Organophosphate Hydrolase in Conductometric Biosensor for the Detection of Organophosphate Pesticides

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ABSTRACT: The research has developed an enzyme biosensor for the detection organophosphate pesticide residues. The biosensor consists of a pair of screen-printed carbon electrode (SPCEs). One of electrodes contains immobilized organophosphate hydrolase (OPH) on a chitosan membrane by cross-linking it with glutaraldehyde. The area of the electrodes was optimized to 3, 5, and 7 mm^2. The OPH was isolated from *Pseudomonas putida*, and was purified by the ammonium sulfate precipitation method, with 6444 ppm (A) and 7865 ppm (B). The organophosphate pesticide samples were 0–100 ppb in tris-acetate buffer 0.05 M, pH 8.5. The results showed that the best performance of the biosensor was achieved by the enzyme A with an electrode area of 5 mm^2. The sensitivity of the biosensor was between 3 and 32 µS/ppb, and the detection limit for the organophosphate pesticides was 40 ppb (diazinon), 30 ppb (malathion), 20 ppb (chlorpyrifos), and 40 ppm (profenofos).

KEYWORDS: organophosphate pesticide, organophosphate hydrolase, conductometric biosensor, screen-printed carbon electrode, chitosan membrane

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Introduction

Organophosphate pesticides are recommended by the Indonesian government, but the level of their residues in agriculture produce is controlled. The maximum level of pesticide residue is 0.1 mg/kg in rice and 0.5 mg/kg in vegetables.[1] Therefore, we need a simple method or an instrument for the detection of organophosphate pesticide residues. Biosensors are a new challenge in developing an instrument that is simple and minimizes the time of detection.

A biosensor is a device that combines a biochemical reaction with a detector or transducer. Because the biosensor response is based on a biochemical reaction, it has high selectivity.[2] Transducers are generally based on optical, electrochemical, or piezoelectric principles.[3] Also, electrochemical transducers are very simple to develop. While conductometry is easier than amperometry, potentiometry is not very sensitive.[4] The conductometric transducer has several advantages: the electrodes are small; it does not require any reference electrode; only a small voltage is required, thus saving energy consumption; and the cost of production is low.[5] Because of these, in this work we have developed conductometric biosensors. In our previous work, we developed a modified Pt electrode—nata de coco—as a conductometric biosensor for the detection of uric acid.[6,7]

Biosensors for organophosphate pesticides have been developed electrochemically, but most of them are amperometric. Enzyme-based biosensors for organophosphate pesticide detection have been developed by using acetilcholinesterase (Ache),[8–13] organophosphate hydrolase (OPH),[13–15] and alkaline phosphatase (ALP).[16] In this work, we developed an organophosphate pesticide biosensor based on the hydrolysis reaction by OPH catalysis.[17] The hydrolysis of organophosphate pesticide produces hydronium ions, which can be detected conductometrically.

Performance of an enzyme-based biosensor is affected by the pH, activity, and mass of the enzyme.[18,19] On the other hand, the conductometric signal depends on the area of the electrode.[20] OPH activity depends on the source of microbes (or others) and the substrate.[3] OPH was isolated from *Pseudomonas putida* by 0%–45% (enzyme A) and 45%–65% (enzyme B) ammonium sulfate fractionation, and the substrates (samples) were diazinone, malathion, chlorpyrofos, and profenofos. We studied the OPH activity for various organophosphate compounds and its relation with the sensitivity of the sensors. Immobilization was through cross-linking, and glutaraldehyde was used as the cross-linker.[17,18,21]

Experimental

Materials. OPH was isolated from *P. putida*. The isolation and purification of OPH used the precipitation method

