X-Ray Studies on Phospholipid Bilayers. V. Interactions with DDT

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The possible interaction of DDT with the lipids dimyristoyl lecithin (DML), dipalmitoylphosphatidylethanolamine (DPPE) and tripalmitin (TP) was studied. The work was carried out on oriented films and crystalline powders of DDT-lipid mixtures at different molar ratios by X-ray diffraction techniques. The diagrams showed only the patterns of pure DDT and that of the corresponding lipid. It is concluded that new phases were not formed and, therefore, no interactions occurred.

Introduction

DDT (1,1,1-trichloro-2,2-bis-(*p*-chlorophenyl)-ethane) is a pesticide which has been widely used as aid in food production. Nevertheless, because of its low biodegradability and its tendency to accumulate, it finally finds its way to human cells through the food chain. Several toxic effects such as hyperexcitability, tremors, convulsions and paralysis have been reported [1]. However, the molecular mechanisms of its toxicity are still unknown. Several investigations have implied that DDT modifies basic membrane mechanism, *e.g.*, permeability to non-electrolytes and transport of cations mediated by ionophores [2–4]. It has been suggested that DDT, because of its lipophillic character, does not act directly on the active sites of the enzymes involved but alters the lipid phase of the membranes which in turn would interfere with the allosteric transitions of the AT-Pases [5].

Information on the interaction of DDT with phospholipids is sparse and contradictory. It has been found by NMR techniques that they interact in chloroform solution, being involved the phosphorylcholine moiety of the lecithin and the benzylic proton of DDT [6]. Another report [7] showed that DDT was barely taken up in the apolar interior of lipid vesicles and, consequently, it could not affect the NMR signals. Contrasting conclusions have also been reported about a "fluidization" effect of the lipid bilayers caused by DDT, which might occur in artificial membranes [4, 8–10] – where DDT would intercalate between the acyl chains [11] – but not in the natural membranes [12]. On the other hand another paper reports that DDT does not appear to disrupt the bilayer structures of dioleoyl – and dipalmitoyl lecithin vesicles [13].

In an attempt to solve these controversies and because of the interest of the subject itself, it was decided to study the possible interaction of DDT with phospholipid bilayers by X-ray fiber and powder diffraction techniques. This was thought to be quite feasible as the molecular structure and packing arrangement of several phospholipid bilayers had already been determined in our lab [14–17]. The possible interaction of DDT with tripalmitin, a long-chain triglyceride, water insoluble neutral lipid usually found in the adipose tissue of animals and plants, where DDT tends to deposit, was also studied.

Materials and Methods

Synthetic L-α-dipalmitoylphosphatidyl-ethanolamine (DPPE) A Grade from Calbiochem (Lot 040025); L-α-dimyristoyl lecithin (DML) from Sigma (Lots 81F-8365 and 65C-8100); DDT 99% purity from Aldrich, and Tripalmitin (TP), a gift from Dra. L. Masson of University de Chile, were used without further purification. Powder mixtures of DDT with each phospholipid and tripalmitin were prepared in the molar ratios of 1:1, 1:2 and 1:4, and dissolved in chloroform:methanol 1:1. Two types of specimens, oriented films and powder samples, were then prepared from different solutions of each mixture. Oriented films were obtained by slow evaporation until supernatants were formed; they were collected

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with the aid of fine metallic rings, allowed to dry and then cut into small rectangular pieces of about 1 mm^2. These specimens were subjected to X-ray diffraction at room humidity and temperature (about 50% r.h. and 20 °C) in flat-plate cameras provided with a 0.25 mm diameter glass collimator using Ni-filtered Cu K_α radiation.

Powder samples were obtained by allowing newly prepared solutions of each mixture to evaporate until complete dryness. The residues, in the form of fine powders, were put into low absorbing 0.5 mm diameter glass capillaries and diffracted in 114.6 mm diameter Debye-Scherrer powder cameras. The relative intensities of the reflections (Io) were measured from the films in a Joyce-Loebl MK IIICS microdensitometer without correction factors.

