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INHIBITION OF SHEEP BRAIN ACETYLCHOLINESTERASE BY MALATHION

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ABSTRACT

The *in vitro* effects of malathion, an organophosphorus insecticide on sheep brain acetylcholinesterase was studied in the present investigation. Malathion exerted a mixed type of inhibition especially showing a tendency towards competitive type of inhibition by a greater per cent increase in Km values and a lesser per cent decrease in V_{max} values. The activation energy values were found to increase, indicating decreased catalytic efficiency of the enzyme.

INTRODUCTION

MALATHION (O, O-dimethyl S-(1, 2-dicarboxyethyl), an organophosphorus insecticide is known to interrupt neural transmission by inhibiting the activity of acetylcholinesterase[1–3]. A decrease in vertebrate brain acetylcholinesterase was known to manifest in several behavioural and physiological modifications in the animal[4–5]. A detailed discussion on acetylcholinesterase has been reviewed by several investigators[6,7]. Since various possibilities exist for the inhibition of an enzyme catalysed reaction with its specific substrate, the present investigation is carried out to have a clear understanding of specificity of the enzyme and the kinetic mechanism involving the inhibition of acetylcholinesterase system in the tissues of mammals, during malathion stress.

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MATERIALS AND METHODS

Procurement of Material

Brains were obtained from healthy sheep after decapitation at the local slaughter house in a clean dry, ice jacketed glass beaker. They were quickly transferred to deep freeze and kept at -5 to $2°C$ in the laboratory until further use. The frozen brains were thawed with repeated washings in mammalian Ringer medium. Meninges over the cortical area were removed with care, so that the exposed cortical surface was free from blood vessels. Requisite amount of cortical tissue was taken, pressed gently between the folds of Whatman No. 1 filter paper and 10% homo-

genate (W/V) was prepared in cold sucrose 0·25 M for the enzyme assay.

The acetylcholinesterase (E.C. 3.1.1.7) activity was estimated by the modified method of Metcalf[8]. Experimental tubes received 200 μg of malathion (technical grade of 95% malathion obtained from Cyanamide India Ltd., Bombay) after due standardization in addition to the contents of reaction mixture, whereas the control tubes received distilled water in place of malathion. The activity levels of AchE in the brain homogenates with and without malathion (experimental and control respectively) were determined at 37° C at varying substrate concentrations (0·5 to 5 mM). The maximal velocities (V_{max}) and Michaelis-Menten constants (Km) were calculated by the method of least squares. The activation energy values were determined following the method of Dixon and Webb[9].

Results and Discussion

An enzyme concentration of 10 mg and 30 minutes of incubation time was selected for the present study after due standardization which ensured initial velocity of the enzyme catalysis. Absence of buffer, substrate and enzyme extract yielded negligible activity indicating specificity of reacting system for the enzyme. Substrate concentration *versus* enzyme activity (velocity) plots revealed that the enzyme activity was linear with substrate concentration upto 4 m Moles following a 1st order reaction and then onwards, the reaction phase entered zero order suggesting the termination of the substrate dependency or saturation of enzyme with the substrate. Lineweaver Burk plot for the AchE activity showed variation in the kinetic parameters like maximal velocity (V_{max}) and the Michaelis-Menten constant (Km). V_{max} value for the experimental enzyme showed a 10·9% decrease (Table I) in the presence of malathion which suggests a decrease in the active site density perhaps by masking some of the active sites. An increase of 113·6% was observed in the Km value of experimental enzyme suggesting decreased affinity of the enzyme for the substrate and also the decreased rate of breakdown of E-S complex. Since these are the two major factors which mainly contribute to the catalytic efficiency of the enzyme, this type of modulation in the kinetic parameters of enzyme activity indicates a mixed type of inhibition in which both V_{max} and Km are affected; and more distinctly in the present investigation, the per cent increase in Km is more than the per cent decrease in V_{max} showing a tendency towards a competitive type of inhibition as reported by several earlier investigators also in different species[3,10,11]. As the competitive inhibitors possess structural similarity with the substrates[12], the competitive inhibition of AchE elicited by malathion in the present investigation might be due to its virtual structural resemblance with the substrate, acetylcholine. This view is further strengthened by the earlier reports that the presence of Ach reduces the inhibitory effect of organophosphorus compounds on AchE[13] suggesting that the competition for the same binding site occurs between the organophosphate and acetylcholine compounds[14].

Table I

In vitro *effect of malathion on substrate dependent kinetics of AchE of sheep brain*

(V_{max} values are represented in μ moles of Ach hydrolyzed/mg protein/hr)

Sl. No.	Sample	Kinetic parameters	
		V_{max}	Km (mM)
1.	Control	30·3	0·909
2.	Experimental	27·0	1·942
	Per cent change	(−10·9)	(+113·6)

The activation energy required by an enzyme is the index of its catalytic efficiency, as the "enzymes enhance the reaction rates by lowering the activation energy[15]". Thus, lower the activation energy, higher is the efficiency of an enzyme and *vice versa*. The increased values of the activation energy, in the presence of malathion in the present findings (Table II), show that the activation energy barrier is increased by malathion suggesting the decreased catalytic efficiency of AchE.

