Pesticide Biosensor for Direct Environmental Analysis

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Introduction

Water is one of the most important resources and quality control requires fast and reliable measurements. However, the large number of pollutants present in surface waters (more than 100.000 substances) make conventional analytical methods inadequate for toxicity measurements and screening of real samples.

One of the most common class of highly toxic pollutants, is organophosphorus pesticides (OPs). These neurotoxic compounds, such as dichlorvos and paraoxon, have found wide application in agriculture[1] the past decades. Their toxicity lays in the inhibition of insect as well as vertebrate AChE and therefore represent a potential hazard for human health and environmental food chains[2].

Acetylcholinesterase (AChE)-based biosensors are ideal systems for screening of total toxicity due to pesticides[8]. The use of inhibitor biosensors for on-line monitoring of surface water and for the determination of intermittent samples offers a powerful and promising tool for water management.

This strategy has been used to detect low levels of these contaminants in crop, water or soil samples[3,4] and is based on the determination of the decay of cholinesterase activity in the presence of OPs.

Biosensors based on the use of immobilized cholinesterases from various sources, for the detection of OPs have been described[5,6]. In this work, the enzyme utilized is a recombinant acetylcholinesterase from *Drosophila melanogaster*[7]. The mutation has proven to increase the sensitivity and selectivity to the organophosphorus pesticide dichlorvos, in biosensor systems.

AChE-Based Biosensors for Measurement of OPs

In the absence of OPs, AChE reacts catalytically according to the following general scheme:

$$\text{Acetylcholine} + H_2O \xrightarrow{\text{AChE}} \text{Choline} + \text{acetic acid}$$

E: AChE, PX: OPs, EP: phosphorylated enzyme, X: residual group, ki: bimolecular rate constant, ki= k_2/Kd

1. Mulchandani et al., *Biosens. Bioelectron*, 16, 2001
2. Bachmann et al., *Anal. Chim. Acta*, 401, 1999
3. Kumaran & Morita, *Talanta*, 42, 1995
4. Albareda-Sirvent et al., *Anal. Chim. Acta*, 442, 2001
5. Gogol et al., *Talanta*, 53, 2000
6. Hart & Collier, *Sens.Actuators B*, 53, 1998
7. Bachmann et al., *Biosens. Bioelectron*, 15, 2000
8. Neufeld T. et al., *Biosens. Bioelectron*., 15, 2000
9. Boublik et al., *Prot. Bioengin*. 15, 2002
10. Gavalas & Chaniotakis, *Anal.Chim. Acta*, 404, 2000
11. Ellman et al., *Biochem. Pharmacol*., 7, 1961
12. Harel et al., *Protein Sci.*, 9, 2000

Aims of the Project

The development of a reusable biosensor system with low detection limit, and high selectivity for the continuous monitoring of dichlorvos and other pesticides in water.

For this, a highly sensitive and selective recombinant AChE mutant is employed[9]. The use of an activated conductive porous carbon as the immobilization matrix, and as transducer allows for the easy and reproducible construction of the AChE biosensor for dichlorvos.

Toxicity Biosensor Based on AChE

Calibration Curve of the AChE Biosensor

After exposure to OPs, enzyme activity is decreased, depending on the concentration and time of exposure.

The quantitative relationship between the inhibition % and the biosensor signal is given below.

$$I(\%) = \frac{i_O - i_1}{i_O} \times 100$$

I (%): inhibition %, $i_{(0)}$,$i_{(1)}$:current signal before and after incubation with the pesticide

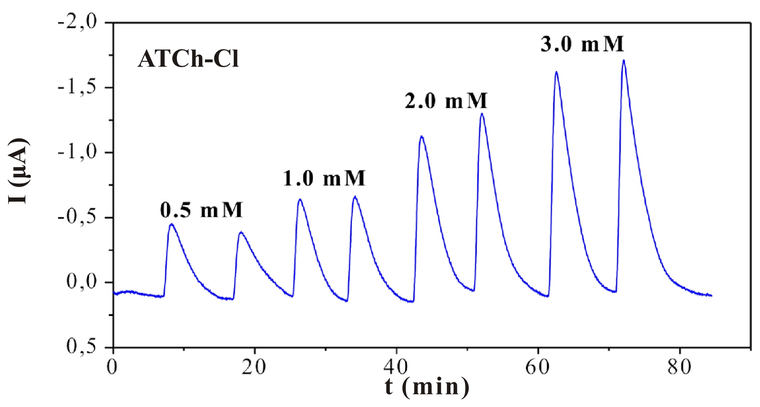
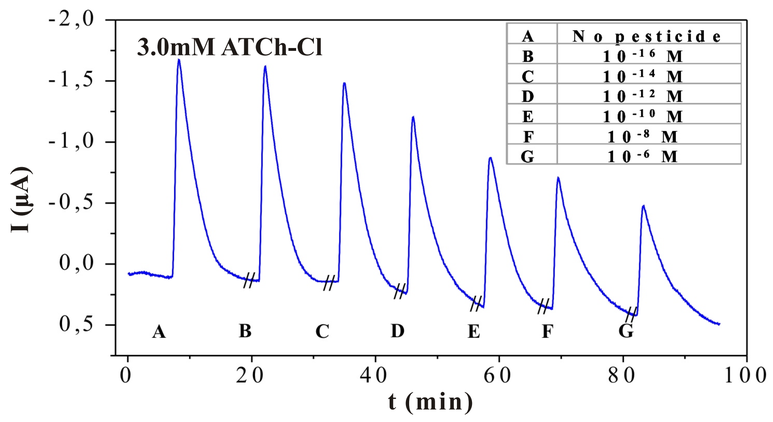


