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INTERACTION OF DDT (1,1,1-TRICHLORO-2,2-BIS(*p*-CHLOROPHENYL)-ETHANE WITH LIPOSOMAL PHOSPHOLIPIDS

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Summary

The influence of 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) and several other pesticides on the physical state of membrane phospholipids was investigated using model lipids. The thermal dependence of fluorescence intensity of the probe parinaric acid in dipalmitoylphosphatidylcholine liposomes and lipid vesicles of mixed composition were recorded. DDT was incorporated into the liposomal bilayer. The insecticide lowered the phase transition temperature and broadened the temperature range of the transition. The effects were concentration-dependent.

The results may be interpreted as a sort of blurred and facilitated phase transition of bilayer lipids caused by intercalation of DDT between fatty acyl chains of membrane phospholipids.

Introduction

Despite low environmental concentrations many abundant and persistent pesticides are harmful for individual organisms [1]. The initial event underlying toxicity can be the accumulation of lipid-soluble chemicals in tissues, cells or subcellular structures of animals, resulting in toxicologically effective concentrations in target organs (for a recent tabulation see Ref. 2). A low environmental level of a given pesticide, for example DDT, may work via bioaccumu-

Abbreviations: DDT; 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane; DDE, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene; *trans*-parinaric acid, all-*trans*-9,11,13,15-octadecatetraenoic acid; *cis*-parinaric acid, *cis, trans, trans, cis*-9,11,13,15-octadecatetraenoic acid; DPPC, glycero-1,2-dipalmitoyl-3-phosphatidylcholine; DMPC, glycero-1,2-dimyristoyl-3-phosphatidylcholine; DOPC, glycero-1,2-dioleoyl-3-phosphatidylcholine.

lation as a continuous supply to various lipid pools in cells. The prime target in organisms for lipophilic pesticides is considered to be the lipid-rich plasma membrane of tissue cells [3].

Biological membranes have a very complex structure. A large number of different proteins and lipids interact in countless variations within the membrane bilayer. Considering only the lipid matrix one can easily figure out hundreds of different lipid species as building blocks of natural membranes. Interaction of pesticides with these structures will certainly produce a wealth of information. In order to facilitate the interpretation of results we thought it would be of advantage to start the investigations with membrane models such as liposomes.

In the form of liposomes, many phospholipids or phospholipid mixtures exhibit marked structural changes at discrete temperatures. At these rather well-defined temperatures thermal reorganization of phospholipids occurs. The fatty acyl chains of the molecules pass from an ordered structure (gel-crystalline phase) to a more disordered one (liquid-crystalline phase) [4]. The temperature midpoint of this transition has been known as 'phase transition temperature' [4]. This structural rearrangement can be influenced by a variety of substances embedded in the membrane. As an example, the influence of lipid soluble drugs on the phase transition of model lipids has been demonstrated [5,6].

To monitor pesticide-induced deviations from the normal physical behavior of liposomes, the naturally occurring fluorescent probe parinaric acid was used in our study. The chemical and physical properties of the this probe and its ability to report on phase transitions in model membranes have been thoroughly investigated by Sklar et al. [7]. In short, the usability of this probe rests – among others – on the fact that the molecule is uniformly dispersed in the lipid bilayer. The hydrophobic fatty acid bearing four conjugated double bonds is intercalated between the fatty acyl chains of the bilayer lipids. Thus, the electronic transitions following radiation excitation occur in the part of the molecule that is anchored in the core of the membrane. Changes of the fluorescence intensity of the probe molecule with temperature monitor, in this way, alterations of the physical state of the bilayer interior [7].

Our work has been directed towards learning about the mode of interaction of pesticides with biological membranes. We have been studying the effect of DDT on the physical state of model liposomes. The interaction of DDT with bilayer lipids gives rise to shifts in spectroscopically determined parameters of the probe parinaric acid. Preliminary results have been published [8].

Materials and Methods

Reagents. The phospholipids were of analytical grade from Sigma Chemie GmbH, Taufkirchen, F.R.G. (DPPC, egg lecithin) or from Natterman, Cologne, F.R.G. (DMPC, DOPC). Purity was checked by thin-layer chromatography. All the pesticides were obtained from Riedel-de Haen, Seelze, F.R.G. ('Pestanal' quality). The parinaric acid isomers were purchased from Molecular Probes Inc., Plano, TX, U.S.A.

