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Membrane fluidity as affected by the insecticide lindane

M C Antunes-Madeira and Vitor M C Madeira

Centro de Biologia Celular Departamento de Zoologia Coimbra (Portugal)

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Fluorescence polarization of 1,6-diphenyl-1,3,5-hexatriene (DPH) was used to study the interaction of lindane with model and native membranes Lindane disorders the gel phase of liposomes reconstituted with dimyristoyl-, dipalmitoyl- and distearoylphosphatidylcholines (DMPC, DPPC and DSPC), since it broadens and shifts the main phase transition, but no apparent effect is detected in the fluid phase These effects of lindane are more pronounced in bilayers of short-chain lipids, e.g., DMPC. In equimolar mixtures containing DMPC and DSPC, lindane preferentially interacts with the more fluid lipid species inducing lateral phase separations. However, in mixtures of DMPC and DPPC, the insecticide only broadens and shifts the main phase transition, i e , an effect similar to that observed in bilayers of pure lipids. Lindane has no apparent effect in DMPC bilayers enriched with high cholesterol content ($\geq$ 30 mol%), whereas disordering effects can still be detected in bilayers with low cholesterol ($<$ 30 mol%) Apparently, lindane does not perturb the fluid phase of representative native membranes, namely, mitochondria, sarcoplasmic reticulum, myelin, brain microsomes and erythrocytes in agreement with the results obtained in fluid phospholipid bilayers, despite the reasonable incorporation of the insecticide in these membranes, as previously reported (Antunes-Madeira, M.C. and Madeira, V M C. (1985) Biochim. Biophys. Acta 820, 165–172).

Introduction

Previous studies in our and other laboratories support the idea that biomembranes are primary targets of insecticide action [2–13] Therefore, the partitions of some popular insecticides, namely, DDT, parathion, malathion and lindane were extensively studied in model and native membranes [1,14–16] Insecticide partitioning does not correlate with toxicity and is affected by multiple parameters, such as, temperature, cholesterol, fluidity and physico-chemical profiles of the membrane components and the insecticides themselves [1,14,15,17] Fluidity and the insecticide character are main parameters affecting incorporation and, probably, insecticide toxicity

To further characterize insecticide interaction with membrane components, we now report the effects of lindane in the fluidity of well defined model and native membranes in terms of fluorescence polarization of the probe 1,6-diphenyl-1,3,5-hexatriene (DPH)

Materials and Methods

Preparation of liposomes and native membranes Solutions of pure phospholipids were taken in round-bottom flasks and the solvent ($CHCl_3$) was evaporated to dryness The lipid film was then hydrated with an appropriate volume of 50 mM KCl, 10 mM Tris-maleate (pH 7 0), and dispersed under N_2 atmosphere by hand-shaking in a water bath, 7–10 Cdeg above the transition temperature of the phospholipids The sample was then shaken vigorously by vortex for 1 min and briefly sonicated (five bursts of 30 s each) Heterogeneous phospholipid bilayers were obtained with equimolar amounts of single components and phospholipid-cholesterol bilayers by supplementing original phospholipid solutions with appropriate amounts of cholesterol The final concentration of lipid was nominally 345 μM, in all cases

Various native membranes, namely, erythrocytes, brain microsomes, myelin, sarcoplasmic reticulum and mitochondria were prepared as described elsewhere [14] Protein concentrations were determined by the biuret

Abbreviations DMPC dimyristoylphosphatidylcholine DPPC dipalmitoylphosphatidylcholine, DSPC distearoylphosphatidylcholine lindane γ-1 2 3 4,5 6-hexachlorocyclohexane, T_m, midpoint temperature of thermotropic phase transition DPH 1 6-diphenyl-1,3,5-hexatriene

Correspondence V M C Madeira, Centro de Biologia Celular, Departamento de Zoologia, 3049 Coimbra Codex, Portugal

method [18] calibrated with serum albumin Membrane suspensions were rapidly frozen in liquid nitrogen and kept at −80 °C