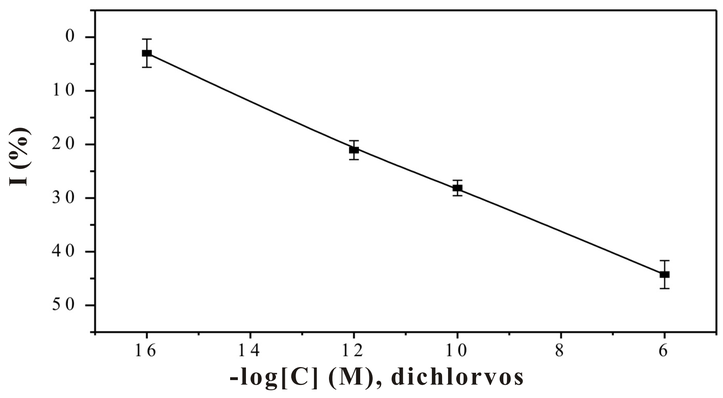
Chart recording for successive incubations with dichlorvos



The break on the chart represents incubation period (10min) and buffer washing (30min). The total enzyme loading was 2U.

Inhibition is clearly observed, after incubation with the pesticide dichlorvos.

Dichlorvos Calibration Curve



Data shown in this curve are the average of three different measurements. The detection limit of the AChE biosensor was calculated to be 22.0 fg/lt.

Recombinant AChE

Drosophila melanogaster AChE is 70-fold more sensitive to dichlorvos than the *Electrophorus electricus* enzyme. Furthermore, mutations in the active site of the Drosophila enzyme increase its sensitivity by 200 fold.

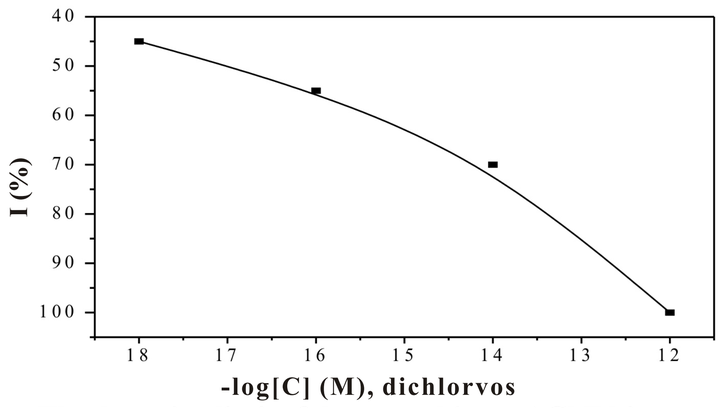
The *kis* of AChE for dichlorvos are presented below:

AchE	*ki* (μM^{-1}. min^{-1})
Electric eel	0.026
Drosophila	1.4
Mutant B4-21	487

B4-21 is a Drosophila enzyme mutated at two positions (E107Y, Y109D). Residue numbering is from Harel et al[12].

Recombinant AChE Biosensor

Dichlorvos Calibration Curve



The detection limit of the recombinant AChE biosensor for dichlorvos was as low as 0.2 fg/lt.

Under the same conditions, the detection limit of the recombinant AChE biosensor for the organophosphorus pesticide paraoxon, was found 9ug/lt, which is approximately 10^{10} times higher than that for dichlorvos.

Conclusions

A very sensitive biosensor based on recombinant AChE has been designed for the detection of dichlorvos. The detection limit of the sensor is 0.2 fg/lt, with detection range between 10E-18 M to 10E-12 M, and excellent measurement-to- measurement reproducibility (CV< 10%). These characteristics allow both the toxicity measurements and the selective detection of pesticides in water.

Electrochemical measurements

In all experiments a three electrode Metrohm 641 VA-Detector, a Ag/AgCl double junction reference electrode and a stainless steel counter electrode were used. Temperature control at 25.0 °C was achieved with a circulating bath. The flow injection system consisted of a wall-jet flow cell, an injection valve with loop volume 182μl, while the solvent delivery was done using a syringe pump (Model 362, ORION Research Inc.). The flow rate was 0,5mL/min.

Experimental Setup

Preparation of the biosensor

The WE was a rod (5,6mm d., 1mm height) of porous conductive carbon. Enzyme immobilization into the porous conductive carbon was done as described in previous work[10].

Acetylcholinesterase from electric eel (EC. 3.1.1.7) was obtained from Sigma.

Recombinant AChE from Drosophila melanogaster very sensitive to dichlorvos (ki=487 μM^{-1} . min^{-1}) was used for improving sensor sensitivity. Recombinant AChE activity was assayed according to Ellman et al[11].

Operational conditions

The system was optimized for the chemical and biochemical conditions. The optimum working conditions are shown in the following table:

Working potential	+100mV
Buffer	Phosphate 25mM + KCl 0.1M
PH	7.0
Enzyme loading	2U
Substrate concentration (acetylthiocholine Chloride) ATChCl	3mM
Incubation time	10min
Buffer washing	30min

Acknowledgements

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