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Cholinesterase sensor based on glassy carbon electrode modified with Ag nanoparticles decorated with macrocyclic ligands

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ABSTRACT

New acetylcholinesterase (AChE) sensor based on Ag nanoparticles decorated with macrocyclic ligand has been developed and successfully used for highly sensitive detection of organophosphate and carbamate pesticides. AChE was immobilized by carbodiimide binding on carbon black (CB) layer deposited on a glassy carbon electrode. The addition of Ag nanoparticles decreased the working potential of the biosensor from 350 to 50 mV. The AChE sensor made it possible to detect 0.4 nM–0.2 μM of malaoxon, 0.2 nM–0.2 μM of paraoxon, 0.2 nM–2.0 μM of carbofuran and 10 nM–0.20 μM of aldicarb (limits of detection 0.1, 0.05, 0.1 and 10 nM, respectively) with 10 min incubation. The AChE sensor was tested for the detection of residual amounts of pesticides in spiked samples of peanut and grape juice. The protecting effect of new macrocyclic compounds bearing quaternary ammonia fragments was shown on the example of malaoxon inhibition.

1. Introduction

The development of instrumental tools for sensitive detection of enzyme inhibitors is of substantial interest in the environmental, food and agricultural areas [1]. Among others, organophosphate and carbamate pesticides exert irreversible inhibition of acetylcholinesterase (AChE). The pesticides or their primary metabolites form a covalent bond between the hydroxyl group of serine residue of the enzyme active site and esteric fragment of an inhibitor molecule [2,3]. The product of the reaction, i.e. phosphorylated or carbamoylated AChE does not react with a substrate. This results in a decrease of the enzyme activity quantified by appropriate transducer. The carbamoylated AChE is spontaneously re-activated in aqueous media so that maximal inhibition levels can be beyond 100%. The product of organophosphate inhibition is commonly stable and the enzyme activity decreases down to zero with an increase of the inhibitor concentration and/or incubation period.

The necessity of the detection of organophosphates and carbamates is related to the AChE biological function. This enzyme is widely present in warm-blooded living beings and is responsible for the nerve impulse transduction by hydrolysis of a natural neurotransmitter, acetylcholine.

$$\underset{\text{Acetylcholine}}{(CH_3)_3N^+CH_2CH_2O\overset{\displaystyle O}{\overset{\|}{C}}CH_3} + H_2O \xrightarrow{\text{AChE}} \underset{\text{Choline}}{(CH_3)_3N^+CH_2CH_2SH} + CH_3COOH \quad (1)$$

The inhibition of AChE in living beings increases the concentration of acetylcholine followed by abdominal cramps, muscular tremor, hypotension, breathing difficulty, slow heartbeat and death [4,5].

The residual amounts of anticholinesterase pesticides in soils, vegetables, biological tissues and food are commonly determined by gas chromatography with mass spectrometry [6,7] and flame photometry detection [7] and liquid chromatography with fluorescence/UV [8], diode-array [9] and mass spectrometry [10] detection. For sample pre-concentration, liquid–liquid [10] and solid-phase extraction techniques are often used [11]. The chromatographic detection of pesticides is summarized in recent review [12]. From other techniques, immunoassay in ELISA [13], fluorescent polarization [14] and lateral flow format [15] can be mentioned.

AChE biosensors are compact devices developed for the fast and sensitive detection of pesticides, preferably in field conditions.

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