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SHORT COMMUNICATIONS

Effects of two biodegradable 1,1,1-trichloro-2,2-bis (*p*-chlorophenyl)ethane (DDT) analogs on cockroach ATPases*

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The precise action of p,p,'-DDT† at the molecular level remains unclear [1], and evidence for its effect on nerve axons [2,3] apparently does not relate to its inhibitory effect on Mg^{2+} ATPases found in several studies [4–7]. Yet, symptomatology in treated insects is neuromuscular, with prolonged muscle tumours as well as trains of increased action potentials in nerves [8]. Related to these effects is the evidence that mitochondrial Mg^{2+} ATPase, an energy-regulating portion of the ATPase system [9,10], is inhibited by several DDT analogs having various structural configurations. The importance of studying the effects of analogs lies in the fact that evidence may be assembled from discrete structural alterations to provide an analysis of adverse biochemical effects at a more precise molecular level. In addition, a possible guide for synthesis of biodegradable analogs of DDT may result.

Our previous reports were on two asymmetrical biodegradable analogs of DDT having the aliphatic moiety of the molecule identical to p,p'-DDT [4], alterations being on the aromatic portion. The effects on the ATPases were less pronounced than with DDT, but qualitatively similar in that oligomycinsensitive (O-S) Mg^{2+} ATPase was inhibited to the greatest extent. The present study compares two additional analogs having structural alterations in the aliphatic moieties *only*. These modifications have been important considerations in describing the mode of action, and will be presented in the discussion.

Both compounds are insecticidally active but have low mammalian toxicity [11,12]. One, monochlorinated, is 1,1-bis(*p*-ethoxyphenyl)-2-chloropropane. The second, non-chlorinated, is 1,1-bis(*p*-ethoxyphenyl)-2-methylpropane. Their structure–activity relationships have been studied with several species [11,12].

The ATPase enzyme from muscle of male American cockroaches was prepared by the procedure of Koch [13]. Brains of both sexes were frozen in liquid nitrogen and stored for a few days before being homogenized and subjected to appropriate centrifugation procedures for isolation of mitochondria. Tissues once frozen averaged somewhat higher (10–20 per cent) in total ATPase activity than fresh preparations, but had consistent activity values. Enzyme activity was determined according to the continuous procedure described by Pullman *et al.* [14] and Fritz and Hamrick [15]. Protein determination was by the method of Lowry *et al.* [16].

A 3-ml reaction mixture employed for the enzyme assay consisted of 100 mM Na^+, 20 mM K^+, 5 mM Mg^{2+}, 135 mM imidazole buffer (pH 8.5), 4.3 mM ATP, 0.14 mM NADH, 0.5 mM phosphoenol pyruvate, 0.02% bovine serum albumin, approximately 9 units of pyruvate kinase, 12 units of lactic dehydrogenase and 100 μl of enzyme preparation. The protein content was between 15 and 20 μg/100 μl homogenate. Absorbance changes were measured at 340 nm using a Beckman DU spectrophotometer provided with thermostatic control.

Total ATPase activity was measured with Mg^{2+}, Na^+ and K^+ in the reaction mixture. Mg^{2+} ATPase was measured using 1 mM ouabain in the reaction mixture, the latter being a specific inhibitor of Na^+–K^+ATPase [17]. Na^+–K^+ATPase activity is total activity minus Mg^{2+}ATPase activity. Mg^{2+} was further differentiated into oligomycin-sensitive and oligomycin-insensitive portions by adding 1 μl of an ethanol solution of oligomycin (0.03 μg) per ml of reaction mixture [18]. The oligomycin-sensitive portion is designated as mitochondrial Mg^{2+}ATPase, or O-S Mg^{2+}ATPase.

The compounds, [A], 1,1-bis(*p*-ethoxyphenyl)-2-chloropropane, and [B], 1,1-bis(*p*-ethoxyphenyl)-2-methylpropane, were dissolved in ethanol, using 1 μl of solution in 3 ml of the reaction mixture for the ATPase test, and agitated immediately. Ethanol had no effect on the enzyme system at this concentration.

Inhibiton was determined at pH 8.5, the optimum for Mg^{2+}ATPase [19]. Specific activity values for Na^{2+}ATPase were approximately 30 per cent lower than at pH 7.5, but inhibition values could be determined accurately.

