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Fatty Acids and Phospholipids of Membranes Isolated from *Escherichia coli* Growing in a Medium with Parathion

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A number of lipid soluble pesticides is widely spread in our environment. The investigation of the interrelationships between these organic chemicals and non-target microorganisms have increased in recent years. Reviews have been published discussing the environmental fate of pesticides and the role microorganisms play in their degradation. Several bacterial enzymes capable of degrading a variety of pesticides have been described (Johnson & Talbot 1983). However, little is known about the effects of pesticides and their degradation products on the chemical composition of microbial membranes. The available data are consistent with the idea that most of the pesticides interact presumably with the bacterial membrane (Antunes-Madeira & Antunes-Madeira 1979). We had observed that cells of Escherichia coli contained an accumulation of phospholipids and an increased saturated fatty acid composition when four insecticides were added to the growth media (Rosas et al.1980). The purpose of the present study was to examine the lipidic composition of membranes isolated from E.coli growing in a medium with the insecticide parathion. The phosphorothioate insecticide chosen is normally short-lived in soil, but can be detected in some soils after sixteen years (Alexander 1977).

MATERIALS AND METHODS

E.coli RC424 cells were grown in a minimal medium containing, in grams per liter: KH_2PO_4,3; Na_2HPO_4,6; NH_4Cl,1; $MgSO_4$,0.2; and glucose,4 (pH 6.8).

Stock solution of parathion (O,O-diethyl O-4-nitrophenyl phosphorothioate) was prepared in absolute ethanol and diluted 1000 fold by volume in the basal medium to give a final concentration of 8.6 µM. The low concentration chosen is representative of pesticide pollution in soil water (Cook et al.1983).

The control had the equivalent volume of absolute ethanol.

After 5 h of incubation at 37°C , the cells were harvested at the exponential phase (Rosas et al.1980), concentrated and washed with 0.89% NaCl. Membranes were isolated using the method detailed by Evans (1969) For dry weight determination of the membranes 4 ml of the membrane suspension was lyophilized for 24 h. Proteins were evaluated as Lowry et al. (1959). Membrane lipids were extracted by the Ames (1968) modification of the Bligh & Dyer (1959) technique, and subjected to thin-layer chromatography on Silica Gel H plates using the previously reported procedures (Rosas et al.1980). Total lipid phosphorus and individual phospholipids were determined as Dodge & Phillips (1967). The methyl esters of the fatty acids were prepared with the Boron trifluoride-methanol reagent, examined in a Varian 2100 gas chromatograph, identified and evaluated as Rosas et al. (1980).

Parathion and p-nitrophenol were extracted from the broth medium and then analyzed by thin-layer chromatography by the technique of Sethunathan (1973). Spots were located by ferric chloride spray and identified by comparison with standards which were spotted alongside samples on the plate.

RESULTS AND DISCUSSION

We have already demonstrated (Rosas et al.1980) that E.coli RC424 cultivated in a medium with 8.6 µM parathion for 5 h at 37°C achieved the exponential phase of growth. In this study, E.coli RC424 was tested for its ability to degrade parathion. We observed that complete hydrolysis of parathion to p-nitrophenol occurred within 5 h of incubation. It is well known that hydrolases are the most important enzymes in bacterial catabolism of organophosphates. Christensen (1976) reported that the hydrolysis of parathion to p-nitrophenol leaded to 22-fold decrease in overall toxicity to rats.

In an attempt to know if cells that were grown on parathion had altered the lipidic composition of their membranes, fatty acids, phospholipids, and proteins were determined in membranes isolated from E.coli at 5 h of growth. Membrane fatty acid profiles of cells that have degraded parathion were quite similar to controls (Table1). The data suggest that membrane fluidity was maintained.

On the other hand, when calculated on a dry weight

Table 1. Fatty acids of membranes isolated from E.coli at the exponential phase of growth with 8.6 µM parathion.