Results

The X-ray diagrams of oriented films prepared from various molar mixtures of DDT with DML, DPPE and TP showed in all cases two superimposed patterns, as can be seen in Fig. 1. One of them corresponded to the oriented diagram of each lipid involved, while the other was that of the powder pattern of DDT. No additional reflections that did not belong to the pure lipid or DDT were ever observed. It was possible, of course, that adducts were formed and remained dissolved in the solutions from where the oriented films were extracted. In order to check out this posibility, fresh solutions of each mixture were prepared and then carefully dried at room temperature. The analysis of their powder diagrams revealed again that the patterns corresponded only to the binary mixtures of pure lipids and DDT without any indication that new phases were formed. Fig. 2 shows comparisons of the densitometric traces

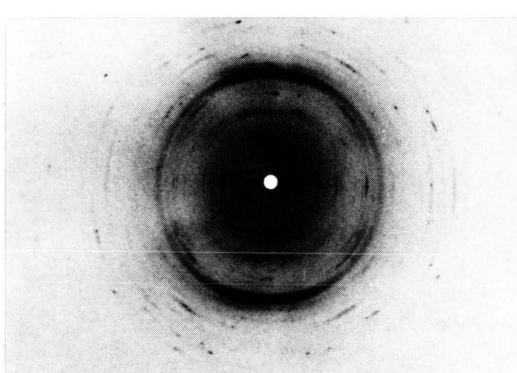
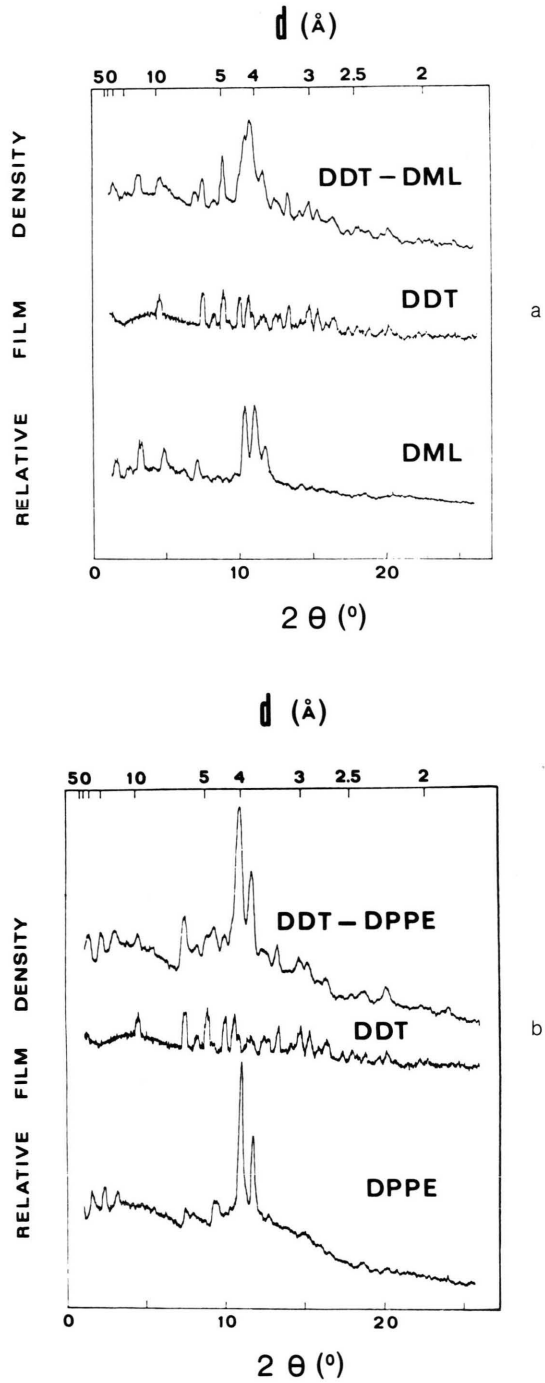


Fig. 1. X-ray fiber diagram of an oriented film of DDT-DML in the molar ratio of 1:4. Spotty reflections correspond to DDT and the oriented arcs to DML.