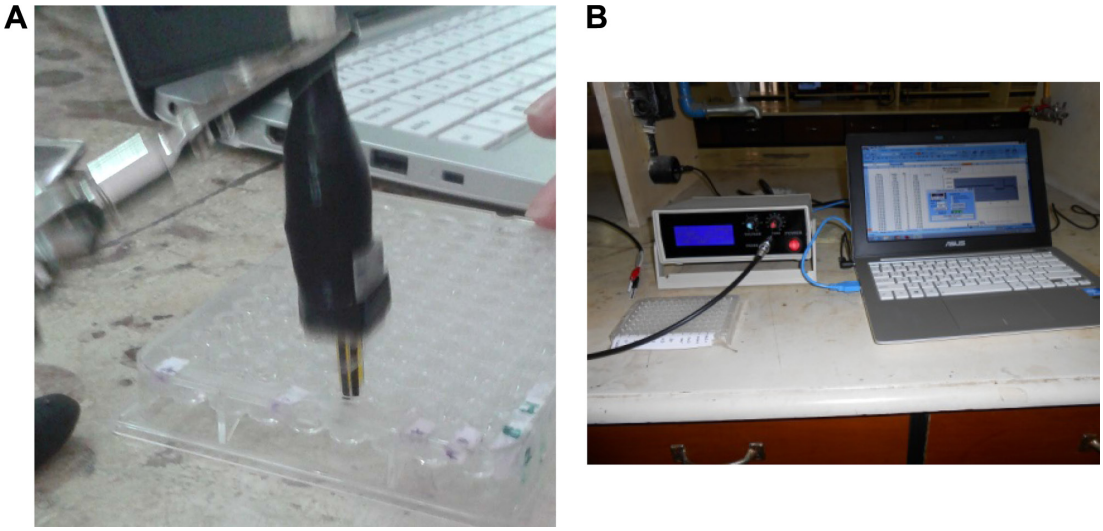

Figure 1. (**A**) Conductivity cell comprising a pair of electrodes with SPCE (without chitosan and OPH) and working electrode. (**B**) Conductometer connected to a computer.

by ammonium sulfate at 0%–45% fraction [OPH (A), 6444 ppm] and 45%–65% [OPH (B), 7865 ppm]. Chitosan, tris, and organophosphate pesticides were purchased from Sigma Aldrich (USA). Tris-acetate buffer was made in deionized water. Fresh stock solutions were made in 0.05 M tris-acetate buffer, pH 8.5. Diazinon (*O*,*O*-diethyl *O*-[4-methyl-6-(propan-2-yl)pyrimidin-2-yl]phosphorothioate), malathion (diethyl 2-[(dimethoxy phosphorothioyl) sulfanyl] butanedioate), profenofos (*O*-(4-bromo-2-chlorophenyl)-*O*-ethyl *S*-propyl ester), and chlorpyrifos (*O*,*O*-diethyl-*O*-3,5,6-trichloropyridin-2-yl phosphorothioate) were used as standard organophosphate insecticides.

Instrumentation. The conductometer (Fig. 1B), consisting of a pair of electrodes (Fig. 1A) modified from screen-printed carbon electrodes (SPCEs), was purchased from QUASENSE Thailand. A pH meter (Schoot-Gerate type CG.820) and common laboratory glass ware were used.

Procedure.

Design of biosensors for organophosphates. On each 1 × 3, 1 × 5, and 1 × 7 mm^2 of the SPCE surface, 10 mL drops of chitosan (1%) in acetic acid (2%) were deposited and dried at 40°C for 1 h. Furthermore, 25 µL of OPH solution was dropped on the chitosan membrane, which was then cross-linked by 10 µL glutaraldehyde (0.5%). The electrode was stored at 4°C for 24 h. OPH immobilization on the electrode surface is illustrated in Figure 2.

Measurement of solution organophosphate conductance. The biosensor consisted of a pair of electrodes, SPCE-chitosan-OPH and SPCE. The biosensor was set up as in Figure 1; the distance between the two electrodes was 0.3 cm. The biosensor was immersed in 0.05 M tris-acetate buffer, pH 8.5,[16] to obtain a readable conductivity. Then, the biosensor was immersed in a solution of the organophosphate pesticide and the conductivity was recorded after the display indicated a constant value.

Results and Discussion

Activity of organophosphate hydrolase. In this study, OPH was isolated from *P. putida*; so it is important to know the activity of OPH on four organophosphate pesticides. OPH activity was determined separately, and the results can be seen in Table 1. OPH activity is different with different organophosphate compounds. OPH activity of (A) was higher than that of (B) except in malathion. The OPH activity is specific for each substrate, that is, diazinon, malathion, chlorpyrifos, and profenofos. Activity is expressed as Units per milligram E, where U is the substrate in micromoles that can be hydrolyzed per minute.

