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Volume I

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TIME – DEPENDENT INHIBITION OF ELECTRIC EEL AChE INDUCED BY CHLORPYRIFOS

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Abstract

The aim of the work was to investigate the influence of contact time between acetylcholinesterase (AChE) and chlorpyrifos, on the sensitivity of earlier developed AChE based bioanalytical method for detection and determination of organophosphates in water samples. The IC_{50} values were obtained from the concentration-dependent responses of AChE activity to chlorpyrifos and they decreased with the increasing the contact time. The results indicated that the sensitivity of AChE based bioassay can be improved by increasing the time of incubation, but this comes at the expense of additional analysis time. In addition, the inhibition parameters of chlorpyrifos induced inhibition of AChE were determined.

Introduction

Organophosphorus compounds (OP), such as chlorpyrifos, malathion, parathion, have been commonly used as insecticides for over 50 years. These compounds are specific irreversible inhibitors of acetylcholinesterase (AChE, EC 3.1.1.7), the enzyme involved in the hydrolysis of the neurotransmitter acetylcholine (ACh) at cholinergic synapses in the central and peripheral nervous systems (cholinergic syndrome) [1].

Organophosphates are preferred in agriculture because of their relatively low persistence in the environment. In recent years various bioanalytical methods and biosensors, based on specific inhibition of AChE by organophosphorus compounds, have been widely developed for the fast screening organophosphates in the environment [2, 3].The aim of this work was to investigate chlorpyrifos-induced inhibition of AChE activity as a function of the contact time between enzyme and inhibitor, in order to improve sensitivity of the recently developed bioanalytical method for detection and determination of OPs [2]. In addition, inhibition parametres, K_I and k_3, of chlorpyrifos-induced AChE inhibition were determined.

Material and Methods

Acetylcholinesterase (AChE, specific activity 288 IU/mg solid) from electric eel, acetylthiocholine iodide (ASChI) and 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB) were purchased from Sigma Chemicals Co. Chlorpyrifos (98% purity) was purchased from Galenika (Zemun, Serbia). The inhibition of enzyme was measured using the Ellman's method [2]. Experiments were performed by *in vitro* exposure of 0.174 μg enzyme to inhibitors in a final volume of 0.650 ml.

Results and Discussion

The influence of chlorpyrifos on AChE activity, was investigated by *in vitro* exposure of the enzyme to the inhibitor in the concentration range from 1 x 10^{-8} to 5 x 10^{-4} M in several incubation times (enzyme-inhibitor contact time): 14, 17, 20, 25 min (Fig.1). The IC_{50} values (inhibitor concentration that produced 50% of enzyme inhibition), determined by sigmoidal fitting of the experimental inhibition curves, are presented in Fig. 1. It is obvious that IC_{50} value decreased for almost one order of magnitude due to the prolonged incubation time. Consequently, the percent of inhibition in the presence of the particular inhibitor concentration decreased as a function of exposure time.

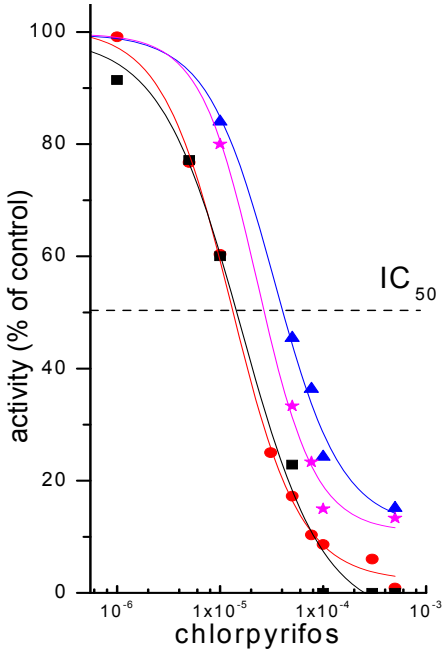

Fig. 1. (a) The concentration dependent irreversible inhibition of AChE activity by chlorpyrifos after 14 min (triangle), 17 min (asterisk), 20min (circle), and 25 min (square) of contact time.