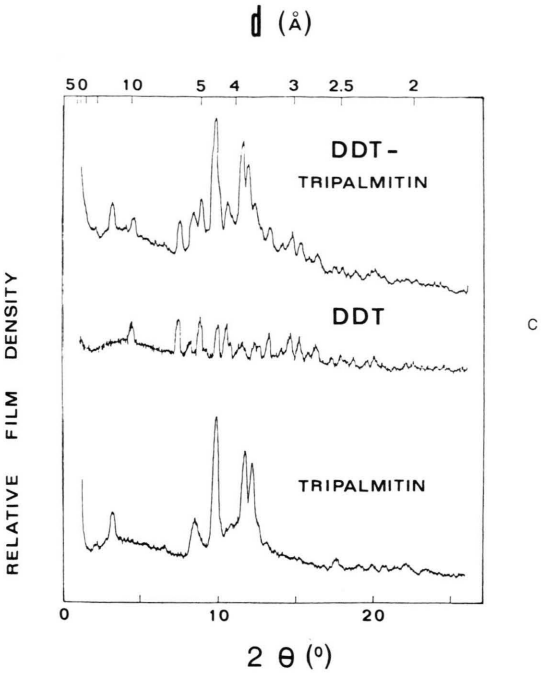


Fig. 2. (a, b, c). Densitometric traces of X-ray powder diffraction patterns of DDT-lipid 1:1 mixtures and of pure DDT and lipids. (a) DDT + DML (b) DDT + DPPE and (c) DDT + DP.

of the powder diffraction patterns of the 1:1 molar components while Table I compares their spacings and relative intensities.

Table I. Comparison of observed spacing (d_o) and relative intensities (I_o) of the DDT-lipid 1:1 powder specimens with those of their pure components. Several additional reflections below 3.0 Å were also observed. A) DDT + DML, B) DDT + DPPE and C) DDT + TP.

(A)

DDT–DML		DDT		DML	
d_o [Å]	I_o	d_o [Å]	I_o	d_o [Å]	I_o
29.4	6	–	–	28.9	4
19.3	3	–	–	19.1	3
13.8	11	–	–	13.8	8
9.61	7	9.61	7	–	–
9.21	6	–	–	9.23	7
8.35	3	–	–	8.41	2
8.01	2	–	–	7.91	1
7.26	3	–	–	7.25	3
6.31	7	–	–	6.28	7
5.87	13	5.88	13	–	–
5.47	1	–	–	5.46	1
5.37	2	5.37	4	–	–
5.25	1	–	–	5.27	1
5.16	1	5.17	1	–	–
4.98	13	4.97	11	4.97	1
4.77	2	4.76	5	–	–
4.69	1	–	–	4.64	3
4.59	1	4.60	1	–	–
4.43	9	4.44	11	–	–
4.35	22	–	–	4.35	26
4.18	13	4.19	10	–	–
4.09	45	4.10	4	4.09	29
3.95	2	3.95	2	–	–
3.84	16	3.84	3	3.83	10
3.73	3	3.76	2	3.75	2
3.60	3	–	–	3.60	1
3.56	2	3.58	2	–	–
3.50	3	3.50	5	–	–
3.44	1	3.43	3	–	–
3.40	1	–	–	3.40	1
3.35	9	3.35	7	–	–
3.26	1	3.26	1	–	–
3.18	2	–	–	3.18	2
3.15	3	3.15	2	–	–
3.06	5	3.06	2	–	–
3.03	9	3.03	4	3.02	2

(B)

