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INHIBITION OF *ESCHERICHIA COLI* Mg^{2+} ATPase

SYNERGISM BETWEEN A FIRE ANT, *SOLENOPSIS RICHTERI* (FOREL) ABDOMEN FACTOR AND A PHOTOREDUCTION PRODUCT OF MIREX*

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Summary

Fire ant, *Solenopsis richteri* (Forel), abdomen was found to contain a water-soluble and heat-stable inhibitor of the ATPase activity in an E-*Escherichia coli* membrane preparation. A photoreduction product of Mirex was also inhibitory toward *Escherichia coli* Mg^{2+} ATPase, but was less effective in total enzyme activity inhibition than the fire ant inhibitor. However, the two compounds were found to be strongly synergistic in their inhibitory action. Surprisingly, Mirex had little or no effect on the bacterial membrane enzyme activity.

Organochlorine pesticides have been shown to be inhibitors of ATPases in vertebrate and invertebrate animals [1—6]. Recently, it has been shown that mitochondrial (oligomycin-sensitive) Mg^{2+} ATPase was more sensitive to DDT and its analogues than Na^+—K^+ and oligomycin-insensitive Mg^{2+} ATPases. The above findings have led us to propose the inhibition of ATPases as a possible mechanism of toxicity for organochlorine pesticides [7]. However, Mirex, which is an organochlorine compound, did not show any effect in vitro on this enzyme system in fish and in fire ants**, although it is toxic to imported fire ants [8], to house flies [9], and to crayfish [10]. This observation prompted us to study the effects of Mirex and its photoreduction prod-

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ucts on fire ant ATPases. Our preliminary results* showed that a photoreduction product of Mirex inhibited the oligomycin-insensitive Mg^{2+} ATPase system from fire ant head homogenate.

In studies on various parts of the fire ant, we observed that the abdomen homogenate had no ATPase activity. The whole body homogenate also had little or no ATPase activity, but head homogenate had the expected ATPase activities*. This suggested the presence of an endogenous inhibitor of ATPase activity in the abdomen homogenate.

A sample of a bacterial membrane preparation was obtained which contained a single type of ATPase activity rather than the three types of activity normally found in animal tissue preparations [1—6]. The sample of *E. coli* cytoplasmic membrane vesicles (prepared according to the method of Kaback [11]) was kindly supplied by Dr E. Thomas, St. Jude Children's Hospital, Memphis, Tenn. The *E. coli* preparation was quick frozen in liquid nitrogen and stored at −20°C. The samples were thawed and used in the assay. Quick freezing apparently ruptured the vesicles and yielded an enzyme active preparation which gave good reproducible results in our procedure for measuring Mg^{2+} ATPase activity. It was found to have only oligomycin-insensitive Mg^{2+} ATPase activity. Thus, this sample provided a convenient source of material for studying the effects of a photoreduction product of Mirex (1,2,3,4,6,7,8, 9,10,10-undecachloropentacyclo [5.3.0.0.^{2,6}.0^{3,9}.0^{4,8}]decane) and the unknown inhibitor from fire ant abdomen on a single type of enzyme activity.

Fire ants, *Solenopsis richteri* (Forel) from the fields around Mississippi State University, were collected and maintained in the laboratory. The abdomens of ants were removed and homogenized in a ground-glass homogenizer and fractionated [12]. The sediment obtained at 13 000 × *g* was resuspended (sediment from 200 abdomen per 2 ml) in 0.32 M sucrose solution containing 10 mM imidazole—Cl, pH 7.5, 1 mM EDTA. Portions of this preparation were added to the reaction mixture for inhibition studies.

