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A Dipstick-Type Electrochemical Immunosensor for The Detection of The Organophosphorus Insecticide Fenthion

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Abstract A dipstick-type immunochemical biosensor for the detection of the organophosphorus insecticide fenthion was developed using a screen-printed electrode system as an amperometric transducer with polyclonal antibodies against fenthion as a bioreceptor. The assay of the biosensor involved competition between the pesticide in the sample and pesticide-glucose oxidase conjugate for binding to the antibody immobilized on the membrane. This was followed by measurement of the activity of the bound enzyme by the supply of the enzyme substrate (glucose) and amperometric determination of the enzyme reaction product (H_2O_2). The activity of the bound enzyme was inversely proportional to the concentration of pesticide. The optimized sensor system showed a linear response against the logarithm of the pesticide concentration ranging from 10^{-2} to 10^3 μg/L.

Keywords: fenthion, organophosphorus insecticide, amperometry, immunosensor, dipstick

Introduction

Fenthion [*O,O*-dimethyl *O*-[3-methyl-4-(methylthio)phenyl] phosphorothioate] is an insecticide used to control fruit flies, leafhoppers, stem borers, cereal bugs, and other insect pests found on fruit, vegetables, cotton, rice, beet, tobacco, and other crops (1). The toxicologically relevant effect of fenthion is inhibition of acetylcholinesterase activity in the target insect (1).

Due to the widespread use of pesticides, there is increasing concern over food contamination caused by their use. Current methods like gas and liquid chromatography have been used successfully for analysis of many pesticides (2). However, these classical methods are costly, require skilled analysts, and involve time-consuming sample preparation steps. Therefore, there is a growing demand for more rapid and economical methods for detecting pesticide residues. One method that has recently emerged as an alternative to these traditional methods is the use of immunoassays (3). Immunoassays such as enzyme-linked immunosorbent assay (ELISA) are usually performed in laboratories using microtiter plates and an ELISA reader, and, thus, are not suitable for on-site screening. An interesting solution to this problem is the use of disposable immunosensors. Their use as a diagnostic tool for detecting pesticides (4-7) and other environmental pollutants (8, 9) is becoming more common.

This paper describes an electrochemical immunosensor for the detection of the organophosphorus insecticide fenthion. The sensor system is composed of an antibody-coated membrane strip and a screen-printed carbon electrode. The principle of the sensor is based on competition between the pesticide and pesticide-glucose oxidase (GOD) conjugate for binding to the antibody. Most electrochemical immunosensors use an enzyme- or antibody-coated electrode, which allows assay protocols simpler than those of ELISAs. However, such a biologically modified electrode often suffers from a large matrix effect (10). In this study, the possibility was explored of fabricating a dipstick-type immunosensor, which allows minimization of the matrix effect through separation of the electrochemical measurement step from the competition step.

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Materials and Methods

Reagents and instruments Glucose oxidase, casein, bis-tris propane, and glucose were purchased from Sigma (St. Louis, USA). Fenthion was obtained from Dr. Ehrenstorfer (Augsburg, Germany). The dialysis membrane (MW cutoff 12,000-14,000) was a Spectra/Por product from Spectrum Laboratories (Rancho Dominguez, USA). Immunodyne ABC membrane was acquired from Pall (Pall filtrationstechnik GmbH, Dreieich, Germany) and nitrocellulose membrane (pore size of 8 μm) was purchased from Whatman (Brentford, UK). Carbon (TU-15ST, lower resistence type) and silver pastes (LS-506J) were obtained from Asahi (Tokyo, Japan), and insulator paste (SCR-505G) was from Seoul Chemical Research Laboratory (Ansan, Korea). Electrochemical measurement was performed using BAS model 100B from Bioanalytical Systems (West Lafayette, USA).