Both DDT analogs, like DDT, reduced the specific activity of O-S Mg^{2+}ATPase in brain and muscle homogenates of the American cockroach. In brain homogenates, 50 per cent reduction was obtained with a 6.7 μM concentration of compound A and a 9.7 μM concentration of compound B, a non-significant difference (Fig. 1). The enzyme sensitivity was eight to ten times greater to DDT, the I_{50} being 0.84 mM (Fig. 2).

O-S Mg^{2+}ATPase has a higher specific activity in muscle homogenates, and its inhibition in coxal muscle by DDT has generally been greater than in nervous tissue [4]. Compound B was a stronger inhibitor in muscle (I_{50} = 2.25) (Table 1) than in brain preparations (I_{50} = 9.7) (Fig. 1) and was more effective than compound A with muscle homogenates. Inhibition by both compounds at 17° was not significantly different than at 37° (Table 1). DDT has been shown to be more effective at the lower temperature using muscle homogenates [5], and a similar negative temperature effect was determined for brain homogenates in this study (Fig. 2). The I_{50} values were 0.27 mM and 0.84 mM at 17° and 37° respectively.

Oligomycin-insensitive Mg^{2+}ATPase from muscle was far less sensitive than O-S Mg^{2+}ATPase to all three compounds. The greatest effect with the analogs occurred with compound B at 17°, which gave 50 per cent inhibition at slightly less than 10 μM. Compound A had an insignificant effect on oligomycin-insensitive Mg^{2+}ATPase at both temperatures. Results are given for 17° in Table 2; the compounds were ineffective at 37°. The specific activities of the controls (untreated) were lower in comparisons using Compound B (Tables 1 and 2). The reason for this is not clear except that they were done at an earlier time with a different sample of BSA (bovine serum albumin) which was used to standardize the protein determinations.

The results provide further evidence for DDT and its

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† DDT = 1,1,1-trichloro-2,2-bis(*p*-chorophenyl)ethane.

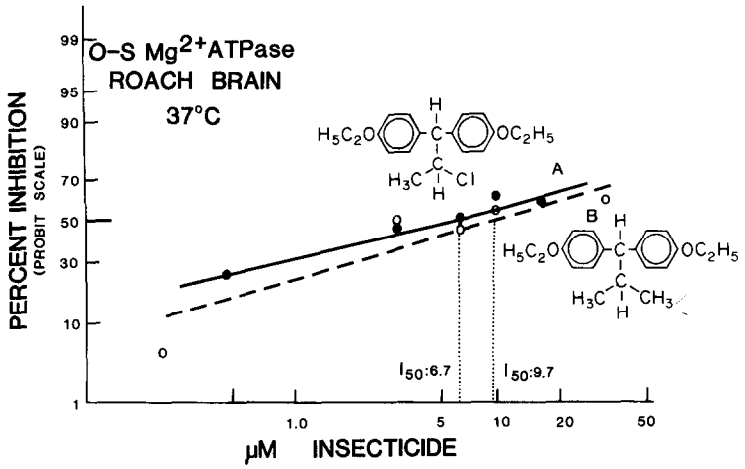

Fig. 1. Comparative inhibition of O-S Mg^{2+}ATPase from American cockroach brain by compounds A and B at 37° and pH 8.5. Specific activity values of controls (untreated) were 29.07 ± 2.75 and 37.59 ± 0.77 μmoles P_i mg^{-1} Protein hr^{-1} respectively. I_{50} values did not differ significantly.

analogs acting most precisely on mitochondrial Mg^{2+}ATPase at nanomolar concentrations, and having no significant effect on Na^+–K^+ATPase. This qualitative similarity of effect is distinctly different from less specific actions of cyclodiene compounds such as aldrin, dieldrin, isodrin and endrin [7], and the chlorinated compound lindane.

The effectiveness of the compounds on O-S Mg^{2+}ATPase is less than that of p,p'-DDT and overall the effectiveness of compounds A and B (symmetrical, but with altered aliphatic moieties) in the present study was less than that of analogs 1 and 2 [4] in which asymmetrical molecules with aliphatic moieties identical to DDT were evaluated. The differences are small, however, and one cannot, without additional compounds, arrive at an explanation of differences in configuration which might support the concept, involving electron-withdrawing groups as aromatic substituents, discussed by Holan [20] and Metcalf [21].