Preparation of liposomes. All the ingredients intended to make up the liposomes under study, including the fluorescent probes, were mixed in ethanolic solution in the proper molar proportions. Probe to lipid ratio was generally

1 : 200. The resultant solution was dried down by a stream of nitrogen at about 40°C. 1.8 ml of pre-warmed phosphate-buffered saline (pH 7.4) was added to a final concentration of 0.6 mM. Multilamellar lipid vesicles (liposomes) were prepared by intermediate mixing (Vortex mixer, 3 × 30 s) while keeping the sample temperature at least 10°C above the respective lipid phase transition for a period of 5 min [9]. All manipulations were carried out under an atmosphere of nitrogen and by red light in order to minimize oxidation of the probe.

Measurement of fluorescence intensity. Following preparation, the sample was briefly cooled to room temperature and pipetted into a cuvette in the water-jacketed cell holder of an Aminco-Bowman Spectrophotofluorometer (American Instruments Inc.). Heating by means of a circulating water bath did not exceed 1 K/min. Temperature was recorded with a thermocouple, sealed in the sample cuvette and connected to a voltmeter (model DMM 8050 A, Fluke GmbH). Precision was ±0.2°C. While the measurements were being made a gentle stream of nitrogen was passed on top of the solution. Excitation and emission wavelengths were set at 319 nm and 411 nm, respectively, with proper adjustment in each assay to the maximum position. Fluorescence intensity was read off a photometer at 0.5°C temperature intervals. Fluorescence efficiency of parinaric acid in water is negligible [7], in our experiments less than 5%.

Results

The fluorescence intensity of the probe *trans*-parinaric acid in DPPC liposomes is temperature-dependent [7]. The response of the fluorescence intensity to the thermally induced phase transition and the effect of DDT are shown in Fig. 1. In pure DPPC liposomes, a sharp decrease of fluorescence intensity centers at the phase transition temperature of 40.6°C, indicating a strong cooperative phase change in the phospholipid bilayer [7]. The incorporation of the insecticide DDT in the vesicle membrane has two effects. The gel-to-liquid crystalline phase transition temperature is decreased and the temperature range in which the transition occurs is broadened. Fluorescence efficiency of the probe is obviously not influenced by DDT at the concentrations examined. This is illustrated by the fact that fluorescence intensity differences of the probe between rigid and fluid phase are identical in control and DDT-containing liposomes.

The data of Table I gives a full account of the DDT effect. The results of two experiments disclose quantitatively the influence of DDT on bilayer phospholipids. The phase transition shift is concentration-dependent and the temperature range of the structural rearrangement is broadened more than 2-fold.

Almost similar results are obtained with the probe *cis*-parinaric acid (Table I). This probe differs from its *trans*-isomer in molecular geometry as well as in its solid/fluid lipid phase partition factor [7]. Generally, transition points are obtained at slightly lower temperatures (Table I and Ref. 7).

The influence of several other pesticides on the physical behavior of DPPC liposomes was tested under the same conditions (Fig. 2). The herbicides atrazine and 2,4,5-trichlorophenoxyacetic acid have no effect on the phase transition temperature at concentrations of 7.5 mol%. 4,4'-Dichlorobiphenyl

TABLE I

EFFECT OF DDT ON FLUORESCENCE INTENSITY OF PARINARIC ACIDS IN DPPC LIPOSOMES

The DDT concentration in liposomes is given as mol% of DDT relative to liposomal phospholipid. Phospholipid concentration is 0.6 mM. Explanation of symbols: T_m, lipid phase transition temperature; $T_m^0 - T_m^{DDT}$, difference of phase transition temperature of control liposomes and of liposomes containing DDT; ΔT, temperature range of phase transition. Experimental details are as in Materials and Methods.

Expt.	Parinaric acid isomer	DDT (mol%)	T_m (°C)	$T_m^0 - T_m^{DDT}$ (°C)	ΔT (°C)
1	*trans*	0	40.6 *		1.8
		2	40.2	0.4	3.4
		5	39.6	1.0	4.2
		10	39.3	1.3	4.4
		20	38.3	2.3	4.7
2	*trans*	0	40.7 *		1.9
		2	40.2	0.5	3.0
		5	39.1	1.6	4.9
		10	38.2	2.5	5.7
		20	38.2	2.5	5.4
3	*cis*	0	39.5		2.5
		5	39.1	0.4	3.3
		10	37.6	1.9	4.6

* Each experiment is representative for one set of liposomal preparations. Error in determination of T_m within one set is ±0.2° C.