DDT–DPPE		DDT		DPPE	
d_o [Å]	I_o	d_o [Å]	I_o	d_o [Å]	I_o
29.6	12	–	–	28.7	9
19.3	14	–	–	19.0	14
14.1	13	–	–	14.1	8
9.61	7	9.61	7	–	–
8.01	3	–	–	8.07	3
5.95	13	–	–	5.95	8
5.87	16	5.88	13	–	–
5.61	5	–	–	5.62	2
5.36	6	5.37	4	5.37	1
5.18	1	5.17	1	5.16	1
4.99	9	4.97	11	–	–
4.79	10	–	–	4.79	7
4.75	4	4.76	2	–	–
4.69	3	–	–	4.69	5
4.60	1	4.60	1	–	–
4.43	8	4.44	11	–	–
4.30	2	–	–	4.31	2
4.21	6	4.19	10	–	–
4.08	100	4.10	4	4.08	78
3.95	6	3.95	2	3.95	2
3.82	48	3.84	3	3.81	37
3.76	3	3.76	2	–	–
3.64	2	–	–	3.64	1
3.58	2	3.58	2	–	–
3.50	3	3.50	5	3.51	4
3.43	1	3.43	3	–	–
3.34	11	3.35	7	–	–
3.25	1	3.26	1	–	–
3.16	1	3.15	2	–	–
3.07	5	3.06	2	–	–
3.02	5	3.03	4	–	–

(C)

DDT–TP		DDT		TP	
d_o [Å]	I_o	d_o [Å]	I_o	d_o [Å]	I_o
20.8	3	–	–	20.8	2
15.5	2	–	–	15.5	1
13.6	13	–	–	13.6	12
10.4	1	–	–	10.4	1
9.61	7	9.61	7	–	–
8.19	1	–	–	8.20	2
6.84	3	–	–	6.83	1
5.88	13	5.88	13	5.91	1
5.62	1	–	–	5.63	1
5.37	3	5.37	4	–	–
5.28	19	–	–	5.28	18
5.17	1	5.17	1	–	–
4.98	11	4.97	11	–	–
4.88	1	–	–	4.90	1
4.75	1	4.76	2	–	–
4.58	65	4.60	1	4.59	56
4.39	5	4.44	11	4.41	1
4.20	9	4.19	10	–	–
4.13	8	–	–	4.13	1
4.10	6	4.10	4	–	–
4.03	5	–	–	4.03	1
3.95	3	3.95	2	–	–
3.86	31	3.84	3	3.85	28
3.73	25	3.76	2	3.71	29
3.61	10	3.58	2	3.60	2
3.49	1	3.50	5	–	–
3.42	1	3.43	3	3.42	1
3.37	7	3.35	7	–	–
3.27	1	3.26	1	3.29	1
3.16	3	3.15	2	3.17	1
3.07	6	3.06	2	–	–
3.03	6	3.03	4	–	–

Discussion

The phospholipids dimyristoyl lecithin (DML) and dipalmitoylphosphatidylethanolamine (DPPE) are known to form well ordered multilayer systems in their crystalline phases, below their transition temperatures.

Their molecular conformations and packing arrangements have been determined in our lab in oriented films and powder specimens by X-ray techniques [14–17]. In view of the biological relevance it was thought of interest to incorporate DDT into these bilayers as well as in tripalmitin. The structure determination of the resulting products was to provide an insight on how these molecules interact and a possible explanation of the effect of DDT on the living cells.

In the light of the results obtained with oriented and powder diagrams of DDT mixtures with DPPE, DML and tripalmitin at different molar ratios it can be concluded that under the conditions the work was carried out no interactions occurred. In fact, in all cases the X-ray diagrams corresponded only to a binary mixture of DDT and a lipid without the indication of a new phase being present. Neither the reported effect of "fluidization" of the lipid bilayers caused by DDT was observed as the X-ray patterns of the lipids in the mixtures remained as crystalline as in their pure forms. These results would tend to support the reports that the effects of the insecticide on biological membranes in vivo are most likely protein related and not lipid mediated [18–19]. It might be possible, however, that DDT can bind to phospholipids under different conditions such as low oxygen levels [20] or to unsaturated phospholipids [21].

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