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INHIBITION OF ACHE BY MALATHION AND SOME STRUCTURALY SIMILAR COMPOUNDS

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Abstract

Inhibition of bovine serum acetylcholinesterase by *in vitro* exposure to malathion, malaoxon, isomalathion and diethyl maleate was investigated to elucidate the mechanism of the enzyme interaction with structurally similar organophosphorus compounds. IC_{50} (half maximum inhibitory concentrations) were determined by Hill analysis of experimentally obtained inhibition curves. The values $(2.87 \pm 0.24)x10^{-6}$ M, $(2.65\pm0.61)x10^{-6}$M, $(3.01\pm0.36)x10^{-4}$ M and $(5.69 \pm0.7)x10^{-2}$ M were obtained for malaoxon, isomalathion, malathion and their hydrolysis product diethyl maleate, respectively. The relationship between the structure of the compounds and their potency to inhibit the enzyme activity was discussed.

Introduction

Organophosphorus compounds are commonly used as insecticides in agriculture[1]. When applied, they are usually completely removed by physico-chemical water treatment methods before getting into the potable water. The survey of pesticides trace amounts usually includes monitoring of parent compounds, but more polar, water-soluble transformation products (oxons) are usually not target analytes in monitoring surveys. The use of oxidation procedures or irradiation methods to remove the pollutants leads to different chemical transformations, e.g. hydrolysis, oxidation and isomerisation [2]. This work deals with the *in vitro* investigation of the mechanism of inhibition of bovine serum acetylcholinesterase (AChE, EC 3.1.1.7) by malathion and its three main degradation products found in irradiated solutions: malaoxon, isomalathion and diethyl maleate. The aim of our work was to investigate the toxic properties of malathion photochemical degradation products, which can be even more toxic than the parent compound.

Experimental

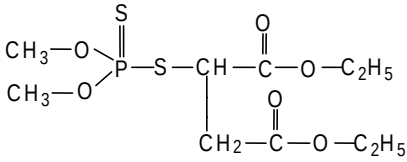
All chemicals were of analytical grade. Standard solutions of malathion, malaoxon, isomalathion and diethylmaleate (0.1 M) were made in ethanol shortly before use. AChE, specific activity 0.28 UI/mg, from bovine serum was purchased from Sigma Chemicals Co. The AChE activity was measured by Ellman procedure [3]. The experiments were performed by single exposure of 200 μg enzyme to inhibitors in final volume 0.634 ml. All measurements were made in triplicate. The control tubes

contained the corresponding concentration of ethanole without organophosphate. Spectrophotometric measurements were performed on Perkin Elmer Lambda 35 UV VIS spectrophotometer.

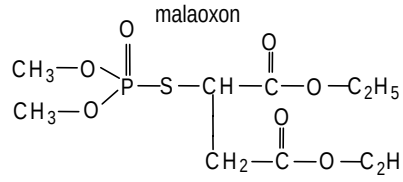
Results and Discussion

The influence of organophosphates on AChE activity was investigated by *in vitro* exposure to the enzyme in the concentration range from $1x10^{-8}$ to $1x10^{-1}$ M. Besides, the inhibiton potency of diethylmaleate was also investigated, since this compound is usually formed due to the chemical conversion or photochemical treatment of the slected organophosphates.