DPH and lindane incorporation into membranes DPH (2 mM) in tetrahydrofuran was injected, while vortexing, into membrane suspensions (345 μM in lipid) to give a final phospholipid/probe molar ratio of about 200 The mixture was then incubated, in the dark, for 18–20 h After this period of incubation, lindane was added from concentrated ethanolic solutions (50 mM) The period of equilibration with lindane varied from 1–2 h according to the concentration used Control samples received equivalent volumes of tetrahydrofuran and ethanol

Fluorescence polarization measurements Fluorescence spectra were measured in a Perkin-Elmer spectrofluorometer, Model MPF-3, provided with a thermostated cell holder The excitation was set at 336 nm and the emission at 450 nm The excitation and emission slits were 4 and 6 nm, respectively The temperature of the sample was checked with an accuracy of ±0 1 °C, using a termistor thermometer The degree of fluorescence polarization (P) was calculated, according to Litman and Barenholz [19] from the equation

$$P = \frac{I_{\parallel} - I_{\perp} G}{I_{\parallel} + I_{\perp} G}$$

where $I_{\parallel}$ and $I_{\perp}$ are the intensities of the emitted light oriented, respectively, parallel and perpendicular to the

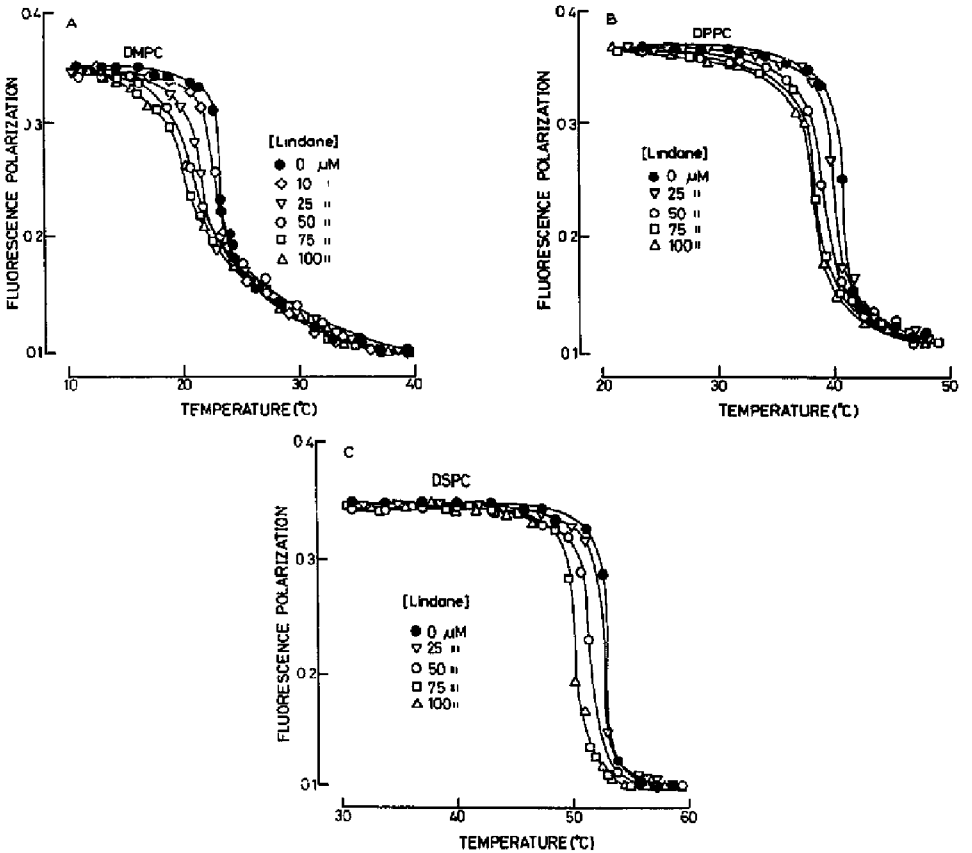

Fig 1 Lindane effects on the thermotropic phase transition of DMPC (A), DPPC (B) nd DSPC (C) bilayers, as determined by fluorescence polarization of incorporated DPH Maximal incorporations were obtained in the temperature range of the thermotropic phase transition (T_m values for DMPC DPPC and DSPC are about 24 41 and 54 °C, respectively, as reported in Fig 3A of Ref 1)

