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Bioelectrochemistry 55 (2002) 75–77

Bioelectrochemistry

www.elsevier.com/locate/bioelechem

Polyaniline-modified cholinesterase sensor for pesticide determination

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Received 1 June 2001; received in revised form 4 September 2001; accepted 25 September 2001

Abstract

Cholinesterase sensors based on glassy carbon and planar epoxy graphite electrodes modified with processed polyaniline have been developed and examined for pesticide detection. The modification of electrode surface with polyaniline provides high operational stability and sensitivity towards the pesticides investigated. The detection limits found (coumaphos, 0.002, trichlorfon, 0.04, aldicarb, 0.03, methiocarb, 0.08 mg l^{-1}) make it possible to detect the pollutants in the waters on the level of limited threshold levels without sample preconcentration.

Keywords: Cholinesterase biosensor; Polyaniline; Pesticide determination

1. Introduction

Organophosphorus and carbamic pesticides are widely used in agriculture as insecticides. Certain amount of pesticides, when transferred into the environment, can be dangerous for human health within several weeks [1,2]. Thus, there is a need for fast and inexpensive testing devices for pesticide detection. One of the approaches to their developments assumes the determination of pesticide inhibition on cholinesterases [3–6] (see also review [7]). Previously, we have shown that the use of processed polyaniline doped with camphorsulfonic acid for the modification of carbon electrodes provides reproducible super-Nernstian pH response of about 87 mV per pH unit over the range of 3–9 pH [8]. In this work, the use of polyaniline-modified electrodes in the assembly of cholinesterase sensors has been explored for the detection of enzyme inhibitors.

2. Experimental

Butyrylcholinesterase from horse serum purchased from JSC Biomed (Perm, Russia), 4.2 U mg^{-1}, and from Sigma (St Louis, MO, USA), 500 U mg^{-1}, as well as acetylcholinesterase from electric eel, 1000 U mg^{-1}, from Sigma, were immobilised by cross-linking with glutaraldehyde on the surface of glassy carbon or planar epoxy graphite electrode (IVA, Ekaterinburg, Russia). Prior to enzyme immobilisation, electrodes were covered with 0.5% polyaniline solution in chloroform containing camphorsulfonic acid and phenol [8] and left to dry at room temperature. Acetylcholine iodide was used as the substrate. Trichlorfon was purchased from Sigma, coumaphos, methiocarb and aldicarb from Riedel-de-Haen (Seelze, Germany). All the measurements were performed in 0.002 M Tris buffer solutions containing 0.1 M NaCl.

The potential shift of polyaniline sensor caused by acid release in enzymatic reaction (1),

$$(CH_3)_3N^+CH_2CH_2OC(O)CH_3 + H_2O \rightarrow (CH_3)_3N^+CH_2CH_2OH + CH_3COOH, \quad (1)$$

was measured vs. Ag/AgCl electrode by digital ionometer Ecotest-001 (Econix, Russia) within 5 min after the substrate injection. Before incubation, coumaphos was preoxidized in a 10-min electrolysis to phosphoryl analog as described in [9].

3. Results and discussion

The immobilisation of cholinesterase on polyaniline-modified sensor does not alter the pH sensitivity of the

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1567-5394/02/$ - see front matter
PII: S1567-5394(01)00163-3