The OPH activity on profenofos and chlorpyrifos are lower than that on diazinon and malathion. Profenofos and chlorpyrifos have a larger geometric structure, so that it is more difficult for them to interact with OPH. Profenofos has a –N– group, which has a free electron pair, and diazinon has the –N= group, which has free electron pair and double bonds, so diazinon is more reactive than profenofos. Based on this, the activity of OPH on diazionon is higher than that on profenofos. OPH activity on malathion is the highest, because malathion has the simplest structure. The hydrolysis reactions of diazinon, malathion, profenofos, and chlotpyrifos are shown in Figure 3.

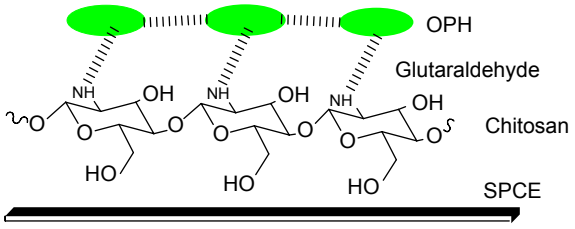

Figure 2. Design of the biosensor. OPH is made to cross-link with chitosan and with glutaraldehyde as a cross-linker.

Table 1. OPH activity for diazinon, malathion, profenofos, and chlorpyrifos as substrates.

NO.	ORGANOPHOSPHATE PESTICIDES	OPH ACTIVITY (U/mgE)	
		A	B
1	Diazinon	95	78
2	Malathion	460	681
3	Profenofos	49	47
4	Chlorpyrifos	36	28

Notes: OPH (A) was isolated by 0%–45% ammonium sulfate precipitation and OPH (B) was isolated by 45%–65% ammonium sulfate precipitation.

Performance of the biosensor. In theory, the conductivity of the solution is affected by the electrode area, as shown in Equation 1.[20] It has been proven that the conductivity of a profenofos solution (as an example) rises corresponding to an increase in the electrode area in the range 0–100 ppb profenofos (Fig. 4). Data relationship between the profenofos concentration and conductivity at various electrode area are shown in Supplementary Table 3. However, the increase in conductivity does not necessarily mean an increase in the sensitivity of the biosensor. The results showed that the highest sensitivity was achieved at the electrode area of 5 mm^2. This phenomenon occurs in all organophosphate compounds, irrespective of whether the biosensor used was OPH (A) or OPH (B) (Figs. 5 and 6).

$$G = \frac{1}{R} = k\frac{A}{l} \quad (1)$$

G = conductivity (siemens, S)
R = resistance (Ω)
k = specific conductivity (S cm^{-1})
A = surface area of the conductivity cell (cm^2)
l = distance between the two electrodes (cm)

Based on Figures 5 and 6, one can see a higher sensitivity in the biosensor that used OPH (A). The sensitivity data are shown in Supplementary Tables 1 and 2. It shows that the sensitivity of the biosensor is affected by the activity of the enzyme. From Figures 5 and 6, one can also see a higher sensitivity in the biosensor that uses OPH (A). It shows that the sensitivity of the biosensor is also affected by the activity of the enzyme. It is interesting to note that in malathion, the activity of OPH (B) is higher than that of OPH (A), but the sensitivity of OPH (B) is the lowest in malathion. It is because

