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ORIGINAL PAPER

Acetylcholinesterase biosensor for carbaryl detection based on interdigitated array microelectrodes

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Abstract In this study, an acetylcholinesterase (AChE) biosensor with superior accuracy and sensitivity was successfully developed based on interdigitated array microelectrodes (IAMs). IAMs have a series of parallel microband electrodes with alternating microbands connected together. Chitosan was used as the enzyme immobilization material, and AChE was used as the model enzyme for carbaryl detection to fabricate AChE biosensor. Electrochemical impedance spectroscopy was used in conjunction with the fabricated biosensor to detect pesticide residues. Based on the inhibition of pesticides on the AChE activity, using carbaryl as model compounds, the biosensor exhibited a wide range, low detection limit, and high stability. Moreover, the biosensor can also be used as a new promising tool for pesticide residue analysis.

Keywords Interdigitated array electrode · Acetylcholinesterase · Electrochemical impedance spectroscopy · Biosensor · Pesticide

Introduction

In recent years, the extensive utilization of pesticides in modern agriculture for insect control will cause potential harm to human health, environment and food safety [1–3]. So it is of great significance to develop fast, sensitive and reliable testing devices for pesticide residue detection. Enzymatic methods have been widely researched for some advantages such as time saving and low cost [4, 5]. Among them, acetylcholinesterase (AChE) biosensors based on the inhibition action of pesticides on AChE are particularly attractive, in which the enzymatic activity is employed as an indicator of quantitative measurement of insecticide residues [6–8]. Impedance biosensors appear very promising due to the relatively simple and compact equipment required. They are more sensitive than biosensors that employ amperometry, where the signal depends critically on the relative proximity of the active site and the electrode surface [9]. Compared to traditional electrodes, interdigitated array microelectrodes (IAMs) have their advantages in impedance measurement, such as low ohmic drop, fast establishment of steady-state, rapid reaction kinetics, and increased signal-to-noise ratio [10–12]. IAMs eliminate the need for a reference electrode and provide simple means for obtaining a steady-state current response, which is comparatively simpler to detect as compared to three or four electrode systems [13, 14]. In particular, IAMs enable the detector device and sample volume to be miniaturized while sustaining its sensitivity and selectivity [15], and their low response time also favors rapid detection [16]. IAMs are increasingly used for impedance measurement of bacterial cells during enrichment growth (impedance microbiology) [17–21] by capturing bacterial cells to the antibodies immobilized on the surface of electrodes (faradic impedance method) [22–26], or by using dielectrophoresis (DEP) for the capture of cells on the surface of electrodes (dielectrophoretic impedance). Characterization of stepwise process of surface modification and bacterial cell capture was studied simultaneously with atomic force microscopy (AFM) and electrochemical impedance spectroscopy (EIS) in the presence of $[Fe(CN)_6]^{3-/4-}$ as a redox probe [26]. However, to the best of our knowledge, there was no report on IAM-based acetylcholinesterase (AChE)

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impedance biosensor applied to the quantitative determination of pesticide residues.

To obtain high performance of the biosensor, it is necessary to choose adequate supporting materials to maintain the inherent nature of enzyme [27–30]. Chitosan (CHIT) has been extensively used for immobilization of enzymes and to construct the biosensors due to its good biocompatibility, excellent film forming ability, and a susceptibility to chemical modifications [31]. Here we used CHIT to immobilize enzymes.

In this study, we demonstrated that IAM-based AChE impedance biosensor could be used as a new approach for the quantitative detection of pesticide residues. The electrochemical impedance spectroscopy biosensor was fabricated using CHIT to modify the microelectrode surface, followed by immobilization AChE on CHIT. Target pesticides in different titers were captured by the immobilized AChE, resulting in a change in the impedance of IAM surface. Finally, nafion film used here to prevent the loss of the enzyme molecules, improved the anti-interference ability of the biosensor and provided a biocompatible microenvironment to maintain enzymatic activity.

Experimental

Apparatus

Impedance measurements were performed using an IM-6 impedance analyzer (BAS, West Lafeyette, IN), with IM-6/THALES software. Interdigitated array microelectrode (IAM) (1550.5-M-Au-U, 25 digital pairs with 15 μm digit width, 15 μm interdigit space) and a clip for collection were from ABTech.

Reagents and materials

Acetylcholinesterase (Type C3389, 500 U/mg from electric eel), acetylthiocholine chloride (ATCl) and 0.1 M pH 7.0 phosphate buffer solution (PB) were used as running buffers and prepared by mixing the stock solutions of NaH_2PO_4 and Na_2HPO_4. Carbaryl was standard product, ethanol and other reagents were of analytical grade. The carbaryl solution was prepared by a step-dilution method. Ultrapure water (18.2 MΩ × cm) produced by PALL Cascada LS ultrapure water system was used throughout.