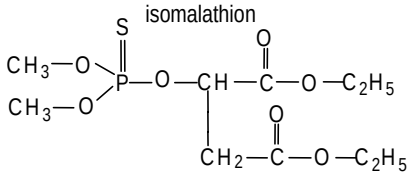
malathion



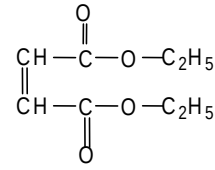
malaoxon



isomalathion



diethyl-maleat



The results show, that malathion and its related compounds inhibit AChE in a concentration-dependent manner. The sigmoid shaped inhibition curves (Fig.1) were obtained in all cases. IC_{50} (half-maximum inhibitory concentration) and Hill coefficient *n* were determined using the Hill method (Fig.2), by linear regression analysis of log [% activity/(100 -% activity)] vs. log $C_{inhibitor}$ plots and are given in Table 1. Although the investigated organophosphates have similar structure (malaoxon and malathion are even isomers), it is obvious that the combination of the substituents at the central phosphorous atom is responsible for the inhibition power. This finding can be ascribed to the polarity of the chemical bond that binds phosphorous to sulfur (malathion, malaoxon) or oxygen (isomalathion) atom. However, the catalytic triad in AchE active site that performs the catalytic functions of the enzyme is composed of three amino acid residues: Ser 200, Glu 327 and His 440. It is generally considered that organophosphorus compounds inactivate the AChE by phosphorylation of serine (Ser 200) hydroxyl group in the active site of enzyme. It seems that the strong covalent character of malathion P-S complicates the nucleofilic attack to serine –OH group of protein. The consequence is the decrease of the fosforilation, i.e. relative high IC_{50} value. On contrary, P-S and P-O bonds in the case of isomalathion and malaoxon molecules are more electronegative, and facilitate the fosforilation. Moreover, oxygen atom in both cases increases the polarity of the central phosphorous atom, and favorises the nucleophilic attack of serine –OH group from AChE. The consequence is the higher inhibi-

tory potency of these compounds. However, the inhibitory power of diethyl maleate is not significant.

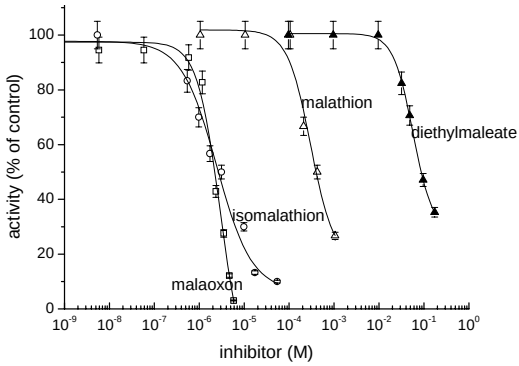


Fig. 1. Inhibition curves of AChE activity in the presence of malathion and its related compounds

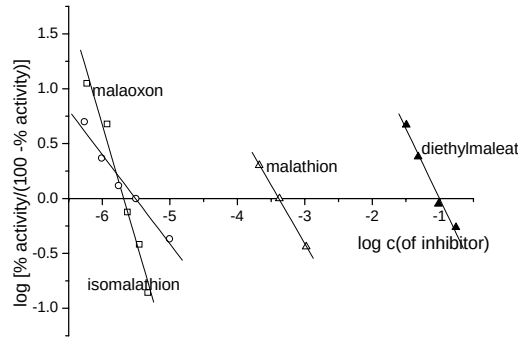


Fig. 2. Hill analysis of inhibition of AChE activity induced by malathion and its related compounds

Table 1. IC_{50} values of malathion and its related compounds obtained by fit of sigmoid inhibition curves and by Hill analysis

compound	IC_{50}, Hill [M]	n	IC_{50}
malaoxon	2.14×10^{-6}	2.12±0.18	$(2.87 \pm 0.24) \times 10^{-6}$
isomalathion	3.18×10^{-6} M	0.81±0.08	$(2.65 \pm 0.61) \times 10^{-6}$
malathion	4.32×10^{-4}M	1.06±0.02	$(3.01 \pm 0.36) \times 10^{-4}$
diethyl maleate	0.10 M	1.28±0.12	$(5.69 \pm 0.7) \times 10^{-2}$

Conclusion

The toxic effects of organophosphorus compounds and their transformation products (oxons, isomers) are primarily based on irreversibile inhibition of acetylcholinesterase (AChE), enzyme which participates in the transfer of impulse in central synapses of the cholinergic nervous system through a chemical mediator, acetylcholine. The inhibition of AChE leads to acetylcholine accumulation and the exhibition of toxic effects, thus poisoning by these compounds is in fact poisoning by endogenous acetylcholine.

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