhibitors such as organophosphorus and carbamate pesticides. Although they normally show low environmental persistence and high effectiveness, some of them exhibit strong dose-related acute and chronic toxicity in human beings by acting on ChE[1]. Until now, organophosphates and carbamates have chiefly been determined by instrumental analyses including GC, HPLC and HPLC coupled with mass selective detector[2,3]. These methods, however, are undertaken in restricted laboratory facilities equipped with complicated instruments, and time-consuming and expensive in analytical cost, thus limiting their utility to high-precision detection and quantification of individual chemicals[4].

As one of rapid screening tools for organophosphorus and carbamate pesticides, a ChE-based biosensor generally determines ChE inhibition as a sum parameter[5,6]. Compared to a ChE inhibition test comprising spectrophotometric assay[7,8], its sensitivity could be enhanced greatly by the introduction of a new transduction principle for sensor response[9,10]. Moreover, it is useful for on-site monitoring and easily applied to a real-time multi-sample analysis using an arrayed system[10].

It has been reported that the sensitivity of a quartz crystal microbalance (QCM)-based enzyme sensor can be amplified significantly by increasing mass deposition by way of the precipitation of enzymatic reaction products[9,11,12]. Karousos *et al*.[13] have applied this fact to the detection of organophosphorus and carbamate pesticides and reported on a QCM sensor detecting acetylcholinesterase (AChE) inhibition by measuring the precipitation degree of an enzymatic reaction product, 4,4′-diimino-3,3′-diaminobiphenyl which is derived from 3,3′-diaminobenzidine substrate, over the QCM electrode. In this study, we measured the sensitivity of a QCM-precipitation sensor for paraoxon-ethyl.

Results and Discussion

The QCM-precipitation sensor of this study measures the inhibition in enzyme activity of the QCM sensor chip, immobilized with AChE on one side and simultaneously exposed to the substrate solution, by an organophosphorus pesticide, paraoxon-ethyl. The properties of the sensor are described briefly as follows. Table 1 shows the sensor responses according to the change in paraoxon-ethyl concentration. As expected, it was the biggest when the inhibitor was not present. When inhibitor concentration increased, sensor response gradually decreased owing to the inhibition of the immobilized biocatalyst[6]. Then, the relationship between inhibitor concentration and %I was plotted in semi-logarithmic scale at the paraoxon-ethyl concentrations of 3.63×10^{-10}-3.63×10^{-6} M. As shown in Figure 1, the increase in inhibitor concentration was correlated with the decrease in frequency shift, and a linear relationship found between inhibitor concentration and %I, with the regression equation of Y (%I)=12.48X ($\log_{10}$ paraoxon-ethyl)+122.30 (r=0.8477). From the results of Table 1 and Figure 1, and the normally accepted criterion of three times of

Table 1. Inhibition degrees of the QCM-precipitation sensor at various concentrations of paraoxon-ethyl.

Paraoxon-ethyl (M)	Frequency shift (ΔF)[a]	%I
3.63×10^{-10}	134.99±1.58[b]	10.61
1.82×10^{-9}	129.12±2.71	14.50
3.63×10^{-9}	120.80±3.12	20.01
1.82×10^{-8}	118.28±2.21	21.68
3.63×10^{-8}	112.59±3.78	25.45
1.82×10^{-7}	111.01±1.93	26.49
3.63×10^{-7}	99.00±1.62	34.45
1.82×10^{-6}	59.99±2.47	60.28
3.63×10^{-6}	57.48±2.66	61.94

[a]The sensor response found in the absence of paraoxon-ethyl was 151.02±1.11 Hz.

[b]Five values of frequency shift at adjacent measuring time were determined, and the mean and standard deviation were shown.

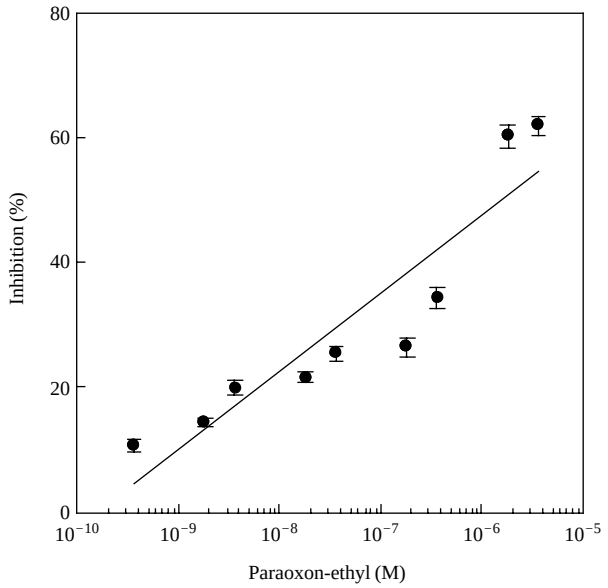

Figure 1. Calibration curve between inhibitor concentration and %I plotted in semi-logarithmic scale. Five values of frequency shift at adjacent measuring time were determined and error bars were inserted.

the standard deviation for baseline drift, the sensor response for paraoxon-ethyl was even meaningful at 1.82×10^{-9} M. The sensitivity obtained in this study was approximately higher by two decades compared to that of a spectrophotometric assay reported[8]. Also, the QCM-precipitation sensor of this study showed better sensitivity in pesticide detection than an inhibition sensor which required multiple enzymes like AChE, choline oxidase and horseradish peroxidase for signal transduction[13].