Fatty acid	Control	Parathion
14:0	2.99 ± 0.60	3.01 ± 0.70
15:0	0.23 ± 0.04	0.14 ± 0.01
16:0	40.47 ± 3.64	42.21 ± 1.87
17:0Δ	1.14 ± 0.16	1.18 ± 0.30
16:1	16.31 ± 2.61	12.76 ± 1.37
18:0	1.09 ± 0.26	not detected
N.I.	0.79 ± 0.40	0.83 ± 0.30
18:1	28.06 ± 1.55	27.27 ± 1.46
N.I.	7.07 ± 1.10	7.27 ± 1.02
19:0Δ	1.89 ± 0.60	2.20 ± 0.80
N.I.	0.89 ± 0.04	0.62 ± 0.03
Saturated	0.95	1.06
Not saturated		

Values are reported as % of total fatty acids. They are the means ± standard errors of five determinations Fatty acids: Myristic 14:0; Methyl-myristic 15:0; Palmitic 16:0; Palmitoleic 16:1; cis-9,10-Methylene-hexadecanoic 17:0Δ; Stearic 18:0; cis-Vaccenic 18:1; Lactobacillic 19:0Δ; Unidentified N.I.

basis, quantitative differences existed in the amount of membrane total lipid phosphorus between controls and cells capable of hydrolyzing parathion (Table 2). Protein concentration did not change, consequently the ratio between phospholipids and proteins increased in membranes of E.coli that have degraded parathion. The accumulation of phospholipids in membranes was due to two-fold increases in both phosphatidyl-ethanolamine (PE) and phosphatidyl-glycerol (PG) contents, accompanied by a three-fold increase in cardiolipin (CL) concentration (Table 3) . In addition, an unidentified phospholipid was present only in controls. When individual phospholipid percentages were calculated, there were increased amounts of PE and CL noticed, and a decreased percentage of phosphatidyl-serine (PS) Nevertheless, PE remained the major phospholipid present in both membrane systems. The ratio among phospholipids synthetized from the liponucleotide cytidine-5'diphosphate-diglyceride (CDP-DG) by separate branchs, was diminished in membranes isolated from cells that were grown in parathion (Table 3). A similar decrease was reported in mutants of E.coli resistant to organic solvents (Clark & Beard 1979).

Table 2. Phospholipids and proteins of membranes isolated from E.coli at the exponential phase of growth with 8.6 µM parathion.

mg/g dry membrane	Control	Parathion
Lipid phosphorus (L)	0.435 ± 0.016	0.894 ± 0.013
Protein (P)	250.1 ± 9.3	253.0 ± 7.2
L/P x 1000	1.81	3.53

Values reported are the means of six experiments ± standard errors.

Table 3. Phospholipidic composition of membranes isolated from E.coli at the exponential phase of growth with 8.6 µM parathion.

Phospho-lipid	Control (")	%	Parathion (")	%
PS	15.27 ± 2.86	27	14.03 ± 0.67	13
PE	31.75 ± 0.87	57	71.12 ± 1.56	67
PG	2.92 ± 0.16	5	5.91 ± 0.30	5
CL	4.48 ± 0.30	8	15.79 ± 0.98	15
NI	1.45 ± 0.14	3	not detectable	
$\frac{PS + PE}{PG + CL}$	6.3		4.0	

(") Results presented as µmole phospholipid / g dry weight of membranes. Data are means ± standard errors from five experiments. PS: phosphatidyl-serine; PE: phosphatidyl-ethanolamine; PG: phosphatidyl-glycerol; CL:cardiolipin; NI:unidentified phospholipid

All the changes induced by the presence of parathion and p-nitrophenol in the growth medium, resulted in a membrane lipid composition that was significantly different from that which existed in control E.coli. Our observation of similar fatty acid but different base profiles of the phospholipids in membranes of cells that have degraded parathion and membranes of controls, leaves open the possibility for differences in the physical chemistry of the two membrane systems. It was demonstrated (Esfahani et al.1977)that critical phospholipid concentrations were specifically required for the expression of membrane-associated functions such as transport and enzymatic activity. Therefore, the reported modifications in individual phospholipid

content could be expected to alter important membrane functions. Whether the observed changes were due to effects of parathion and p-nitrophenol on synthesis or degradation of phospholipid species remain to be clarified by further biochemical and genetic studies.

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