plane of the excitation beam G is the correction factor for instrument polarization, given by the ratio of vertical to the horizontal components when the excitation light is polarized in the horizontal direction [19] High degree of fluorescence polarization (P) represents high structural order or low membrane fluidity, and vice-versa

Reagents Cholesterol, dimyristoyl-, dipalmitoyl- and distearoylphosphatidylcholines, at least 98% pure and DPH were obtained from Sigma Lindane was obtained from Supelco, Inc

Results and Discussion

Fluidity of model membranes of pure phospholipids

Lindane lowers the phase transition temperature midpoint (T_m) of DMPC, DPPC and DSPC bilayers (Fig 1 and Table I) and broadens the transition, i e, expands the temperature range at which two-dimensional domains of fluid and gel phase coexist (Fig 1A, B and C) These effects depend on lindane concentration up to about 100 μM Furthermore, the insecticide interacts more effectively with short-chain rather than long-chain lipids, since larger shifts in T_m were observed in DMPC as compared with DPPC and DSPC bilayers Thus, for 50 μM lindane, T_m of DMPC, DPPC and DSPC are shifted by 2 5, 1 7 and 1 3 Cdeg, respectively (Table II) The shift and broadening of the transition in pure lipid bilayers induced by lindane is consistent with other results reported previously [20,21]. Furthermore, similar effects have been described for other insecticides and other drugs [11,20–25]

Apparently, lindane disturbs significantly the bilayer order in the temperature range of the cooperative phase transition in DMPC, DPPC and DSPC bilayers, but no disordering effects are noticed at temperatures 10 Cdeg below T_m. This may partially reflect the relative exclusion of lindane from the bilayers (especially, DPPC and DSPC) at low temperatures (Fig 3A of Ref 1) Nevertheless, significant incorporation occurs in DMPC bilayers, at temperatures 10 Cdeg below the T_m, without a corresponding effect in membrane fluidity Apparently, thermotropic geometrical factors imposed by the molecular structure of this lipid permit accommodation of the insecticide without perturbation of membrane order

Also, a reasonable incorporation of lindane occurs in the fluid phase of DMPC DPPC and DSPC bilayers Nevertheless, the insecticide fails to disorder the fluid phase of the above lipids as concluded from the thermotropic data of Fig 1 Hence, it appears that the fluid phase holds enough free volume to accommodate this small insecticide molecule (4 Å, cf Ref 26), without perturbation of the bilayer structure This conclusion is reinforced by the fact that lindane has almost no effect in the permeability of fluid egg-PC membranes to non-electrolytes and to ion-ionophore complexes [10]

TABLE I

The effects of various lindane concentrations (0–100 μM) on the midpoint temperature transitions (T_m) of bilayers reconstituted with DMPC DPPC and DSPC lipid species

Type of bilayers	Added lindane (μM) and respective T_m values (°C)					
	0	10	25	50	5	100
DMPC	23 2	22 8	21 7	20 7	20 5	20 5
DPPC	40 7	–	40 1	39	38 5	38 1
DSPC	52 9	–	52 5	51 6	50 5	50 5

TABLE II

Effects of lindane (50 μM) on the midpoint temperature transition of bilayers reconstituted with pure lipids or with binary mixtures of lipids