Interdigitated array microelectrodes

IAMs consist of a series of parallel microband electrodes in which alternating microbands are connected together, forming a set of interdigitating electrode fingers. Metal interconnects or bonding pads are for connecting electrodes to the impedance analyzer and the interdigitated microelectrodes are for sensing. The area around electrodes is passivated to collect the electrical response of the interdigitated microelectrodes only. In this study, each IAM had 25 digital pairs with 15 μm digit width, 15 μm interdigit space, and a digit length of 4,985 μm. The IAM used in this study was shown in Fig. 1.

Configuration of the biosensor

The IAM was soaked with acetone, ethanol and ultrapure water for 3 min, respectively, then gently cleaned with lens paper, which was dipped with absolute ethanol, and washed with ultrapure water, finally dried carefully with nitrogen stream.

CHIT solution (0.5 %) (w/v) was prepared by dissolving 50 mg CHIT powder in 100 mL 1.0 % acetic acid solution and stirring it until CHIT powder dissolved completely. Then 20 μL CHIT solution was added onto IAM surface and incubated for 30 min. After washing, 10 μL AChE solution (200 mU) was added onto IAM surface and incubated for 1 h. Finally, the AChE/CHIT/IAM was coated with an extra 20 μL 0.5 % nafion to maintain the stability of modified electrode. The obtained biosensor was stored at 4 °C when not in use.

Impedance measurement

Impedance measurement was performed using an IM-6 impedance analyzer (BAS, West Lafayette, IN) with IM-6/

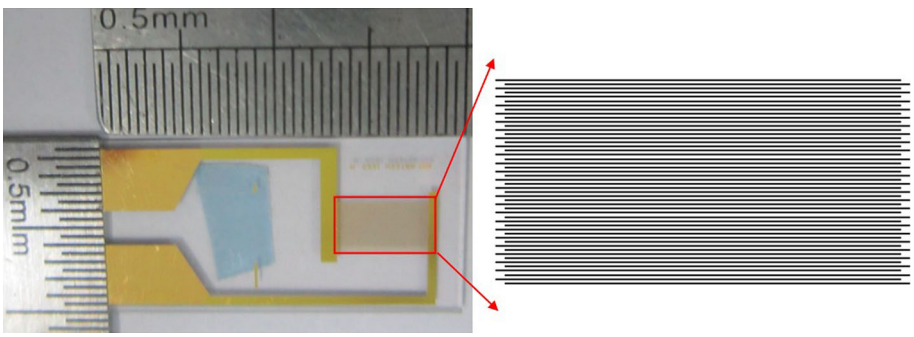

Fig. 1 The IAM used in this study

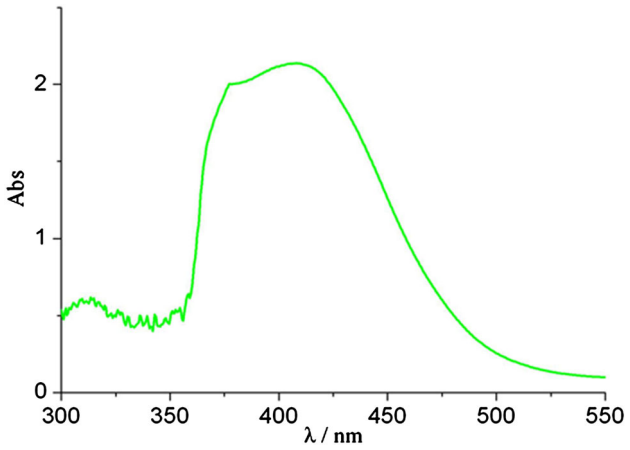

Fig. 2 UV spectra of AChE

THALES software. For all impedance measurements, a sine-modulated AC potential of 55 mV was applied across the IAM and the magnitude and phase angle of impedance were measured for a frequency range from 1 Hz to 1 MHz with an amplitude of 5 mV. One electrode set of the IAM chip was connected to the test and sense probes, and the other was connected to the reference and counter electrodes of the impedance analyzer. All impedance measurements were used 0.1 M PB (pH 7.0) containing 5 mM $[Fe(CN)_6]^{3-/4-}$ and 1 M KCl.

