ORIGINAL ARTICLE

AMPEROMETRIC BIOSENSOR FOR PESTICIDE METHAMIDOPHOS ASSAY

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Summary: Amperometric biosensor based on enzyme acetylcholinesterase (AChE; EC 3.1.1.7) was tested for pesticide methamidophos assay. Biosensor consists from four screen printed platinum electrodes on ceramic strip. AChE was physically adsorbed onto the electrode surface. The measuring principle was based on the inhibition of AChE activity in the presence of methamidophos. The proposed method limit of detection was 2.45 nM, responding to 3.46 pg of methamidophos detected absolutely when we consider the sample volume.

Key words: *Methamidophos; Pesticide; Amperometric; Electrochemical; Biosensor; Acetylcholinesterase; Assay*

Introduction

Organophosphorous pesticides are a large group of compounds exerting an important role in different human activities. Their common toxic effect is based on the inhibition of enzymes with cholinesterase activity: acetylcholinesterase (AChE; EC 3.1.1.7) and butyrylcholinesterase (BChE; EC 3.1.1.8) that play important roles in human and animal bodies (10). Their mechanism of inhibition is based on phosphorylation of the physiologically active serine hydroxyl group in the active centre of the enzyme (8). Poisoning by organophosphates leads to malfunction of the cholinergic nervous system. Although poisoning could be a serious fulmination, there are several ways of treating it. The first one is based on the physiological suppression of the cholinergic crisis; drugs with anticholinergic activity such as atropine are convenient for this purpose (1, 7). Another method of treatment is based on reactivators. For these purposes a large group of oximes is suitable, such as obidoxime, pralidoxime, and HI-6 (2, 8, 9).

Methamidophos ((RS)-O,S-dimethyl phosphoramidothioate) is one of the currently used pesticides; it is commercially available under product names such as Monitor, Nitofol, Tamaron, Swipe, Nuratron, Vetaron, Filitox, Patrole, and Tamanox for suppression of chewing and sucking insect populations. Methamidophos is able to affect AChE in the same manner such as other organophosphorus pesticides (5). Methamidophos exerts high toxicity towards large groups of animals. Per oral LD_{50} value ranges from 10 (rabbits) to 50 mg/kg (guinea pigs); methamidophos intake by humans leads to paralysis of limbs and respiratory muscles within four days after exposure (16, 17).

One of the important tasks in suppressing the harmful effects of methamidophos is timely detection. Widely available techniques are based on mass spectrometry and/or liquid chromatography (6, 15). Biosensors could be an alternative to the classical analytical methods. It was described as a useable tool for the analysis of different analytes, including microorganisms (13) and toxic compounds (11, 14). The suitability of biosensors for organophosphates and carbamates detection was extensively reviewed (18). Biosensors harboring cholinesterase attached to the electrode surface are the most typical; an assay is based on a decrease in cholinesterase activity due to inhibition by analyte (organophosphate) during the measuring procedure. Different electrodes and immobilization techniques can be applied. A platinum electrode covered with a composite, including AChE and graphite, cobalt phthalocyanine and acetylcellulose (20), an adsorbed mixture of cholinesterase, glutaraldehyde and albumin onto a screen printed platinum surface (12, 19), were found to be convenient. Furthermore, graphite rods and nylon fibres were also considered suitable as a biosensor with cholinesterase construction (3, 4, 21). The presented study is focused on the preparation and performance of amperometric biosensor for methamidophos assay. The suitability of the developed biosensor for assay will be the object of discussion.

Material and methods

Chemicals

Lyophilized human recombinant AChE, acetylthiocholine chloride (ATChCl), glutaraldehyde and methamidophos (analytical standard, 98.4 %) were purchased from

Sigma-Aldrich. Deionized water was obtained by Millipore system. All other chemicals were in the standard analytical quality.

Immobilization procedure

AChE was suspended into deionized water: final activity 0.05 nkat/µl. 1 µl of AChE solution was injected on one out of every four platinum electrodes printed onto ceramic strip (BVT, Brno, Czech Republic) and the solution was allowed to dry at the laboratory temperature. In the second step, a whole ceramic strip was placed into the chamber filled with glutaraldehyde vapor and 1 µl of AChE solution per electrode was applied again and allowed to dry (about half an hour). Finally, the created biosensor was gently washed with phosphate buffered saline (PBS).