Two additional comparative areas deserve mention. Toxicity values, more complete on the mosquito, *Culex fatigans*, also show small differences, with DDT and compound B being slightly superior. The present enzyme study also showed a somewhat greater effect from compound B.

In support of the importance of the enzymatic effects as contrasted or related to neurotoxic effects [2,3], one must keep in mind that insect symptoms produced by DDT and analogs include tremors with a neuromuscular pattern. Such disturbances could certainly be the result of an imbalance of mitochondrial Mg^{2+}ATPase involved in energy regulation in muscle tissues and does not rule out adverse neurophysiological effects.

The two symmetrical biodegradable analogs of DDT, nearly equal in insecticidal activity, produced qualitatively

Table 1. Per cent inhibition of O-S Mg^{2+}ATPase in American cockroach coxal muscle tissue homogenates by DDT analogs A and B*

	Per cent inhibition of O-S Mg^{2+}ATPase			
Conc.	A		B	
(μM)	17°	37°	17°	37°
0.25	14.9 ± 2.33	28.3 ± 1.19		
0.50			43.0 ± 3.5	
1.75	38.4 ± 2.8		41.9 ± 3.5	45.1 ± 2.6
3.30	45.4 ± 2.9	41.6 ± 1.89	60.5 ± 0.5	52.3 ± 3.0
6.67	45.7 ± 11.4	43.9 ± 5.11	64.1 ± 1.9	67.3 ± 2.5
10.00			66.5 ± 3.0	76.7 ± 2.8
16.17	50.5 ± 1.78	47.9 ± 4.08		
33.00	59.2 ± 14.3	45.1 ± 4.33		
I_{50}	11.4	>33.0	1.90 (0.89 – 3.10)	2.25 (1.15 – 3.24)
Sp. act.	13. 16 ± 0.74	152.74 ± 5.17	6.88 ± 2.8	61.9 ± 3.4

* Tested at pH 8.5 and temperatures of 17° and 37°. Specific activity = μmoles P_i mg^{-1} protein hr^{-1}.

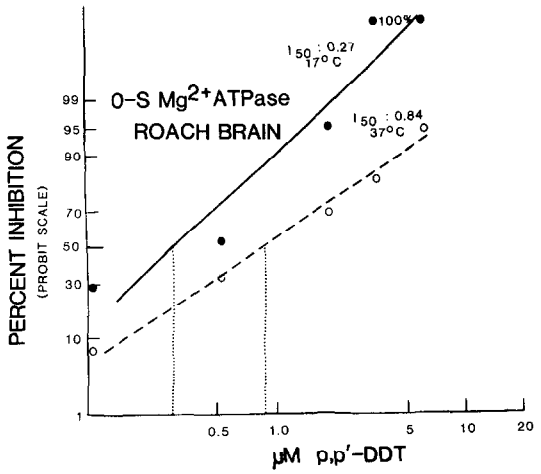

Fig. 2. Per cent inhibition of O-S Mg^{2+}ATPase from American cockroach brain by p,p'-DDT at two temperatures, 17° and 37° and at pH 8.5. Specific activities of controls were 2.34 ± 0.19 (17°) and 29.07 ± 2.75 (37°) μmoles P_i mg^{-1} protein hr^{-1} respectively. Differences in inhibition were statistically significant at the I_{50} and I_{95} points.

similar effects on the ATPase enzyme system. They were, however, about ten times less effective, and did not have a greater effect at a temperature of 17° than DDT. The most effective of the compounds was non-chlorinated, supporting the importance of considering structural configuration in analyzing modes of action.

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Table 2. Per cent inhibition of oligomycin-insensitive Mg^{2+}ATPase in American cockroach muscle tissue homogenates by DDT analogs A and B*

Conc (μM)	Percent inhibition oligomycin-insensitive Mg^{2+}ATPase	
	Coxal muscle	
	A	B
0.3	4.76 ± 1.73	
0.5		10.83 ± 3.23
1.7		22.40 ± 1.82
3.3	18.78 ± 3.68	33.21 ± 2.76
6.7	12.38 ± 6.09	44.67 ± 8.70
10.0		71.81 ± 13.90
16.7	29.25 ± 10.24	74.84 ± 12.18
33.0	32.11 ± 3.1	
Sp. act.	7.35 ± 0.36	2.53 ± 0.94

* Tested at pH 8.5 and at 17°. Specific activity = μmoles P_i mg^{-1} protein in hr^{-1}.

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