The AChE/CHIT/IAM biosensor was employed for the determination of carbaryl using impedance measurement with an IM-6 impedance analyzer. The performance of the biosensor was tested in pH 7.0 PB containing 5 mM $[Fe(CN)_6]^{3-/4-}$, 1 M KCl and 1.0 mM ATCl. Then the electrode was rinsed with water and incubated in pH 7.0 PB containing just desired concentration of carbaryl for 14 min [There was no $[Fe(CN)_6]^{3-/4-}$, KCl and ATCl in the PB]. Finally, it was transferred into pH 7.0 PB containing 5 mM $[Fe(CN)_6]^{3-/4-}$, 1 M KCl and 1.0 mM ATCl for impedance measurement at the same condition. The frequency associated with the largest difference in absolute impedance between the AChE/CHIT/IAM with carbaryl inhibition and the control without carbaryl inhibition was chosen. The inhibition rate of carbaryl was calculated as follows:

$$\text{Inhibition } (\%) = \left(Z_{\text{sample}} - Z_{\text{control}}\right) / Z_{\text{control}} \times 100\ \%$$

where Z_{control} is the absolute impedance of the control and Z_{sample} is the absolute impedance of the sample with carbaryl inhibition. All the absolute impedance was under the chosen frequency.

Results and discussion

UV–vis spectrum

The position of Soret absorption band can provide detailed information about conformation of protein [32]. As shown in Fig. 2, solution-phase AChE displayed a Soret band at about 420 nm, indicating that AChE has activity and no significant denaturation occurred.

Impedance measurement

Electrochemical impedance spectroscopy is a versatile and popular technique used to describe the response of an electrochemical process taking place at the electrode solution interface consequent to a low amplitude sinusoidal perturbation as a function of frequency applied through a capacitive probe. Characterization of stepwise changes in electrochemical properties upon the formation of each layer was carried out using the magnitude and phase angle of impedance measurement in the presence of $[Fe(CN)_6]^{3-/4-}$ as a redox couple in PB containing 1 M KCl. Figure 3 presented the magnitude and phase angle of

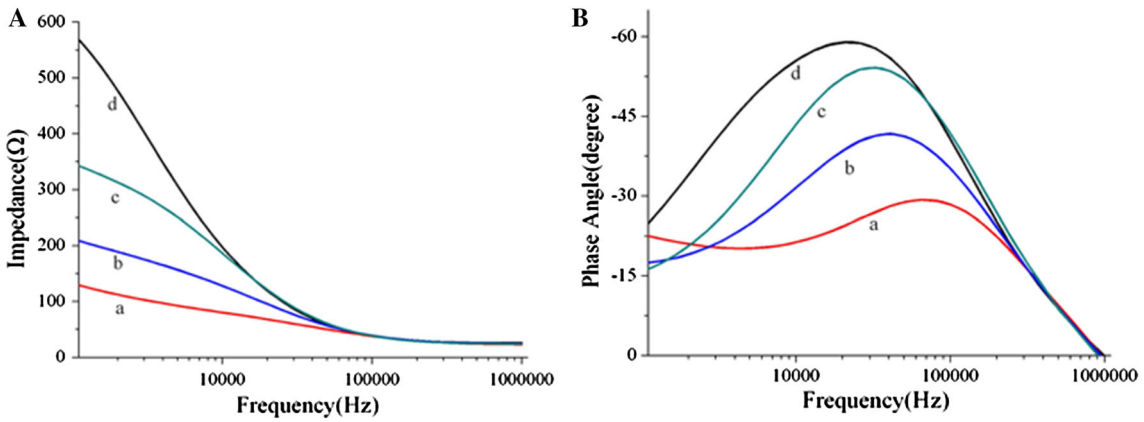

Fig. 3 The magnitude and phase angle of impedance spectra of **a** bare IAM, **b** CHIT/IAM, **c** AChE/CHIT/IAM and **d** nafion/AChE/CHIT/IAM