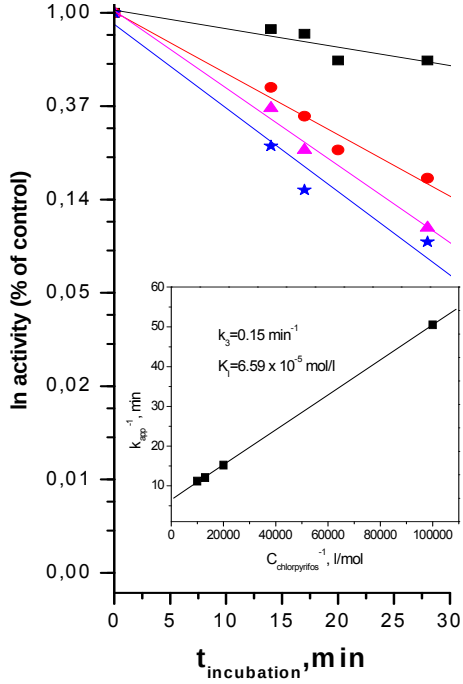

Fig 2. Progressive development of AChE inhibition by different chlorpyrifos concentrations (M):
1 x 10^{-5} (square), 5x10^{-5} (circle), 7.7x10^{-5} (triangle) and 1x10^{-4} (asterisk). The dependence of $1/k_{app}$ upon $1/C_{inhibitor}$ (inset).

Moreover, the chlorpyrifos concentration which induced 10% inhibition of enzyme activity, the statistically significant value to indicate the presence of inhibitor using AChE based bioanalytical method [2,3], decreased from 1x10^{-5}M to 1x10^{-6} M by prolonged incubation under the experimental conditions.

These results are in accordance with the previously reported for malathion and its degradation and oxidation products [2]. However, it can be noticed, based

on IC_{50} values obtained in this work, that chlorpyrifos is more potent AChE inhibitor compared to malathion, but weaker compared to the malathion degradation products (malaoxon and isomalathion). Besides, the reaction between chlorpyrifos and AChE is much slower compared to malathion group of compounds, since a significant improvement in inhibitory power of these compounds, about one order of magnitude, was observed after 5 min incubations of the enzyme with inhibitors.

Inhibition parametres, K_I (the dissociation constant for the initial reversible enzyme inhibitor complex) and k_3 (the first order rate constant for the conversion of the reversible complex to the irreversibly inhibited enzyme) were calculated, according to the method reported by Kitz et.al [4]. Fig. 2 represents the progressive development of inhibition produced by reaction of AChE, lnE /E_o *vs*. t, with different concentrations of chlorpyrifos. E/E_o represents here the percent of the remining activity. From the slope and intercept of the dependence of $1/k_{app}$ *vs*. $1/C_{inhibitor}$ (Fig. 2, (inset)). k_{app} was obtained from the slope of the dependence of lnE /E_o *vs*. t (Figure 2), where E /E_o represents the percent of the remaining enzyme activity in relation to the initial activity, E_o, and *t* is incubation time [4]. The inhibition parameters are given in Fig.2(inset)

Conclusion

The dependence of AChE inhibition on the time of exposure to the chlorpyrifos is in agreement with results reported earlier [2, 3]. This finding is important from the analytical point of view and shows that the sensitivity of used bioassay towards the investigated compound can be improved by increasing the contact time between the enzyme and organophosphate, but this comes at the expense of additional analysis time. The inhibition parametres for chlorpyrifos induced inhibition of AChE obtained in this work show that chlorpyrifos, although structurally different, inhibits the activity *via* the same mechanism as previously investigated irreversible organophosphorous AChE inhibitors, malathion and its related compounds [3].

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