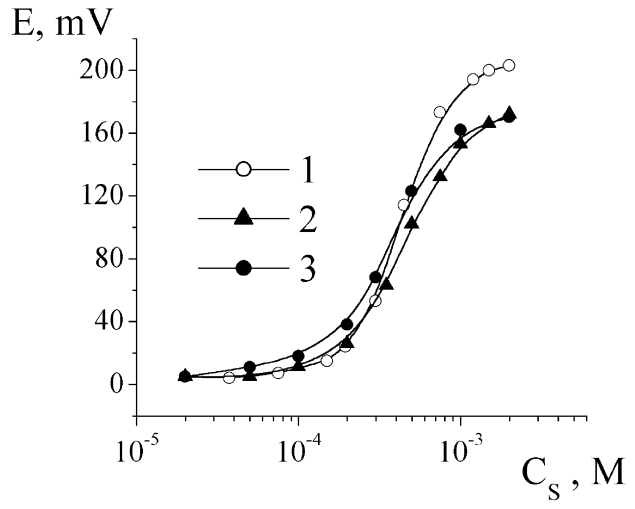

Fig. 1. Acetylcholine calibration curve. Polyaniline-modified carbon glass electrode covered with acetylcholinesterase (1), highly active (Sigma, 2) and low active (Biomed, 3) butyrylcholinesterases. Tris buffer solution, pH 7.5.

response. It was found to be equal to 87 and 84 mV/pH unit before and after enzyme immobilisation, respectively. The graduation curves of the substrate obtained for different cholinesterase preparations are presented in Fig. 1. The slope of a linear part of the curves is equal to 120 ± 5 mV/decade for acetylcholinesterase sensor and 95 ± 5 mV/decade for butyrylcholinesterase sensors. A higher slope of the curves in comparison with that of pH response is probably due to the limitation of the acid escape from the enzymatic layer. The enzyme immobilisation does not significantly alter the pH maximum of enzyme activity as well as their dependence on the buffer capacity of the working solution.

All the pesticides tested showed a strongly irreversible inhibitory effect on immobilised cholinesterases. The results of pesticide determination are summarised in Table 1. The polyaniline modification provides better characteristics of pesticide determination in comparison with other detection systems reported. The appropriate detection limits are much lower and the slopes of calibration curves are higher by 20–40% than those obtained earlier [7]. The higher the peptide loading in the enzymatic layer, the greater the difference in analytical characteristics. Inert peptides in the enzymatic layer probably increase the sensitivity of an enzyme toward hydrophilicity of the peptides determined. The use of planar epoxy graphite electrodes results in the decrease of a response reproducibility and hence in the increase of the detection limits by 20–30%. Meanwhile, the response drift and the changes in the sensitivity toward pesticides were found to be lower for planar sensors than for the sensors based on carbon glass electrodes.

4. Conclusion

The modification of carbon electrodes with polyaniline improves both the analytical and operational characteristics of cholinesterase sensors. This results in the decrease in the

Table 1
Analytical characteristics of pesticide determination with polyaniline-modified cholinesterase sensors, inhibition, percentage, vs. log(C_I, mg l^{-1}), incubation 10 min

	Enzyme immobilised on electrode surface		
	Acetylcholinesterase (Sigma)	Butyrylcholinesterase, highly active (Sigma)	Butyrylcholinesterase, low active (Biomed)
Coumaphos			
Sensitivity, inhibition per decade (%)	60 ± 2	67 ± 3	70 ± 5
Detection limit	0.030	0.015	0.002
Concentration range	0.10–0.90	0.050–0.75	0.015–0.20
Trichlorfon			
Sensitivity, per decade (%)	50 ± 3	700 ± 5	65 ± 5
Detection limit	0.25	0.20	0.04
Concentration range	0.50–4.0	0.50–2.50	0.05–0.60
Aldicarb			
Sensitivity, inhibition per decade (%)	44 ± 4	35 ± 4	65 ± 10
Detection limit	0.03	0.10	0.15
Concentration range	0.045–1.20	0.15–1.0	0.60–5.0
Methiocarb			
Sensitivity, inhibition per decade (%)	40 ± 2	30 ± 3	33 ± 2
Detection limit	0.08	0.25	0.40
Concentration range	0.2–5.7	0.3–20	1.0–10.0

detection limits and increases the sensitivity of pesticide determination. This can be related to the higher pH response of polyaniline films as well as to the limitations of the mass transfer between the peptide layers and bulk solution.

Acknowledgements

Financial support of INTAS (grant 00-273) is gratefully acknowledged. The work was partially financed by the Russian Foundation for Basic Research (projects 00-03-32605, 00-03-33194a).

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