Fabrication of electrode strips Screen-printed electrode strips were constructed, as shown in Fig. 1, by first printing on polyethylene film the reference and counter electrode. Conducting paths were printed with silver paste and then carbon paste was used for printing a working electrode. This was then followed by the printing of the insulating layer. The electrodes were heated to 120°C for 5 min and then treated with 10% $FeCl_3$ for 30 min to convert Ag to Ag/AgCl. The electodes were then stabilized by applying a potential of 1.2 V against the reference

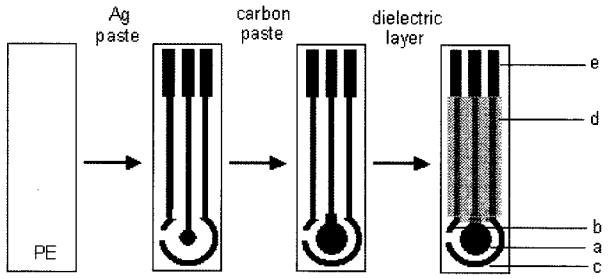


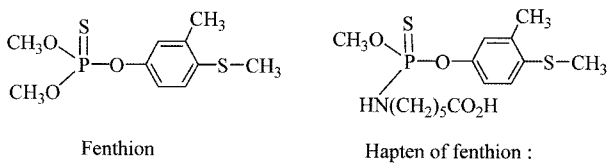
Fig. 1. The steps in the fabrication of electrode strips based on screen printing techniques. PE: Polyethylene film, a) working electrode, b) reference electrode, c) counter electrode, d) insulating layer, e) electrical contact.

electrode in a 0.5 M carbonate-bicarbonate buffer (pH 9.6).

Characterization of the electrode strips To determine the potential to apply to the working electrode, cyclic voltammograms for the buffer (0.1 M PBS, pH 7.4), fenthion-GOD conjugate, and H_2O_2 were obtained. Based on the cyclic voltammograms obtained, potentials of 0.9 and 1.0 V were chosen to be compared. The effect of the concentration of GOD and glucose on electrode response was examined by spotting 5 μL of GOD and 20 μL of glucose on the electrode strip to allow them to react for 1 min and then measuring the current change at 1.0 V for 100 sec. The concentrations tested were 10^{-5}-10^{1} M for GOD and 10^{-3}-10^{-1} M for glucose. The electrode response at different concentrations of H_2O_2 (0-10 mM) was also measured.

Preparation of immunoreagents The antibody used was the one prepared previously in our laboratory from the hapten shown in Fig. 2 (11). The hapten used to prepare fenthion-GOD conjugate was the same as the immunizing hapten. The method of conjugation used was the active ester method, i.e., the hapten was converted to *N*-hydroxysuccinimide ester to attach the hapten to GOD (11). The procedure of the conjugation was as follows. GOD (9.2 mg) was dissolved in 0.45 mL of bis-tris-propane buffer (0.05 M, pH 6.5). A solution of the *N*-hydroxysuccimide ester (0.218 mg, 0.5 μmol) dissolved in 0.05 mL of DMF was stirred into the protein solution. Stirring was continued for 24 h at 4°C and the conjugate was purified by dialysis using Tris buffer (0.05 M, pH 7.4) and triply distilled water. The concentration of the purified conjugate after dialysis was 3.6 mg/mL.

Construction of immunosensor The format adopted for the immunosensor is illustrated in Fig. 3. It was similar to



Fenthion Hapten of fenthion :

Fig. 2. Structures of fenthion and hapten of fenthion.

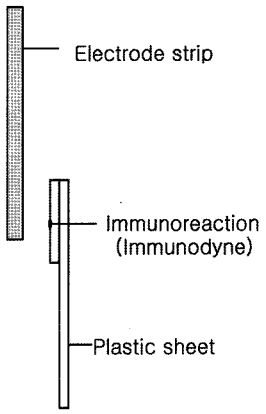


Fig. 3. Format of the dipstick-type amperometric immunosensor.

the one that we adopted to develop a dipstick-type ELISA for fenthion (12). To build the dipstick-type immunosensor, Immunodyne ABC membrane was mounted onto a polystyrene plastic film using a double-sided adhesive tape and 5 μL of diluted antiserum was spotted on the membrane. The residual binding sites of the membrane were then blocked by incubating the membrane in 2% casein solution for 30 min. The assay procedure consisted of addition of fenthion-GOD conjugate to the sample solution and incubation of the antibody-coated membrane in the sample solution for 30 min. The next step was addition of 10^{-2} M glucose to the bound conjugate, followed by attachment of the strip electrode on the membrane to make an amperometric measurement. The concentrations of the antiserum and fenthion-GOD conjugate were optimized.