Table II

In vitro *effect of malathion on activation energy of AchE of sheep brain*

(Values are expressed as calories/mole).

Sl. No.	Temperature range (° C)	Activation energy values	
		Control	Experimental
1.	20–25	15,758	22,446
	% change		+ 42·44
2.	25–30	11,313	16,213
	% change		+ 43·31
3.	30–37	4,791	10,135
	% change		+111·54

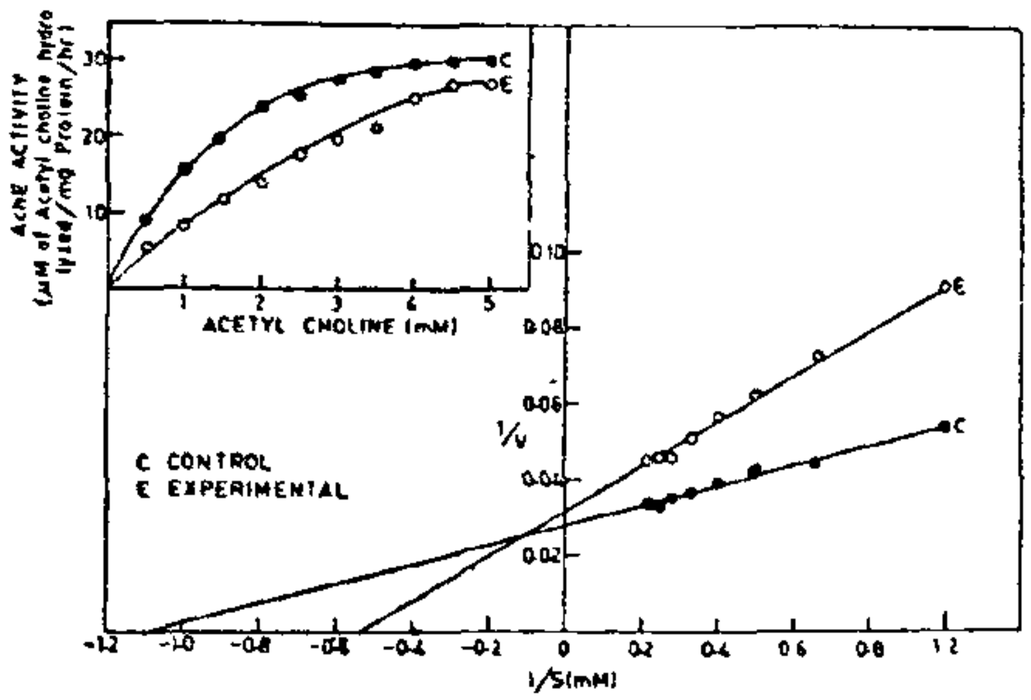

FIG. 1. Double reciprocal plots of substrate (Acetylcholine) *versus* velocity of AchE of control and experimental (malathion) brain homogenates at pH 7·2 along with the substrate *versus* activity curves.

From the above kinetic studies it may be presumed that the organophosphorus compounds exert a mixed type of inhibitory modulation on Acetylcholinesterase tending towards competitive type of inhibition and decrease the overall catalytic efficiency of the enzyme. Thus this type of inhibition elicited by malathion suggests that the inhibition by this pesticide is decreased during the availability of higher concentration of the substrate.

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AWARD OF RESEARCH DEGREES

Sri Venkateswara University, Tirupati, has awarded the Ph.D. degree in Chemistry to Shri G. Rama Krishna Naidu, Shri G. Siva Reddy and Shri V. S. Ramachandran; Ph.D. degree in Zoology to Shri K. R. Purushotham.

The Karnatak University, Dharwar, has awarded the Ph.D. degree in Physics to Shri G. Basavarajappa; Ph.D. degree in Chemistry to Shri S. T. Nandibewoor; Ph.D. degree in Mathematics to Shri B. A. Uralegaddi.

Kakatiya University, Warangal, has awarded the Ph.D. degree in Physics to Shri G. Ramachandra Reddy; Ph.D. degree in Chemistry to Shri G. Punnaiah, Shri S. Jagannatha Swamy; Ph.D. degree in Botany to Shri S. Rama Reddy.

The Maharaja Sayaji Rao University of Baroda has awarded the Ph.D. degree in Chemistry to Shri Babubhai Kalidas Patel; Ph.D. degree in Geology to Shri Toran Sharma; Ph.D. degree in Biochemistry to Shri N. Haridas; Ph.D. degree in Physics to Shri Jayant Ramanlal Otia and Shri Philip Mathew; Ph.D. degree in Geology to Shri Nikhilkumar Dhirajlal Desai; Ph.D. degree in Biochemistry to Shri P. E. Thomas.

Utkal University, Bhubaneswar, has awarded the Ph.D. degree in Botany to Shri B. Prakash Rao and Shri J. K. Johri.