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Short communication

A novel, sensitive, reusable and low potential acetylcholinesterase biosensor for chlorpyrifos based on 1-butyl-3-methylimidazolium tetrafluoroborate/multiwalled carbon nanotubes gel

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ABSTRACT

A novel, low potential and highly sensitive acetylcholinesterase (AChE) biosensor was developed based on 1-butyl-3-methylimidazolium tetrafluoroborate/multiwalled carbon nanotube composite gel thiocholine sensor. Composite gel promoted electron transfer reaction at a lower potential (+50 mV) and catalyzed electrochemical oxidation of thiocholine with high sensitivity. AChE was immobilized in sol–gel matrix that provides a good support for enzyme without any inhibition effect from the ionic liquid. The amount of immobilized enzyme and incubation time with chlorpyrifos were optimized. Chlorpyrifos could be determined in the range of 10^{-8}–10^{-6} M with a detection limit of 4 nM. Fast and efficient enzyme reactivation was obtained at low obidoxime concentration (0.1 mM). Moreover, the biosensor exhibited a good stability and reproducibility and could be use for multiple determinations of pesticide with no loss of the enzyme activity.

1. Introduction

The measurement of pesticides in water and food is of great importance. The major concern about pesticides relates to acute intoxication due to the fact that these compounds are found at low levels in the environment. These compounds inhibit the acetylcholinesterase by phosphorylation of its active site. The current produced by the electrochemical oxidation of enzymatically generated thiocholine can be used as a quantitative measure of the enzyme activity, which is a marker for the biological effect of the pesticides.

Thiocholine (TCh) is oxidized at relative high potential on the surface of classical electrode. Reducing of the working potential was achieved by using chemically modified electrodes based on mediators or multiwalled carbon nanotubes (MWCNT). Anyway, the lowest potential achieved was +150 mV (Liu et al., 2005). Recently, ionic liquid (IL)–MWCNT modified carbon paste (CP) electrodes have been shown to improve the detection of thiocholine by catalyzing the oxidation of thiocholine at very low potentials between 0 and 50 mV (Rotariu et al., 2010).

The immobilization of the enzyme onto the electrode surface represents a crucial step in the development of the biosensor (Arduini et al., 2010). Various methods have been used, from direct adsorption (Marty et al., 2003), cross-linking with glutaraldeyde on chitosan–MWCNT composite (Du et al., 2007b) to mixing the enzyme in polymers (Du et al., 2010), MWCNT/sol–gel (Du et al., 2007a) or use of chelate-functionalized magnetic microbeads (Istamboulie et al., 2007). The sol–gel silicate network provides a biocompatible microenvironment around the enzyme that stabilizes its biological activity and prevents it from leaking out of the catalytical layer (Andreescu et al., 2002).

Chlorpyrifos is a common pesticide widely used in agriculture but few groups focused their research on this compound probably because it is a weak AChE inhibitor (Cetinkaya and Baydan, 2010). Detection limit achieved are comprised between 1 μM (Halamek et al., 2005) and 1 pM (Viswanathan et al., 2009).

There are few papers about use of ionic liquids in development of pesticide biosensors. Direct detection of methyl-parathion was performed with 1-butyl-3-methylimidazolium hexafluorophosphate–single-walled carbon nanotube paste coated glassy carbon electrode (Fan et al., 2008). A biosensor for detection of paraoxon that use immobilized organophosphate hydrolase on MWCNT/IL-modified Au electrodes has also been described (Choi

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et al., 2009). Anyway, no biosensor based on AChE and IL–carbon nanotubes gels was reported in the literature.

Pyridine-2-aldoxime methochloride (PAM) was exclusively used for AChE biosensor regeneration achieving a reactivation degree between 90 and 95% (Du et al., 2007b, 2010). The reactivation potency of various pyridinium oximes was also investigated and obidoxime was found to be more efficient than PAM in the recovery of AChE activity (Jokanovic and Maksimović, 1995; Kuca et al., 2004).

In this paper a new AChE biosensor for chlorpyrifos was developed based on IL–MWCNT gel thiocholine sensor previously reported. Due to the highest sensitivity presented at a low applied potential (+50 mV) 1-butyl-3-methylimidazolium tetrafluoroborate ([BMIM][BF_4]) ionic liquid was chosen for this study (Rotariu et al., 2010).

Sol–gel immobilization of AChE was used in this work providing an excellent protection of the AChE molecule from the potential inhibitory effect of IL. Obidoxime was tested as reactivator of the enzyme activity and proved to be more effective comparing with pralidoxime. This is the first report about use of obidoxime for an AChE biosensor regeneration.