Detection of fenthion using the immunosensor The calibration curve for fenthion was obtained using fenthion standards (0-10^{3} μg/L) prepared in 10% methanol-PBS. The signal of the electrode was normalized by the equation

$$\text{Normalized signal} = \frac{S_X - S_0}{S_{100} - S_0} \times 100$$

where S_x, S_0, and S_{100} denotes the signal with the standard, excess fenthion, and no fenthion, respectively.

Results and Discussion

Performance of the electrode strips The principle of the dipstick immunosensor is illustrated in Fig. 4. The cyclic voltammograms for the buffer solution (0.1 M PBS, pH 7.4), fenthion-GOD conjugate, and H_2O_2 are presented in Fig. 5. The buffer and the conjugate gave weak responses only at high potentials, and H_2O_2 began to give a response at 0.7 V. The current resulting from H_2O_2 increased rapidly with increasing potential. Of the two potentials (0.9 and 1.0 V) tested in an additional experiment, 1.0 V gave a much higher current. The current change caused by the H_2O_2 concentration change from zero to 10^{-2} M was 6.7 μA at 0.9 V. At 1.0 V, the current change from the same H_2O_2 concentration increase was 11.9 uA. Thus, the

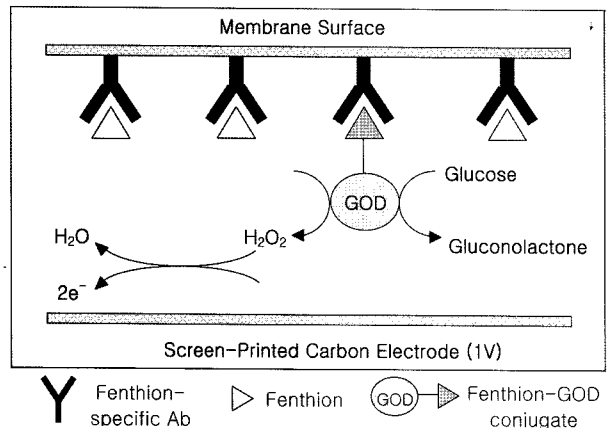


Fig. 4. Schematic diagram of the principle of the immunosensor.

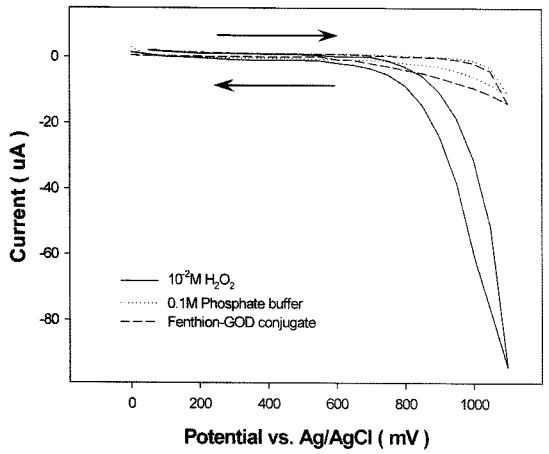


Fig. 5. Cyclic voltammograms for H_2O_2, phosphate buffer, and hapten-GOD conjugate (72 μg/mL).

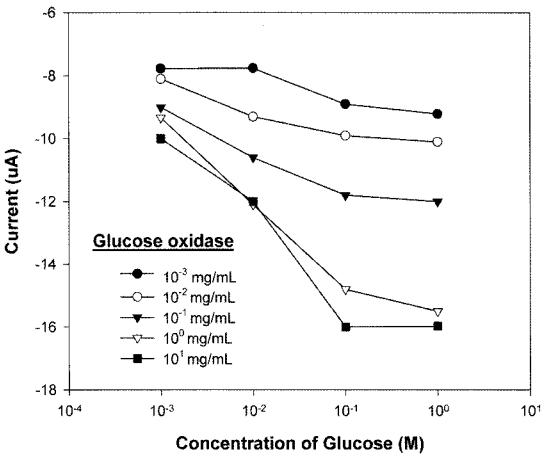


Fig. 6. Effect of the concentration of glucose on sensor response at working potential of 1 V vs Ag/AgCl.