Protein concentration of the *E. coli* preparation was measured by the method of Lowry et al. [13]. ATPase activity was measured by a continuous method described by Pullman et al. [14] and Fritz and Hamrick [15]. Absorbance was measured at 340 nm using a Gilford 2400 spectrophotometer with automatic recorder. The temperature of the cuvettes was held at 37°C. The photoreduction product of Mirex (P-M) and Mirex were dissolved in acetone and the desired volumes were injected below the surface of the reaction mixture being rapidly stirred on a Vortex Junior mixer. The *E. coli* membrane ATPase activity was not sensitive to ouabain in the presence of Na^+ and K^+ and was not sensitive to oligomycin.

The results in Table I show that 1 to 2 μl of the fire ant abdomen fraction alone had little or no effect on *E. coli* Mg^{2+} ATPase activity, but that further progressive increase in fire ant abdomen fraction addition caused a progressive increase in inhibition of the enzyme activity. Also, P-M when used alone at 1 μM concentration had little or no effect on Mg^{2+} ATPase activity (Table I), but further addition of P-M caused definite inhibition (20

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TABLE I

RESPONSE OF *E. COLI* MEMBRANE ATPase ACTIVITY* TO FIRE ANT ABDOMEN FRACTION, PHOTOREDUCTION PRODUCT OF MIREX (P—M), AND MIREX

Amount	Abdomen fraction**		Concn	P-M***		Concn	Mirex†	
(μl)	Spec. Act.††	% Inhibn	(μM)	Spec. Act.	% Inhibn	(μM)	Spec. Act.	% Inhibn
0	48.2							
1	47.8	0.7	1	47.6	1.1	5	44.5	7.7
2	49.4	+2.5	5	42.2	12.4	10	44.3	8.0
5	44.8	6.9	10	34.9	27.4			
10	42.4	12.0	20	30.2	37.3			
25	34.4	28.6						
50	22.1	54.2						
100	14.3	70.3						
200	11.1	76.9						

* A 3 ml reaction mixture contained: 4.3 mM ATP, 5 mM Mg^{2+}, 100 mM Na^+, 20 mM K^+, 120 mM imidazole buffer (pH 7.5), 0.19 mM NADH, 0.5 mM phosphoenol pyruvate, 0.02% bovine serum albumin, 9 units of pyruvate kinase, 12 units of lactate dehydrogenase, and 100 μl of membrane preparation (0.16 mg/ml protein). 1 mM ouabain was used to differentiate Na^+—K^+ and Mg^{2+} ATPases from total ATPase activity. Oligomycin (0.15 μg/ml reaction mixture) was used to delineate oligomycin-sensitive (mitochondrial) and insensitive Mg^{2+} ATPases. Additional studies using the above reaction mixture minus the Na^+, K^+, and ouabain gave essentially identical results. The abdomen fraction did not show any inhibition of the ADP measuring system pyruvate kinase, lactate dehydrogenase in the reaction mixture.

** Fire ant abdomen fraction obtained from 13 000 × *g* sediment.

*** P-M, Photoreduction product of Mirex-undecachloropentacyclo [5.3.0.0.^{2,6}.0^{3,9}.0^{4,8}] decane.

† Mirex, dodecachloropentacyclo [5.3.0.0^{2,6}.0^{3,9}.0^{4,8}] decane.

†† Spec. Act. = μmoles $P_i \cdot mg^{-1}$ protein$\cdot h^{-1}$. Untreated specific activity of *E. coli* membrane ATPase replicated 13 times, most replicate values agreed within 4% of the average. Inhibition values, for the most part, represent at least averages of duplicate determinations.

μM was the highest concentration that could be used in our test procedure). Mirex had little or no effect on the enzyme activity (Table I). This is borne out in Table II which shows that the inhibition values for the abdomen fraction—Mirex combination were substantially the same as the values obtained for abdomen fraction used alone (compare with 5,10,50 μl abdomen fraction, see Table I).

However, the combination of abdomen fraction and P-M were synergistic in their inhibition of *E. coli* Mg^{2+} ATPase at least up to the 50 μl abdomen fraction—10 μM P-M combination (Table II) compared to individual values for the above additions (Table I). The most dramatic evidence of the synergism between abdomen fraction and P-M is shown for 1 μl and 1 μM addition, respectively. This combination (Table II) showed 26.7% inhibition, while the two components added separately at equivalent levels (Table I) had little or no inhibitory action.