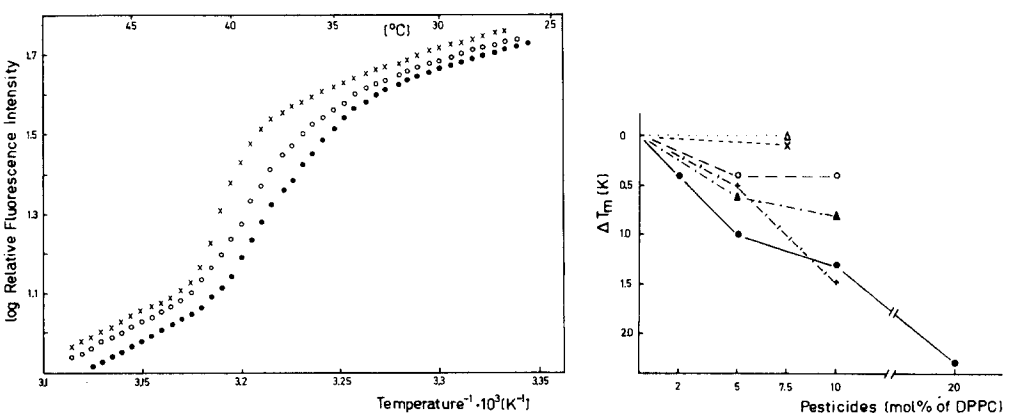

Fig. 1. The influence of DDT on the response of fluorescence intensity of *trans*-parinaric acid to the phase transition of DPPC liposomes. Each point represents the fluorescence intensity recording read off a photometer at intervals of 0.5°C (corresponding to time intervals of about 0.5 min). Heating curves are shown. The concentration of phospholipids was 0.6 mM. The concentration of DDT is given as mol% relative to liposomal phospholipid. Experimental details are as in Materials and Methods. X, DPPC; ○, DPPC + 5 mol% DDT; ●, DPPC + 10 mol% DDT.

Fig. 2. The influence of various pesticides on the phase transition temperature of DPPC liposomes. The phase transition temperature of control liposomes and of liposomes containing pesticides was determined as illustrated in Fig. 1. The difference of transition points is plotted against pesticide concentration. Symbols are: △, 2,4,5-trichlorophenoxyacetic acid; ○, 4,4′-dichlorobiphenyl; ▲, pentachlorophenol; X, atrazine; +, DDE; ●, DDT. Experimental details are as in Materials and Methods.

and pentachlorophenol produce an intermediary effect, the insecticide DDT and its metabolite DDE show the most pronounced influence on the structural order of liposomes (Fig. 2).

The magnitude of the effects appears to be related to a great extent to the molar concentration of the pesticides examined in the vesicle bilayer. Although there have been no reports on water-bilayer partition coefficients of all these compounds under standardized conditions, their potency to interact with bilayer lipids is evidently reversely related to their water solubility [10].

Turning to more complex model systems, the investigations were extended to liposomes of mixed composition. DDT was incorporated into a variety of liposomes composed of two or three phospholipid species. The binary or ternary lipid dispersions thus prepared had all the same 'head group' (namely choline) but differed in the chain length of their fatty acids. These vesicles also exhibit a single phase transition temperature located between the transition temperatures of the individual components.

The results obtained with these mixed liposomes are shown in Table II. In addition to the decrease of transition temperature and the broadening of the transition width by incorporated DDT some other findings merit attention. Going from simple one-compound liposomes (DPPC) to more complex vesicles (DPPC/DMPC/DOPC) several conclusions can be derived from the data: (i) even in untreated liposomes the range of the phase transition broadens from 2.4 to 5.3°C (column 3); (ii) the difference of transition temperature between control liposomes and DDT-containing liposomes tends to become smaller (column 2); (iii) the same finding holds for the width of transition: the differences between control and DDT-treated samples decrease (column 3).

Natural membranes usually contain appreciable amounts of cholesterol. It

TABLE II

EFFECT OF 10 mol% OF DDT ON FLUORESCENCE INTENSITY OF *trans*-PARINARIC ACID IN DPPC LIPOSOMES AND IN LIPOSOMES OF MIXED COMPOSITION

THe liposomes were composed of 60 mol% DPPC/40 mol% second phospholipid species or 40 mol% DPPC/30 mol% DMPC/30 mol% DOPC, respectively. Symbols are as described in Table I. Experimental details are as in Materials and Methods.

Liposomal composition	DDT	T_m (°C)	$T_m^0 - T_m^{DDT}$ (°C)	ΔT (°C)
DPPC	—	40.2 *		2.4
	+	38.5	1.7	3.6
DPPC/DMPC	—	35.4		3.2
	+	33.8	1.6	5.1
DPPC/DOPC	—	33.0		4.2
	+	31.7	1.3	5.3
DPPC/egg lecithin	—	34.1		4.8
	+	33.1	1.0	5.2
DPPC/DMPC/DOPC	—	31.6		5.3
	+	31.0	0.6	5.7

* See footnote to Table I.

has already been established that cholesterol in concentrations exceeding 33 mol% of phospholipids abolishes phase transitions in liposomes [11]. It may be appropriate within this context to mention that pronounced changes of fluorescence intensity of parinaric acid at defined temperatures tend to disappear with increasing concentrations of cholesterol (unpublished results). Consequently, it has not been possible to detect clear effects of DDT in liposomes containing considerable amounts of cholesterol by measuring fluorescence intensity changes with temperature.