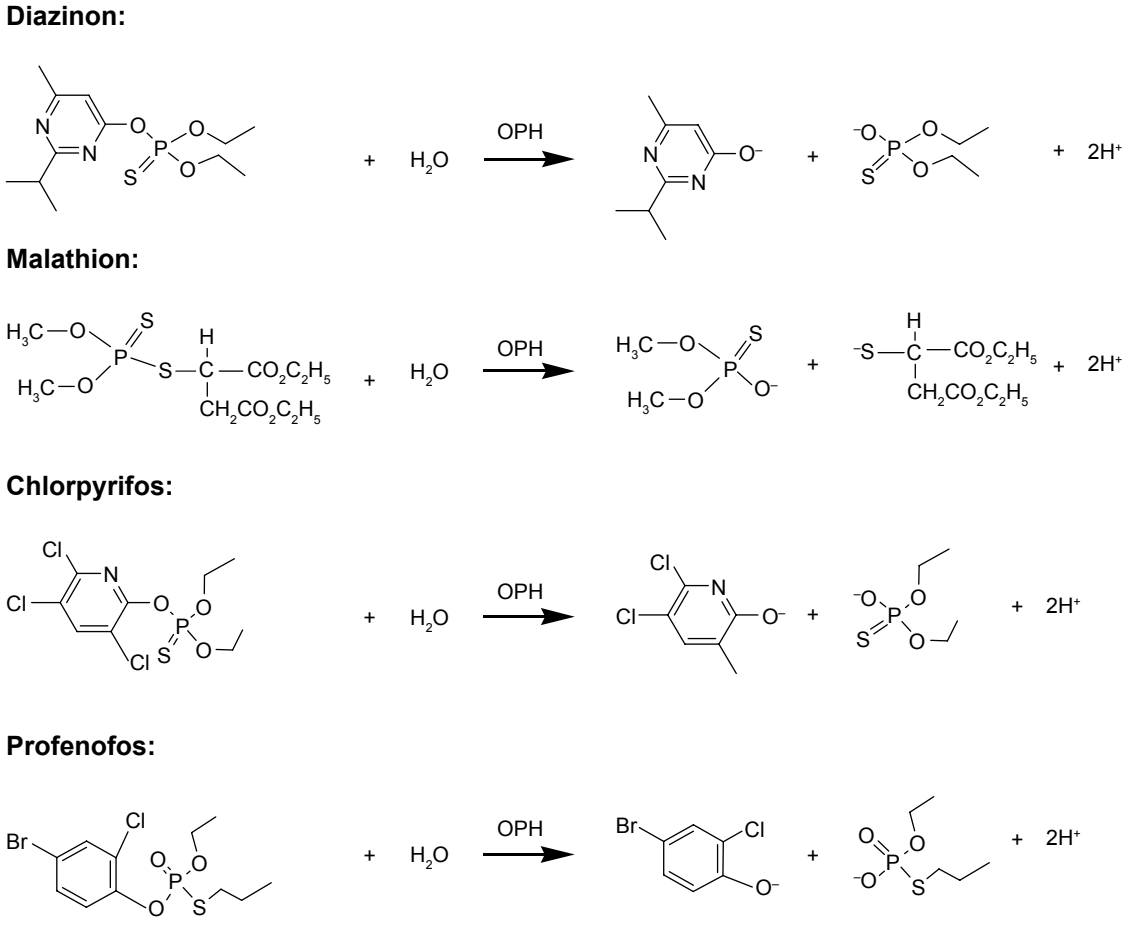

Figure 3. Organophosphate hydrolysis reaction for diazinon, malathion, chlorpyrifos, and prophenophos.

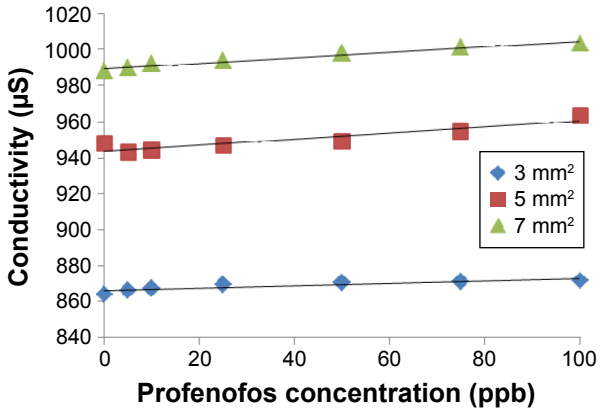

Figure 4. Relationship between profenofos concentration and conductivity at various electrode areas. The biosensor made from OPH was isolated by 0%–45% ammonium sulfate precipitation.

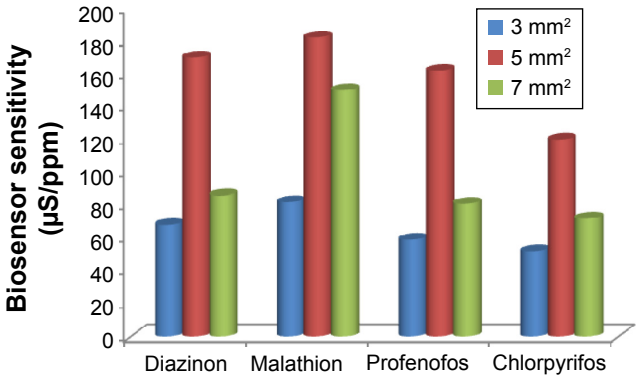

Figure 6. Sensitivity of biosensor for diazinon, malathion, chlorpyrifos, and profenofos at various areas (3, 5, and 7 mm²) of the electrodes. The biosensor made from OPH was isolated by 45%–65% ammonium sulfate precipitation.

the concentration of enzyme OPH (B) is higher, so that the membrane pores are clogged by the enzyme, thus inhibiting the diffusion of ions to the surface of the SPCE.