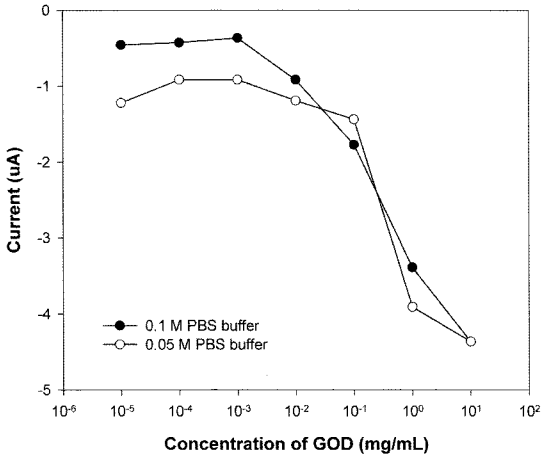


Fig. 7. Effect of the concentration of GOD on sensor response at 1 M glucose and working potential of 1 V vs Ag/AgCl.

potential was set to 1.0 V in the following experiments. The influence of the concentration of glucose on electrode response is presented in Fig. 6. The current increased with increasing concentration of glucose up to 10^{-1} M, after which current increase was negligible. The concentration of glucose had to be high enough to minimize its influence on sensor response. Thus, the optimum concentration of glucose selected was 10^{-1} M. Of the two concentrations of PBS tested (0.1 and 0.05 M), 0.1 M gave better responses, i.e., a wider range of linear response (Fig. 7). The electrode response with different concentrations of H_2O_2 is presented in Fig. 8. The response was linear over 0-10 mM concentrations of H_2O_2.

Performance of the immunosensor In an experiment carried out to select the most suitable dilution ratio for the antiserum, the current increased with decreasing dilution ratios up to the ratio of 1:10 and then remained unchanged. Therefore, the antiserum dilution ratio selected was 1:10. Since the current increased with decreasing dilution ratios of fenthion-GOD conjugate up to the ratio of 1:50 and then remained unchanged, the dilution ratio selected for the enzyme tracer was 1:50. The calibration curve for fenthion obtained using prepared fenthion standards (0-10^3 μg/L) is presented in Fig. 9. The sensor system showed a linear response against the logarithm of the pesticide concentration ranging from 10^{-2} to 10^3 μg/L. The sensitivity of the sensor system was similar to that of the previously-

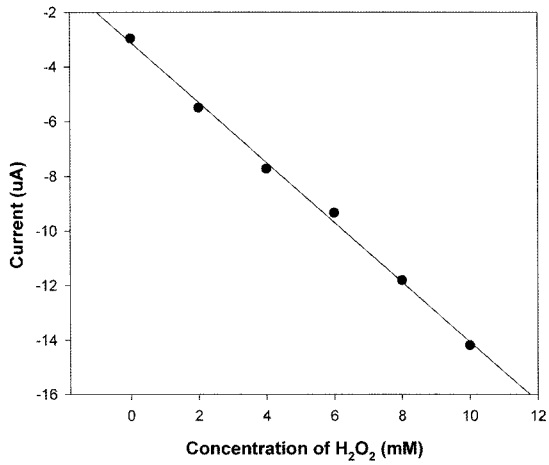


Fig. 8. Dependence of the immunosensor response on H_2O_2 concentration: 0.1 M phosphate buffer, pH 7.4; working potential of 1 V vs Ag/AgCl.

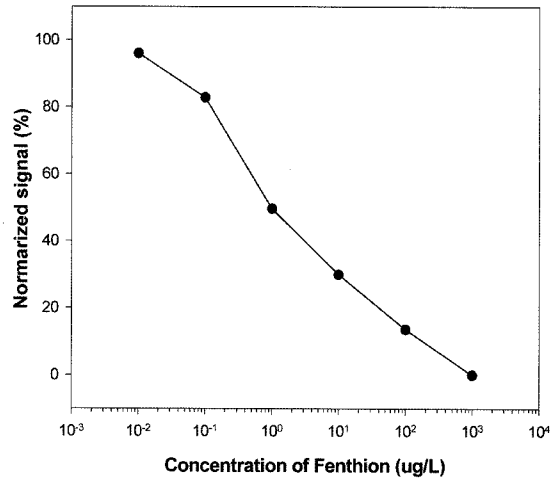


Fig. 9. Standard curve for fenthion : 0.1 M phosphate buffer, pH 7.4 ; working potential of 1 V vs Ag/AgCl.

developed microtiter plate-based ELISA for the same pesticide, which showed a working range of 10^{-2}-10^{2} μg/L (12).

