Determination of organophosphorous pesticide in fruits samples using a nanostructured acetylcholinesterase amperometric biosensor

Yavor Ivanov, Katya Gabrovska, Tzonka Godjevargova

Abstract: *Organophophorous pesticides are some of the most widely used insecticides in citrus fruit, tomato and apple cultures. Biosensors based on the inhibition of acetylcholinesterase have been used for detection of pesticides in different samples. Due to its permitted use in such cultures, the methodology proposed was applied in order to evaluate the occurrence of matrix effects in the electroanalytical determination of paraoxon residues directly in the samples from apple, tomato and orange, without pre-treatment or clean up steps. The results show that the biosensor has the potential for monitoring of pesticides in foods.*

Key words: paraoxon, amperometric analysis, AChE biosensor, fruits

INTRODUCTION

The presence of pesticide residues and metabolites in food, water and soil currently represents one of the major issues for environmental chemistry. Pesticides are, in fact, among the most important environmental pollutants because of their increasing use in agriculture. These pesticides are toxic because they act as inhibitors of acetylcholinesterase, an enzyme that catalysis in a very efficient way the hydrolysis of the neurotransmitter acetylcholine. This enzyme is present in vertebrates and insects and its inhibition can disrupt the transmission of nerve impulses [1,6]. Therefore, the presence of residues of such pesticides in natural waters and in foodstuffs is of major concern for public health reasons. Biosensors for detection and quantification of pollutants have attracted extraordinary interest in recent years, because of the key role they play in the development of highly sensitive, selective chemical analysis, low cost and short analysis time associated with these devices. Biosensors based on the inhibition of acetylcholinesterase have been used for detection of pesticides in different samples [4-5] some different strategies being devised for enzyme immobilization.

MATERIALS AND METHODS

Materials

Acrylonitrile-methylmethacrylate-sodium vinylsulfonate membranes (PAN) were prepared without support according to a methodology described in [2]. The ternary copolymer (acrylonitrile—91.3%; methylmethacrylate—7.3%, sodium vinylsulfonate—1.4%) was a product of Lukoil Neftochim, Burgas.

Ultrafiltration membranes of acrylonitrile copolymer were measured to be 4μm thick and could retain substances with molecular weight higher than 60 000 Da. Multi wall carbon nanotubes- MWCNs (diameter 2–6 nm; length 0.1–10_m, >90% purity) was purchased from Sigma–Aldrich (St. Louis, USA). Acetylthiocholine chloride (ATCh) and acetyl cholinesterase- AChE (Type C3389, 500U.mg^{-1} from electric eel) were purchased from Sigma–Aldrich and used as received. Bovine serum albumin (BSA), glutaraldehyde (GA) and Concanavalin A (Con A) were also purchased from Sigma–Aldrich. Phosphate buffer solution (PBS, pH 7.6) and other reagents were of analytical reagent grade. All solutions were prepared with double distilled water.

Instruments

PalmSens Electrochemical Instrument - Electrochemical measurements were performed on a PalmSens Electrochemical Instrument (Palm Instruments BV, The Netherlands) with a conventional three-electrode system comprising of platinum wire as a counter electrode, Ag/AgCl reference electrode and a platinum working electrode with attached AChE immobilized membrane.

HPLC Value System - Quantitative determinations of paraoxon were also carried out by HPLC-UV technique. A model Agilent 1100 Series HPLC Value System was used. The HPLC conditions were: a Li-Chrosorb RP-18 column (250 mm, 4.6 mm, 5 mm, Merck), with a RP-18 pre-column (30 mm, 4 mm, 5 mm, Merck); the mobile phase was 70/30 v/v acetonitrile/water with 1% v/v acetic acid, at a flow-rate of 1.0 ml min^{-1}. The injection volume was 20 µL and detection was performed at a wavelength of 270 nm for paraoxon. The standard addition method was used for analytical determinations in real samples. The photometric UV/Vis detector was used in these experiments.