Based on these results, it can be seen that the sensitivity is influenced by the area of the biosensor electrode, the enzyme activity, and the enzyme concentration. Therefore, validation is performed on a biosensor with an area of 5 mm and using both OPH (A) and OPH (B). Validation is based on the sensitivity and detection limits of the individual biosensor for organophosphate compounds. The range of concentrations of organophosphate pesticides that provide a linear relationship to the conductivity is 0–100 ppb. Sensitivity is determined based on a linear equation (standard curve) on the relationship between the concentration and the conductivity; this is done for each organophosphate compound at the range of concentrations as above. The limit of detection is the smallest concentration equivalent to 3SD (standard deviation) of the blank. As the blank solution is tris-acetate buffer, pH 8.5, SD is calculated from 20 conductivity readings of the blank solution. Detection limit was calculated for each organophosphate compound based on each standard curve.

Sensitivity and detection limits of the biosensors for each organophosphate compound are shown in Table 2. From the table, it is seen that the sensitivity of the biosensor OPH (A) is higher than that of the biosensor OPH (B). This corresponds with the OPH activity. The limit of detection of the biosensor is 20–40 ppb and 40–60 ppb. The detection limit of the biosensor OPH (A) is lower than the concentration of maximum limit residues (MLR) of pesticide in vegetables, so that the biosensor has the potential to be applied to the determination of organophosphate pesticide residues in vegetables.

Conclusion

The activity of OPH from *P. putida* is affected by the precipitation with ammonium sulfate fraction. OPH of precipitation results in 0%–45% ammonium sulfate fraction has a higher activity than the 45%–65% ammonium sulfate fraction. OPH can be applied to the development of biosensors for organophosphate pesticides. The performance of the biosensor

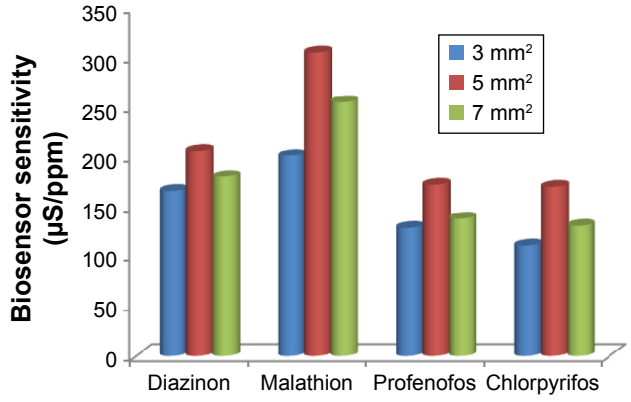

Figure 5. Sensitivity of the biosensor for diazinon, malathion, chlorpyrifos, and profenofos, at various electrode areas (3, 5, and 7 mm²). The biosensor made from OPH was isolated by 0%–45% ammonium sulfate precipitation.

Table 2. Sensitivity and limit of detection of the biosensor for detection of diazinon, malathion, profenofos, and chlorpyrifos.

PESTICIDES	SENSITIVITY (µS/ppb)		LIMIT OF DETECTION (ppb)	
	A	B	A	B
Diazinon	10	7	40	40
Malathion	25	12	30	40
Profenofos	5	4	40	40
Chlorpyrifos	4	3	20	60

Notes: The biosensor used OPH (A) was isolated by 0%–45% ammonium sulfate precipitation; OPH (B) was isolated by 45%–65% ammonium sulfate precipitation.

is directly proportional to the OPH activity. The area of the electrode is proportional to the conductance, but not directly proportional to the performance of biosensor.

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Author Contributions

Both authors were involved in all aspects of this research, including the review and approval of the final manuscript.

Supplementary Materials

Supplementary table 1. Sensitivity of biosensor for diazinon, malathion, chlorpyrifos, and profenofos at various area electrodes (3, 5, and 7 mm^2). The biosensor made from OPH was isolated by 0%–45% ammonium sulfate precipitation.

Supplementary table 2. Sensitivity of biosensor for diazinon, malathion, chlorpyrifos, and profenofos at various (3, 5, and 7 mm^2) area electrodes. The biosensor made from OPH was isolated by 45%–65% ammonium sulfate precipitation.

Supplementary table 3. Relationship between profenofos concentration and the conductivity at various electrode areas.

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