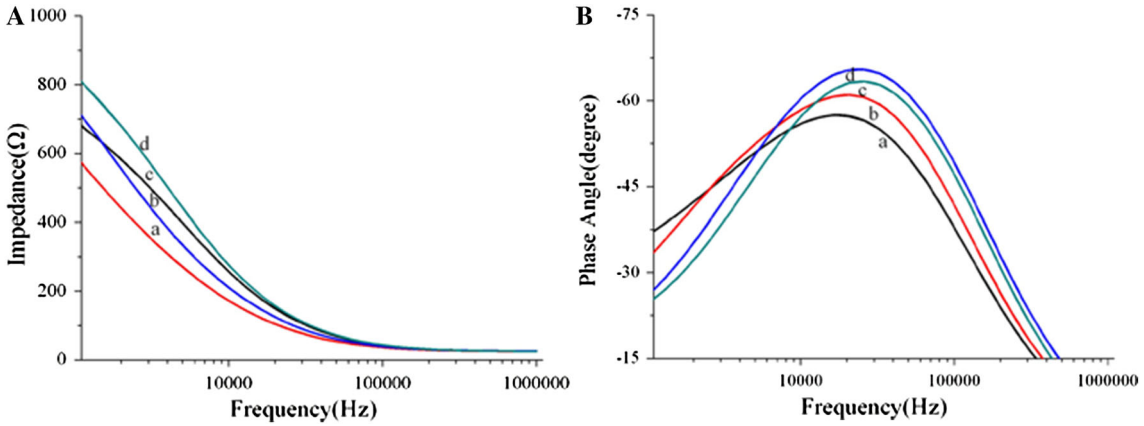

Fig. 4 The magnitude and phase angle of impedance spectra of nafion/AChE/CHIT/IAM after inhibition by carbaryl. **a** Blank, **b** 1 ng/mL, **c** 10 ng/mL and **d** 100 ng/mL

Table 1 The experiment data between inhibition rate and carbaryl concentration

Carbaryl concentrations (ng/mL)	Inhibition (%)	Standard deviation (%)
1	20.83	2.09
10	40.28	2.92
100	58.33	5.73

Table 2 Comparison of analytical characteristics with other reported biosensors for the detection of carbaryl

Electrode	Liner range	Detection limit (ng/mL)	References
AChE/carbon paste electrodes	1–15 μg/mL	400	[34]
GC/PANI/MWCNT/AChE	1.98–9.92 μg/mL	280	[35]
Nafion/AChE/CHIT/IAM	1–100 ng/mL	0.78	This work

impedance spectra of (a) the bare IAM, (b) the CHIT modified electrode, (c) the AChE immobilized electrode, and (d) nafion modified electrode. The frequency range is from 1 Hz to 1 MHz. Each step was measured three times. In a wide frequency range, the impedance value at a given frequency increased upon the addition of (b) CHIT, (c) the AChE layer, and (d) nafion modified to the electrode surface compared to (a) the bare IAM. At higher frequencies, no significant difference was observed between spectra a, b, c and d. The result clearly indicated that the change of the IAM-based impedance biosensor was evident for the stepwise assembly.

Detection of carbaryl

The detection signals for different concentrations (1–100 ng/mL) of pesticides on AChE modified onto the electrode surface were recorded in impedance magnitude of bode amplitude plots as shown in Fig. 4a. Maximum change in impedance magnitude was seen in the frequency range from 1 to 8,000 Hz. The response generation with different concentration of carbaryl was obviously caused. As the concentration of carbaryl increased, there was a further increase of impedance. This result might be ascribed to the fact that carbaryl exhibited fairly high toxicity and performed irreversible inhibition action on AChE, which reduced the enzymatic activity to its substrate. The phase angle was also recorded for all impedance measurements as shown in Fig. 4b. The phase angle describes the contributions of the resistive and capacitive portions to the impedance magnitude. The capacitive portion of impedance is dominant at the high and low ends of the frequency range while the resistance dominates the mid frequency range, where the largest amount of impedance magnitude change is seen [33].

The carbaryl at different concentrations (1, 10 and 100 ng/mL) was investigated to study the performance of the biosensor. In Table 1, the impedance magnitude at 3,000 Hz was plotted for each concentration, showing the increase of the inhibition rate along with the increase of carbaryl's concentration. A corresponding equation was described as: $I\ \% = 21.063 + 18.75\ \mathrm{lgC}$ (ng/mL), has a correlation coefficient of 0.9995.

A comparison of experimental data of nafion/AChE/CHIT/IAM with related literatures for the detection of carbaryl was summarized in Table 2. Information of the performance of the biosensor was summarized in Table 3.

Table 3 Performance of the biosensor

Liner range	Detection limit	RSD (inter-assay precision)	RSD (intra-assay precision)	Stability	Reproducibility
1–100 ng/mL	0.78 ng/mL	4.8 %	4.6 %	84 % (30-day)	87 %

Conclusion

The objective of this study was to develop an IAM-based AChE impedance biosensor for a new application of sensitive and rapid detection of pesticide residues. It was based on the combination of IAM with AChE and impedance spectroscopy, one of the most effective transduction techniques. The results demonstrated that the IAM-based AChE impedance biosensor was able to detect carbaryl, and a linear range was found from 1 to 100 ng/mL. In this electrochemical detection system, CHIT was used to immobilize AChE for carbaryl detection. This biosensor system could be used as a pre-scanning method in pesticide determination for the analysis of environmental, agricultural and pharmaceutical samples after further optimized, due to its rapidity, sensitivity and low cost.

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