Measuring setup and data processing

The biosensor was dipped into a reaction cell (polypropylene, shaped as flat bottom cylinder with maximal volume 2 ml) filled with 990 µl of reaction medium (1 mM ATChCl in PBS). The reaction cell was stirred with a teflon-coated magnetic agitator (length 5 mm). A silver wire covered by silver chloride was used as a reference electrode. The applied potential was set on +450 mV for oxidation of the forming thiocholine (followed parameter). The current (i_0) was measured by the amperometric detector MEB (MultiLab, Brno, Czech Republic). The background current (i_b) was measured in the same manner as i_0; however, the biosensor was displaced by a sensor (strip without immobilized AChE). The current after inhibition (i_i) was obtained when 10 µl of methamidophos solution in water per one electrode was applied for 10 min. Incubation with methamidophos was realized out of the reaction chamber. After that, the biosensor was dipped into the reaction chamber and i_i was read. The percent of inhibition (I) was calculated in the following way:

$$I = (1 - \frac{i_i - i_b}{i_0 - i_b}) \times 100 \qquad (1)$$

Results and discussion

The biosensor was prepared as described above. The i_0 was evaluated as 212 nA during the first observations. The sensor strip without the immobilized AChE tested in the same manner provided the signal 76 nA. Methamidophos was dilluted with deionized water into five calibration concentrations: 1; 10; 100; 1000 and 10000 nM. Every concentration was assayed by the biosensor and the I parameter was calculated according equation 1. The calibration curve (Fig. 1) is presented in the form of I vs. log M, where log M is the logarithm of methamidophos concentration in nM.

PBS applied in the very same manner as methamidophos was used for blank purposes. No relevant false positive result was obtained from blank application. The limit of detection (LOD) was the point at the calibration scale equal to the triplicate of the blank standard deviation (S/N=3); the calculation found LOD 2.45 nM responding to absolute 3.46 pg when we considered the sample volume of 10 µl. The calibration allows for quantification of the measured methamidophos up to a concentration 1 µM. The most important analytical parameters are summarized in Tab. 1.

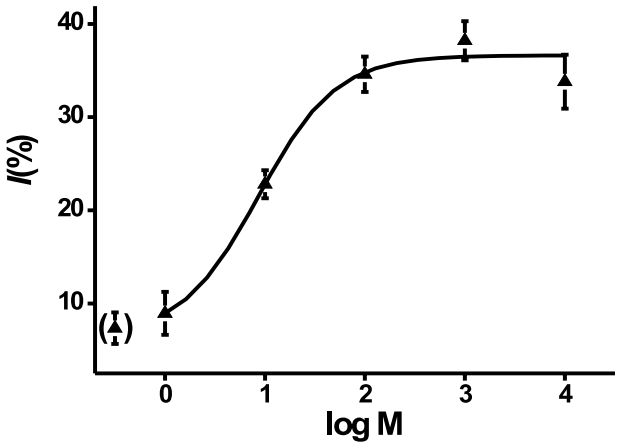

Fig. 1: Calibration curve for methamidophos. The x-axis (log M) value means the logarithm of methamidophos concentration in nanomoles. The y-axis [I (%)] value means the percent of inhibition according to equation 1. The blank is expressed as a value in brackets. Error bars indicate standard deviation (n=3).

Tab. 1: The most important analytical parameter expressions.

Limit of detection (molar scale)	2.45 nM
Limit of detection (parts per million scale)	0.0035 ppm
Limit of detection absolutely	3.46 pg
Needed sample volume per electrode	10 µl
Maximal time per one measuring cycle	20 min

The obtained results point to the feasibility of the biosensors. In particular, biosensors seem to be suitable as a low cost device for methamidophos analysis and in this way diminish fulmination of methamidophos as residuum in the environment. The U.S. Environmental Protection Agency (EPA) on its website proposes decreasing the tolerance limit for methamidophos as residuum in vegetables from 1 to 0.5 ppm (http://www.epa.gov/fedrgstr/EPA-PEST/2007/May/Day-23/p2561.htm). The LOD of developed biosensor is 0.0035 ppm, so not only the original but also the EPA proposed tolerance limit can be detected with this arrangement.

Conclusions

The presented work focused on the development of a simple method for low level methamidophos concentra-

tion detection. The proposed biosensor is able to detect methamidophos in concentrations approximately three hundred times lower than admissible as residuum. We are encouraged by the obtained results and we expected further complex studies focused on the testing of biosensor applicability for pesticides assay.

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