Discussion

As described by Sklar et al. [7], temperature-dependent changes of fluorescence intensity of parinaric acid probes embedded in phospholipid bilayers mirror structural rearrangements of surrounding lipid molecules. Gel-to-liquid crystalline phase transitions in the lipid matrix are accurately indicated by marked changes of the slope of the temperature-dependence [7].

Incorporation of DDT did not grossly alter these spectroscopically determined parameters (Fig. 1). Apparently, DPPC vesicles can accommodate up to 20 mol% of DDT in their lipid bilayer without attenuation of probe fluorescence efficiency. However, the insecticide does decrease the phase transition temperature and expand the temperature range in which the structural rearrangement takes place. These phenomena are concentration-dependent. The pesticide induced effect resembles the 'fluidizing' effects of drug molecules on liposomes [5,6]. In a broad temperature range around the phase transition the presence of DDT causes a greater portion of bilayer lipids to be in the liquid crystalline state than in the absence of the pesticide (Fig. 1). Thus, DDT incorporation results in an apparent 'fluidization' of bilayer lipids. Intercalation of DDT molecules between the fatty acyl chains of phospholipids may weaken hydrophobic forces in the lipid core which contribute to the stabilization of bilayer structure.

Similar conclusions may hold for the effect of other lipophilic pesticides in DPPC liposomes (Fig. 2). The influence of the chemicals examined on the structural order in lipid vesicles can be inversely related to the water-solubility of the compounds (Ref. 10, but cf. Ref. 14).

In order to mimic the structural organization of biological membranes a series of mixed liposomes was prepared. If the liposomes are formed from two or three components the influence of DDT on the thermally induced phase transition becomes less and less evident: transition point differences become smaller (Table II, column 2) as it is the case with the differences of transition range (Table II, column 3). Preliminary results obtained with biological membrane preparations disclose similar features: recordings of fluorescence intensity of parinaric acid in Chang liver cell membranes and in vesicles derived from the membrane total lipid extract do not indicate any marked change of slope between 15°C and 45°C (unpublished results). It seems that the effect of DDT on the lipid structure of biological membranes will not become visible through intensity measurements of probe fluorescence in the way described here.

There have been already several reports on the interaction of DDT and

related pesticides with phospholipid vesicles [12—17]. It may be instructive to focus on the results only as far as the effect of DDT on the physical state of bilayer lipids is concerned.

The results of NMR studies could be interpreted as increased viscosity (decreased fluidity) of membrane bulk lipids following DDT-lecithin interaction [12]. Another report showed that DDT did not quench fluorescence efficiency of a cyanine dye in lecithin vesicles, in contrast to the behavior of the same compound in flat bilayers [13]. An unsufficient concentration of the insecticide in the bilayer could, however, not be ruled out by the authors. Similar problems were encountered in another NMR study [14]. DDT, the most nonpolar of the chemicals examined, was barely taken up in the apolar interior of lipid vesicles and, consequently, was without effect on NMR signals [14]. Two abstracts of ESR studies which were carried out with unspecified model lipids suggested a DDT-induced decrease of lipid fluidity [15,16]. Contrasting conclusions were derived from permeability experiments [17]. Increased permeability of lecithin liposomes to small molecules in the presence of DDT was interpreted as a consequence of increased bilayer fluidity [17].

These conflicting results reveal that no clear idea has yet emerged how DDT might act on biological membranes. Even experiments with rather simple vesicles such as DPPC liposomes or egg lecithin vesicles yield opposite conclusions. Part of the discrepancies may certainly be reduced to inherent limitations of the various physical methods used.

Our work reported here was designed as an approach to the understanding of DDT-triggered events in biological membranes. The use of defined liposomes enabled us to detect concentration-dependent effects of DDT on the physical state of bilayer lipids. Thermally induced reorganization of phospholipid structure ('phase transition') is influenced by DDT embedded in the lipid matrix. It is concluded that the pesticide molecules intercalate between fatty acyl chains of adjacent lipids, thus weakening interchain hydrophobic forces which contribute to the stabilization of membrane structure. Hence, DDT increases membrane fluidity. Although it seems doubtful to us to apply the method used here directly to biological membranes, we feel that our results give substantial evidence that similar disturbances of lipid structure by pesticides occur also in natural membranes.

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