The maximum residue limit (MRL) of fenthion permitted in Korea is between 50 and 500 ppb (μg/Kg). Therefore, the dipstick sensor can be used for screening food samples to evaluate if they are in violation of MRL. The most notable advantage of the new sensor is the much shorter assay time (30 min) compared to those of the current chromatographic methods (several hours). Relatively low cost for large sample loads would be another advantage. However, the immunosensor shows relatively poor reproducibility in signal production due to variability in the fabrication process for both the screen-printed electrode and sensor strip. This newly-developed sensor also may suffer from problems related to oxidation of electroactive interferents in food samples at the potential applied (1.0 V). Therefore, it is desirable to operate the immunosensor at a potential well below the potential employed. Such a low potential could be employed by using an artificial redox mediator. Since the immunosensor that we developed performs competitive assay and electrochemical detection separately, a more integrated fabrication of immunosensor is desirable. The two steps could be combined into a single step by constructing an immunochromatographic sensor in which the electrode strip is mounted on the antibody-coated membrane. This will be the theme of our future work.

Acknowledgments

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References

1. Tomlin CDS. The Pesticide Manual: A World Compendium. 11th ed. The British Crop Protection Council, Surrey, U.K. pp. 514 (1997)
2. AOAC. Official Methods of Analysis, 18th ed. Section 970.52. Association of Official Analytical Communities, Arlington, VA, USA (1995)
3. Hennion MC, Barcelo D. Strengths and limitations of immunoassays for effective and efficient use for pesticide analysis in water samples: A review. Anal. Chim. Acta. 362: 3-34 (1998)
4. Kaláb T, Skládal P. A disposable amperometric immunosensor for 2,4-dichlorophenoxyacetic acid. Anal. Chim. Acta. 304: 361-368 (1995)
5. Bäumner AJ, Schmid RD. Development of a new immunosensor for pesticide detection: a disposable system with liposome-enhancement and amperometric detection. Biosens. Bioelectron. 13: 519-529 (1998)
6. Keay RW, McNeal CJ. Separation-free electrochemical immunosensor for rapid determination of atrazine. Biosens. Bioelectron. 13: 963-970 (1998)
7. Fernandez Romero JM, Stiene M, Luque de castro, MD, Bilitewski, U. Application of screen-printed electrodes as transducers in affinity flow-through sensor systems. Biosens. Bioelectron. 13: 1107-1115 (1998)
8. Laschi S, Mascini M, Scortichini G, Franek M, Mascini M. Polychlorinated biphenyls (PCBs) detection in food samples using an electrochemical immunosensor. Agric. Food Chem. 51: 1816-1822 (2003)
9. Pemberton RM, Hart JP, Foulkes JA. Development of a sensitive, selective electrochemical immunoassay for progesterone in cow's milk based on a disposable screen-printed amperometric biosensor. Electrochim. Acta 43: 3567-3574 (1998)
10. Darain F, Park DS, Park JS, Chang SC, Shim YB. A separation-free amperometric immunosensor for vitellogenin based on screen-printed carbon arrays modified with a conductive polymer. Biosens. Bioelectron. 20: 1780-1787 (2005)
11. Kim YJ, Cho YA, Lee HS, Lee YT, Gee SJ, Hammock BD. Synthesis of haptens for immunoassay of organophosphorus pesticides and effect of heterology in hapten spacer arm length on immunoassay sensitivity. Anal. Chim. Acta 475: 85-96 (2003)
12. Cho YA, Kim YJ, Hammock BD, Lee YT, Lee HS. Development of a microtiter plate ELISA and a dipstick ELISA for the determination of the organophosphorus insecticide fenthion. J. Agric. Food Chem. 51: 7854-7860 (2003)