Preparation of acetylcholinesterase polymer membrane

The chemical modification of MWCNs and chemical modification of PAN membranes was described in detail in our previous paper [2]. The immobilization of enzyme was carried out in seven steps. The first procedure included the activation of the amino groups of modified membrane with glutaraldehyde (10%, PBS, for 1 h at room temperature). The next step was to immerse the activated membrane in a mixture of modified MWCN and BSA (from 11 mg MWCN-NH_2 in 1 mL 10% BSA solution). Then the membrane was immersed in a glutaraldehyde solution (10%, PBS) once more. After being carefully washed with bidistilled water the activated membrane was immersed in Con A solution – 1.5 mg/mL for 1 h at room temperature, then the non-reacted aldehyde groups and the adsorption sites were blocked by incubating the membrane in a solution of BSA (10%, PBS). After that the membrane was immersed in an enzyme solution (70 U/mL) in PBS (pH 7.6) for 24 h at 4 °C. Finally the enzyme membrane was washed with bi-distilled water. Each enzyme carrier was attached to a platinum working electrode, using a plastic ring, with the non-selective side (contained pores larger than pores of the selective layer) of the membranes facing the platinum surface of the electrode.

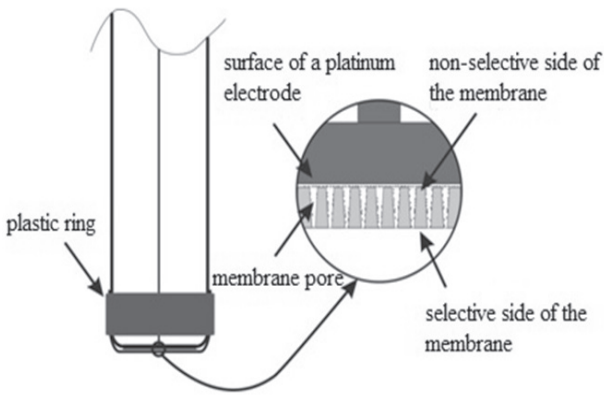

Fig. 1. Constriction of AChE biosensor

Electrochemical measurements of the nanostructured AChE biosensor

Each enzyme carrier was attached to a platinum working electrode, using a plastic ring, with the non-selective side (contained pores larger than pores of the selective layer) of the membranes facing the platinum surface of the electrode, which was then placed in an electrochemical cell containing 40mL 0.1M PBS solution under stirring at 38°C. A potential of 0.8V was applied to the working electrode and the electrochemical current was awaited to become stationary. Then a series of 100µL from a 2mM solution of ATCh were added to the cell and the resulting current was recorded.

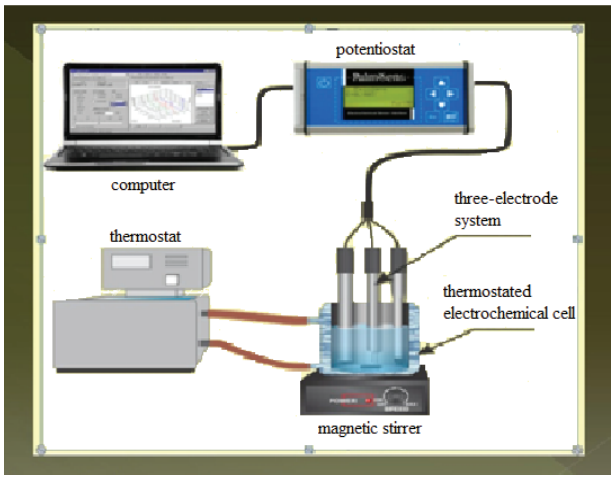

Fig.2. Electrochemical apparatus

Paraoxon concentration measurements

The degree of inhibition (I%) of the organophosphorous insecticide on the enzymatic activity of immobilized AChE was measured as a relative decrease of the amperometric response after a contact of the enzyme carrier with Paraoxon. The initial amperometric response I_0 of 100μL 50mM ATCh was first measured. After washing the membrane with 0.1M PBS (pH 7.6), it was incubated in a Paraoxon solution with a given concentration for 20 min. This was again followed by washing the membrane with PBS and measuring the response to 100μL 50mM ATCh as I_t. The inhibition I% was calculated according to the following Eq.:

$I\% = (I_0 - I_t) / I_0 \times 100$

The concentration of paraoxon was varied and the standard curve was plotted: paraoxon concentration, $g.L^{-1}$/inhibition, I%.

Extraction step in fruits

Sample Treatment for Electrochemical Measurements in Fruits

40 g of tomato and apple samples were directly transferred to an electrochemical cell after triturated and spiked with different amounts of paraoxon. Then, the mixture was mechanically stirred and electrochemical measurements were performed under the conditions described in the Experimental Section. Orange juices were extracted from their respective fruits and 20 mL of the juice were transferred to an electrochemical cell and artificially contaminated with known amounts of paraoxon.