The %I values found for newly prepared AChE-immobilized sensor chips in the presence of 3.63×10^{-7} M paraoxon-ethyl were determined. As shown in Table 2, the coefficient of variability for six measurements was found as 5.46%. This value was as comparable as those reported for EPN and carbofuran[4].

Table 2. Repeatability of measurement for six new chips of the QCM-precipitation sensor at the paraoxon-ethyl concentration of 3.63×10^{-7} M.

Chip number	%I
1	39.49
2	38.99
3	33.99
4	34.89
5	36.22
6	37.42
Mean±S.D. (n=6)	36.83±2.01

Conclusion

The QCM-precipitation sensor of this study shows a possibility as one screening tool for ChE-inhibiting pesticides which are possibly present at very small amounts in agricultural products such as fruits and vegetables.

Materials and Methods

Reagents and Transducer

AChE from *Electrophorus electricus* (an electric eel) that was used as the biocatalyst for signal amplification and a histological substrate for the biocatalyst, 3-indolyl acetate, were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). A heterobifunctional cross-linker for AChE immobilization over the QCM surface, sulfosuccinimidyl 6-[3-(2-pyridyldithio) propionamido]hexanoate (sulfo-LC-SPDP), was supplied from Pierce Biotechnology Inc. (Rockford, IL, USA). The analyte of the sensor, paraoxon-ethyl (M.W. 275.20, $C_{10}H_{14}NO_6P$), was obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany). The other reagents for buffer preparation, and substrate dissolution like Tween 80 and dimethylformamide (DMF) were purchased from Sigma-Aldrich Co. Doubly distilled water was used throughout this research. A 9 MHz AT-cut piezoelectric quartz wafer (QA 9RP-50, Seiko EG and G, Tokyo, Japan), showing a reproducibility of ±0.1 Hz in frequency response, was used as the transducer.

Enzyme Immobilization

AChE immobilization was undertaken according to

the antibody immobilization procedure of Park and Kim[14] with a slight modification. The QCM was soaked in 1.2 M NaOH for 5 min, washed with distilled water and immersed in 1.2 M HCl for 5 min in sequence. After washing with distilled water, it was treated with 20 μL of conc. HCl for 1 min with a special care to keep the acid from touching the electrode leads, washed again with distilled water and finally dried in a convection oven for 20 min. Three microliters of the enzyme solution including 5 units of AChE and the same volume of 20 mM sulfo-LC-SPDP dissolved in distilled water were mixed and the resulting mixture was incubated at room temperature for 1 h. To reduce disulfide bonds of the thiolated AChE, 2 μL of dithiothreitol dissolved in 0.1 M sodium acetate (pH 4.5) containing 0.1 M NaCl was added and reacted for 30 min. The resulting solution was spread over the entire surface of one side gold electrode on the crystal, followed by drying for 1 h at room temperature. The AChE-coated sensor chip thus prepared was consecutively washed with distilled water and 0.1 M potassium phosphate (pH 8.0).

Sensor System and Analytical Procedure

The batch-type sensor system of this study was constructed using a dip holder containing a sensor chip immobilized with the biocatalyst, an oscillator module (QCA 917-11, Seiko EG and G), a quartz crystal analyzer (QCA 917, Seiko EG and G) and an IBM-compatible PC installed with a GPIB interface, as reported previously[4]. As the enzymatic reaction product is precipitated over the QCM surface after dimerization, the degree of sensor response can be traced, in real-time scale, by determining the frequency decrease caused by mass deposit over the QCM surface[9]. To measure a sensor response, the dip holder was first inserted into a small beaker filled with 19.5 mL of 0.1 M potassium phosphate (pH 8.0) containing 0.01% Tween 80, followed by the measurement of resonant frequency of the sensor chip until a steady-state baseline was attained (F_1). Then, 500 μL of 50 mg/mL 3-indolyl acetate dissolved in DMF as the substrate solution was injected into the reaction cell including the above reaction buffer, with a simultaneous stirring for 3 min to induce complete substrate dissolution in the aqueous buffer. The steady-state resonant frequency (F_2) was read again to calculate the frequency shift ($\Delta F = F_1 - F_2$).

As an organophosphate is normally reported to show a noncompetitive inhibition pattern[15], paraoxon-ethyl was added to the reaction cell simultaneously with the substrate solution to measure AChE inhibition. In this case, the presence of this analyte can be determined by tracing the diminution of sensor signal from the level obtained in its absence. The %I was calculated as follows.

$$\%I = \frac{(\Delta F_o - \Delta F_p)}{\Delta F_o} \times 100, \%$$

Where, ΔF_o is the frequency shift obtained in the absence of pesticide and ΔF_p the frequency shift obtained after the addition of a known amount of inhibitor.

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