Effects of Some Organophosphorous Compounds and their Metabolites on Sorghum-Grain Esterase and Certain Insects Attacking Sorghum Grain[1]

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Malathion (O,O-dimethyl dithiophosphate of diethyl mercaptosuccinate), because of its low toxicity on mammals, has practical significance in controlling insect pests in stored grain. Rowlands (1, 2), who studied the effect of malathion and its metabolic products on esterases of wheat grain, observed no inhibition of carboxylesterase. This investigation dealt with the inhibitory effects of malathion, parathion, diazinon, diisopropylphosphorofluoridate (DFP), and their metabolic products on esterases of sorghum grain, rice weevil (Sitophilus oryzae) and granary weevil (Sitophilus granarius), and horse-serum cholinesterase.

Experimental

Organophosphorous compounds. Malathion, malaoxon, K-demethyl malathion, malathion half-ester, malathion dicarboxylic acid, Na-dimethyl phosphate, K-dimethyl phosphorothioate, K-dimethyl phosphorodithioate and paraoxon were obtained from American Cyanamid Co., Princeton, N. J. Diazinon and diazoxon were obtained from Geigy Chemical Corporation, Ardsley, N. Y. and diisopropylphosphorofluoridate (DFP) from Mann Research Laboratory, Inc., N. Y.

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The organophosphorous compounds were dissolved separately in redistilled acetone, 10 ug/ul. However, DFP was used as 1% solution in deionized water.

Enzyme. Cholinesterase (horse serum) CHE 6254 was obtained from Worthington Biochemical Co., Freehold, N. J. It was dissolved in 0.1 M Tris buffer pH 8.0 for use.

Preparation and assaying sorghum grain crude homogenate. To each 40 g portion of sorghum grain, 100 ml ice-cold 0.1 M potassium acetate buffer of pH 5.5 were added. The mixture, placed in an omnimixer container and submerged in an ice bath, was homogenized by operating the omnimixer at top speed for 1 minute. The homogenate was then centrifuged at 10,000 x g for 30 minutes and the supernatant filtered through glass wool and kept cool until used. Esterase activity was assayed by colorimetric method modified after that of Kramer and Gamson (3). A mixture of 4.6 ml 0.1 M phosphate buffer (pH 8.0) and 0.1 ml of indophenyl acetate (1×10^{-3}M in 95% ethanol) in an 18-mm test tube was preincubated at 30°C for 5 minutes, and organophosphorous compounds were added before the addition of 0.1 ml enzyme solution. The reaction was run at the same temperature and the absorbance was measured with a Spectronic 20 spectrophotometer at 625 mμ. Absorbance at 625 mμ was found to be proportional to the amount of enzyme in the range of 0 to 0.5 A after 10 minutes of incubation. Further incubation, up to 120 minutes, did not increase the percentage of inhibition. Organophosphorous compounds were also added to the crude homogenates (3×10^{-5}M/0.1 ml) and preincubated for 10 minutes at 30°C before being assayed for esterase activity. Percentage of inhibition was defined as

$$\frac{A_{625} \text{ of control} - A_{625} \text{ of treated}}{A_{625} \text{ of control}} \times 100$$

Weevil homogenate preparation. Weevil homogenate was prepared by grinding in a cold mortar one gram of frozen insects of the same age with about 0.5 g of 60-mesh alundum (Fisher Scientific Co., New Jersey) and 5 ml of cold 0.1 M potassium acetate buffer (pH 4.0). The homogenate was centrifuged at 10,000 x g for 30 minutes. The supernatant solution was assayed by the indophenol acetate colorimetric method as described for the sorghum-grain homogenate.

Cholinesterase assay. Horse-serum cholinesterase was dissolved in 0.05 M Tris buffer, pH 8.0, and assayed by the method used for the sorghum-grain esterase.

Results

The effects of DFP, malathion, malaoxon, parathion, paraoxon, diazinon, and diazoxon on the esterases studied are given in Table 1.

All esterases were inhibited by paraoxon and DFP; the esterases of the weevils and the horse-serum cholinesterase were also susceptible to malathion and diazinon, respectively.

Because stored sorghum grain commonly is treated with malathion, the inhibitory effect of this compound and its possible metabolites was tested on esterases of the sorghum grain and on the rice and granary weevils. Horse-serum cholinesterase, presenting a mammalian source of esterase, was also used in the comparative study. The results, expressed as percentage of inhibition of indophenyl acetate hydrolysis, are compiled in Table 2. Paraoxon was used to insure positive inhibitory response from these esterase systems.