Extraction for Matrix Solid-Phase Dispersion analysis

Untreated fruit samples were processed as received in their unprocessed and unwashed form and kept in a freezer. After the sample treatment previously described they were spiked with different amounts of paraoxon and introduced into a 15 mL cell containing 3.0 g of anhydrous sodium sulfate placed over 0.5 g of silica gel. The column was prepared in the laboratory and conditioned with 10 mL of ethyl acetate. A 10 mL round-bottomed flask was positioned below the column to collect the eluate. The eluate was then concentrated using a rotary vacuum evaporator (40-45 ^{o}C water bath, reduced pressure), and the final volume was adjusted to 1.0 mL. A 20 μL portion of the extract was analyzed by HPLC.

RESULTS AND DISSCUTION

Organophosphate pesticides are some of the most widely used insecticides in citrus fruit, tomato and apple cultures. Due to its permitted use in such cultures, the methodology proposed was applied in order to evaluate the occurrence of matrix effects in the electro-analytical determination of paraoxon residues directly in the samples, without pre-treatment or clean up steps. The suitable performance of the electrochemical biosensor was investigated by analyzing several food samples to which different amounts of pesticide (paraoxon) were added to give concentrations in the range between $2.0x10^{-8}$ and $2.0x10^{-9}$ $g.L^{-1}$. The inhibition of AChE caused by the incubation in the spiked food was compared with the inhibition observed when equivalent pesticide concentrations were present in the buffer solution. The experiments were carried out in triplicate. The extract of the juices was prepared according to procedures described in the experimental section and had their pH values adjusted to 7.2 with appropriate volumes of NaOH solution. 20 mL aliquots of each sample were added to the electrochemical cell for the recovery experiments. The concentration of pesticide in the extract was determined by amperometry using the standard addition method and the results obtained are presented in Table 1.

Table 1. Recovery values of Paraoxon in fruit samples

Sensor	*Sample*	*Concentration added, $g.L^{-1}$*	*Concentration found, $g.L^{-1}$*	*Recovory,%*
AChE biosensor	Tomato	$2.0x10^{-9}$ $2.0x10^{-8}$	$1.9x10^{-9}$ $1.8x10^{-8}$	95 ± 2 90 ±3
HPLC	Tomato	$2.0x10^{-9}$ $2.0x10^{-8}$	$1.7x10^{-9}$ $1.8x10^{-8}$	85 ±2 90 ±1
AChE biosensor	Apple	$2.0x10^{-9}$ $2.0x10^{-8}$	$2.1x10^{-9}$ $1.9x10^{-8}$	105 ± 3 95 ± 3
HPLC	Apple	$2.0x10^{-9}$ $2.0x10^{-8}$	$1.8x10^{-9}$ $1.8x10^{-8}$	90 ± 1 90 ± 2
AChE biosensor	Orange	$2.0x10^{-9}$ $2.0x10^{-8}$	$1.9x10^{-9}$ $1.92x10^{-8}$	95 ± 3 96 ± 2
HPLC	Orange	$2.0x10^{-9}$ $2.0x10^{-8}$	$1.7x10^{-9}$ $1.8x10^{-8}$	85± 3 90 ± 2

In order to validate the previous electroanalytical results, HPLC quantification experiments were carried out using 20 µL aliquots of the appropriate extract obtained from the different samples (see experimental section). The experiments were carried out in triplicate and the pesticide recovery concentration was determined (Table 1). The obtained values ranged from 85-105 % and were slightly higher than those obtained with the biosensor conditions (see Table 1), suggesting that the biosensor has the same better efficiency as compared with HPLC in fruits analysis. Results of paraoxon determinations in food samples by using the proposed biosensor and HPLC were in close agreement. The major advantage of the biosensor is that it allows the analysis of a large number of samples with no need of clean-up steps such those required in chromatographic methods. Frequent routine analyses can be safely carried out using this simpler and less expensive electroanalytical method without losses in either reliability or precision.

CONCLUSION

A pesticide biosensor was successfully developed. This proposed analytical tool is suitable be to employed for rapid judging of the quality of fruit. The results show that the biosensor has the potential for monitoring of pesticides in foods.

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