The results in Table III show that the fire ant inhibitor was heat-stable. Both unheated and heated samples of abdomen fraction at two concentrations gave identical inhibition of *E. coli* membrane ATPase activity.

The data in Table IV shows that abdomen fraction extracts treated with proteolytic enzymes (papain, chymotrypsin, and trypsin) have reduced inhibitory activity on *E. coli* membrane ATPase activity. Among the three enzymes tested, trypsin was found to be more effective (Table IV).

TABLE II

RESPONSE OF *E. COLI* ATPase ACTIVITY TO A COMBINATION OF FIRE ANT ABDOMEN FRACTION, AND PHOTOREDUCTION PRODUCT OF MIREX (P-M), AND MIREX

See the footnotes to Table I for details

Amount abdomen fraction (μl)		Concn P-M (μM)	Spec. Act.	% Inhibn	Amount abdomen fraction (μl)		Concn Mirex (μM)	Spec. Act.	% Inhibn
0			48.2						
1	+	10	28.9	39.9	1	+	10	50.8	+5.5
5	+	10	21.2	56.0	5	+	10	44.6	7.3
10	+	10	19.2	60.0	10	+	10	42.4	12.0
25	+	10	14.1	70.7	50	+	10	19.4	59.7
50	+	10	11.3	76.6					
100	+	10	11.7	75.7					
1	+	1	35.3	26.7					
2	+	1	33.0	31.5					
10	+	1	26.5	45.0					
10	+	5	21.9	54.6					
10	+	10	19.2	60.0					

Our results demonstrate that the factor(s) present in the fire ant abdomen inhibits the membrane ATPase activity of *E. coli*. The results further show that this water-soluble factor of fire ant abdomen enhances the inhibition produced by the highly non-polar photoreduction product of Mirex. However, the inhibition of *E. coli* ATPase by the fire ant abdomen fraction was not increased or decreased in the presence of Mirex. Our present efforts are being directed toward the isolation and identification of the fire ant ATPase inhibitor component.

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TABLE III

RESPONSE OF *E. COLI* MEMBRANE ATPase ACTIVITY TO UNHEATED AND HEATED FIRE ANT ABDOMEN FRACTION

See the footnotes to Table I for details

Abdomen fraction Amount (μl)	Condition	Spec. Act.	% Inhibition
0	—	48.2	—
50	unheated	22.1	54.2
50	heated*	22.8	52.8
100	unheated	14.3	70.3
100	heated	14.5	69.9

* Fire ant abdomen fraction extract was heated in a boiling water bath for 3 min. The heated extract was cooled to 37°C and filtered.

TABLE IV

RESPONSE OF *E. COLI* MEMBRANE ATPase ACTIVITY TO FIRE ANT ABDOMEN FRACTION TREATED WITH PROTEOLYTIC ENZYMES

Abdomen fraction Amount (μl)	Proteolytic Enzymes*	Spec. Act. (Remaining)	% Inhibition from the control
0	0	48.2	
100	0	14.3	70.3
200	0	11.1	76.9
150	papain	25.2	47.6
150	chymotrypsin	19.6	59.3
150	trypsin	28.9	39.9

* 150 μl of fire ant abdomen fraction extract was added to reaction mixtures (without ATP, pyruvate kinase, lactate dehydrogenase, and bovine serum albumin). To these reaction mixtures (pH 7.5) papain (10 μl of 26.7 mg/ml), chymotrypsin (1 mg/reaction mixture), and trypsin (1 mg/reaction mixture) were added, set for about 10 min, and heated in boiling water bath for 3 min. The reaction mixture was cooled to 37°C and ATP, pyruvate kinase, lactate dehydrogenase, and bovine serum albumin, were added.

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