Our data showed that esterases from the weevils are significantly more sensitive to inactivation by malathion and its metabolites than are sorghum-grain esterase and horse-serum cholinesterase. However, weevils and horse-serum cholinesterases were more sensitive toward malaoxon than that of sorghum-grain esterase. A slight increase in esteratic activity was observed when sorghum-grain esterase was incubated with potassium dimethylphosphorothioate and with sodium dimethyl phosphate.

TABLE 1

Effects of some organophosphates on esterases[a]

Compound 3×10^{-5}M/5 ml of assay mixture	Sorghum-grain esterase	Rice & granary-weevil esterases	Cholinesterase (horse serum)
Malathion	-	+	-
Malaoxon	-	+++	+++
Parathion	-	+	++
Paraoxon	++	+++	+++
Diazinon	-	-	++
Diazoxon	-	++	+++
Diisopropylphosphoro-fluoridate (DFP)	++	++	+++

[a] All esterases were adjusted to approximately the same activity level, using indophenyl acetate as a substrate before adding to the assay medium containing the organophosphorous compounds: (-) refers to <10% inhibition; (+) to 10-30%; (++) to 30-70%; (+++) to >70% inhibition.

TABLE 2

Effects of malathion and its metabolites on esterases of sorghum grain, weevils and horse serum[a]

Compounds 3×10^{-5}M/0.1 ml of enzyme	Per cent inhibition		
	Sorghum-grain esterase	Weevil esterase	Cholinesterase (horse serum)
Malathion	0.5 ± 2.3[b]	27.2 ± 1.5	3.7 ± 1.1
Malaoxon	7.1 ± 2.8	80.3 ± 2.7	97.6 ± 1.1
Malathion half-ester	0.5 ± 1.4	49.4 ± 2.3	3.2 ± 1.0
Malathion dicarboxylic acid	5.4 ± 2.3	11.0 ± 2.0	4.4 ± 1.3
K-demethyl malathion	1.0 ± 2.3	48.8 ± 2.0	6.8 ± 2.1
K-dimethylphosphorothioate	7.6 ± 1.8[c]	19.1 ± 1.3	1.4 ± 1.0
K-dimethylphosphorodithioate	3.2 ± 3.4	36.0 ± 1.7	1.9 ± 1.6
Na-dimethyl phosphate	1.0 ± 1.6[c]	7.7 ± 1.7	3.0 ± 2.0
Paraoxon	66.6 ± 2.4	96.4 ± 2.5	97.6 ± 1.5

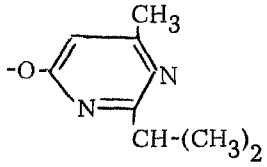
[a] Enzyme was preincubated with organophosphorous compounds for 10 min at 30°C.

[b] Standard deviation.

[c] Per cent activation = $\frac{A_{625} \text{ of treated} - A_{625} \text{ of control}}{A_{625} \text{ of control}} \times 100.$

Discussion

The inhibition of sorghum-grain esterase by various organophosphorous compounds indicated specificity toward paraoxon and DFP; but not toward malathion and its related dithioates, malaoxon, parathion, diazinon, and diazoxon. The weevil esterase and horse-serum cholinesterase, however, were inhibited by paraoxon, DFP, malaoxon and diazoxon under similar conditions, suggesting that diazoxon and malaoxon failed to approach or interact with the active site of the sorghum-grain esterase. That failure might have been the result of the bulky



group of diazoxon and $-S-\underset{H}{C}-(\overset{O}{\overset{\|}{C}}OC_2H_5)_2$ group of malaoxon

(4); or these compounds could have been hydrolyzed by other hydrolases in the sorghum-grain homogenate. Such an effect being observed when the homogenate and the organophosphorous compounds were added simultaneously to the assay mixtures. If these compounds had acted as inhibitors, the initial hydrolysis rate of acetate should have been lowered, because the hydrolases and the esterase competed for the organophosphates. But that effect was not observed. Parathion, malathion and diazinon were less active than malaoxon and diazoxon, owing to the lower electrophilicity of =S compared with =O. The observed slight inhibitory effect might have been due to impurities, in that commercial malathion and parathion which contained about 1% of malaoxon and of paraoxon, respectively, as verified by gas-liquid chromatography. Such specificity of interaction between the esterases and the organophosphorous compounds could be useful in developing selective insecticides.

The failure of malathion and its metabolites to inhibit sorghum-grain esterase does not exclude the possible interaction of these compounds with sites other than the active site of the enzyme protein. Slight enzyme activity when those compounds were added might have been a net result of such allosteric binding. Under present methods of residue analysis of organophosphates seldomly account for residues stably bound to proteins, thus becoming insoluble.

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