2. Experimental

2.1. Materials

Acetylcholinesterase (AChE, from electric eel, 425.94 IU/mg) acetylthiocholine chloride (ATCh, purity ≥99%), multiwalled carbon nanotubes (MWCNT, purity ≥95%, outside diameter = 40–60 nm, inside diameter = 5–10 nm, length = 0.5–500 μm), 1-butyl-3-methylimidazolium tetrafluoroborate ([BMIM][BF_4]), PAM, obidoxime (1,1′-[oxybis(methylene)]bis{4-[(*E*)-(hydroxyimino)methyl]pyridinium}), acetonitrile (purity ≥99.9%), tetramethoxysilane (TMOS), methyltrimethoxysilane (MTMOS), polyethylene glycol (PEG600) were purchased from Sigma–Aldrich. Chlorpyrifos (O,O-diethyl-O-3,5,6-trichloro-2-pyridyl phosphorothioate, purity ≥99.9%) were purchased from Riedel-de Haën. The carbon paste (66% graphite, 34% paraffin) was supplied by Metrohm. The AChE stock solution (66 IU/mL) was prepared in 0.1 M PBS, pH 8. The chlorpyrifos dilutions were prepared in ultrapure water from a 10^{-4} M stock solution, prepared in acetonitrile.

2.2. Instruments

Electrochemical measurements were carried out using a potentiostat AUTOLAB PGSTAT12 (Eco Chemie BV, Utrecht, Netherlands). A three-electrode cell was used: mini carbon paste electrodes (diameter 3.0 mm) as working electrode, an Ag/AgCl 3 M KCl reference electrode and a Pt auxiliary electrode, all produced by Metrohm. All determinations were performed at +50 mV vs. Ag/AgCl reference electrode. All experiments were carried out at room temperature (22 °C), using a 5 mL cell.

2.3. Preparation of AChE biosensor

2.3.1. Preparation of thiocholine sensor

100 μL of [BMIM][BF_4] was sonicated with 5 mg MWCNT for 45 min in a 1.5 mL tube. The composite gel is centrifuged at 6400 rpm and the excess of ionic liquid was removed. Preparation and modification of the mini-carbon paste electrode with composite gel was performed according to the procedure described by Rotariu et al. (2010).

Table 1
Response characteristics of AChE biosensors for different enzyme loading.

AChE (IU/electrode)	Linear range (mM)	Sensitivity (μA mM^{-1})	Correlation (R^2)	LOD[a] (mM)
0.96	0.3–7	2.42	0.9992	0.12
0.24	0.2–5.5	1.92	0.9988	0.10
0.13	0.2–4	1.12	0.9972	0.10
0.066	0.1–3	0.81	0.9890	0.08
0.033	0.1–3	0.55	0.9735	0.07
0.017	0.05–4	0.17	0.9946	0.03

[a] LOD was calculated for S/N = 3.

2.3.2. AChE immobilization procedure

The sol–gel matrix was prepared following a protocol previously published (Andreescu et al., 2002). 100 μL TMOS and 100 μL MTMOS were mixed with 400 μL of ultrapure water, 440 μL of HCl 1 mM and 40 μL PEG600 in order to hydrolyze the monomers and to obtain the "*sol*". After sonication for 5 min, the mixture was stored overnight at 4 °C. The AChE solution was mixed with "*sol*" in a ratio of 50:50 (vol.) and 5 μL of this mixture are dropped on the modified CP electrode. The biosensor is left to dry for 3–4 h at 4 °C and kept afterward in phosphate buffer pH = 8 at 4 °C.

3. Result and discussion

3.1. Study of the effect of ionic liquid on the enzyme kinetic

Arning et al. (2008) reported the ILs potential inhibitory effect on the activity of AChE. ILs have also the capability to disperse the carbon nanotubes and form composite gels (Fukushima and Aida, 2007). Consequently, the mobility of the [BMIM][BF_4] is reduced in the composite gel and it is expected to have a low accessibility to the enzyme cataytical site.

Immobilization of the AChE in sol–gel matrix intended to prevent the loss of the enzyme from the catalytical layer and to protect it from the potential inhibitor effect of the IL. In order to verify the efficiency of the immobilization procedure two biosensors were prepared: one based on a CP electrode and another one based on [BMIM][BF_4]–MWCNT gel-modified CP electrode. 0.96 IU AChE were immobilized on both sensors. Enzyme reaction rate was determined amperometrically. The Lineweaver–Burk linear representation of the kinetic data is presented in Fig. S1 and the calculation of the apparent Michaelis–Menten constant gives similar results, 2.1 mM in the absence of IL and 6.6 mM in the presence of [BMIM][BF_4] gel. These results lead us to the conclusion that no inhibition from IL could be distinguished and AChE immobilized in sol–gel matrix could be successfully used for determination of pesticide without any interference from the IL.

3.2. Optimization of the amount of immobilized AChE

The amount of AChE immobilized onto the electrode surface is crucial for pesticide determination. A compromise between a high enough analytical signal and a highest possible inhibition degree should be realized.

Amounts of AChE between 0.96 and 0.017 IU were immobilized onto the thiocholine sensor. The optimum ATCh concentration and biosensors sensitivity were determined from calibration curves. To determine the enzyme activity, an excess of substrate is necessary in order to ensure a saturation of the enzyme catalytical sites but it has to be avoided the inhibition by excess of substrate. This is why the ATCh concentration should be very carefully settled.

For 17 mIU AChE the plateau is reached at about 5 mM ATCh while for higher amount of enzyme the saturation is observed at approximately 10 mM ATCh. Table 1 presents the main response