Type of bilayers	Control T_m (°C)	Lindane (50 μM) T_m (°C)	ΔT_m (Cdeg)
DMPC	23 2	20 7	2 5
DPPC	40 7	39	1 7
DSPC	52 9	51 6	1 3
DMPC + DPPC	30 8	28 6	2 2
DMPC + DSPC	32 6	32 25 6	0 6 7 0

Fluidity of model membranes of binary mixtures

Lindane affects the thermotropic behavior of bilayers containing equimolar mixtures of DMPC plus DPPC similarly as described for single DMPC or DPPC (Fig. 2A) Lindane (50 μM) lowers the phase transition midpoint of the mixture by about 2 2 Cdeg, a value close to that observed in pure DMPC bilayers (Table II) This indicates a preferential interaction with DMPC lipid species, but a biphasic transition is not observed However, a clear biphasic transition is observed in mixed bilayers of DMPC plus DSPC upon interaction with lindane (Fig 2B and Table II) The higher temperature profile is only slightly modified by the insecticide, but the lower temperature component, i e the new transition, is broadened and shifted to lower temperatures relatively to the control This new transition is centered at 25 6°C, i e, at a T_m close to that of pure DMPC bilayers, which is about 24°C This property of lindane, as promoter of phase separations is similar to that previously described for the organophosphorus compounds parathion and azinphos [11] Therefore, lindane interaction with membranes depends on the physicochemical character of membrane lipids and is facilitated by species which form domains with higher fluidity in agreement with the conclusions previously achieved by Omann and Lakowicz [27] Since native membranes consist of domains or patches of lipids differing in composition and fluidity [28–30], it can be predicted

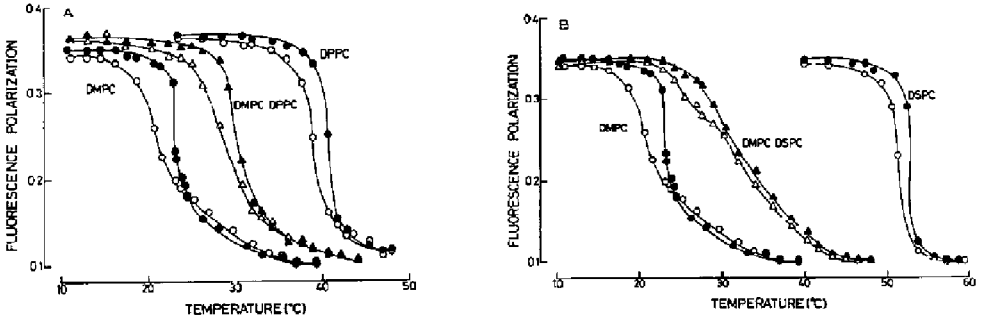

Fig 2 Fluorescence polarization of DPH in bilayers reconstituted with equimolar mixtures of DMPC DPPC (A) and DMPC DSPC (B) in the absence (solid symbols) and presence (open symbols) of 50 μM lindane as a function of temperature Lindane converts the single transition of the binary mixture of DMPC plus DSPC into a biphasic transition The new transition (lower temperature component) has a midpoint at 25 6 °C a value close to the T_m of DMPC, which is about 24 °C

that lindane is not homogeneously distributed, but preferentially associated with fluid zones Furthermore, upon interacting with more fluid lipid species, lindane may cause phase separations in microdomains with the consequent physical [31,32] and physiological [31–34] implications

Fluidity of bilayers containing cholesterol

Cholesterol progressively increases the molecular order in the fluid phase of DMPC bilayers and equimolar mixtures of DMPC plus cholesterol are devoid of thermotropic phase transitions (Fig. 3), according to classical observations [35] Disordering effects of lindane can be detected in DMPC bilayers with low cholesterol content (< 30 mol%) However, the disordering effects of lindane are gradually depressed and vanish at cholesterol concentrations higher or equal to 30 mol% (Fig 3) Apparently, cholesterol counteracts insecticide interaction, either by modifying the membrane structural organization or by competitively opposing the incorporation of lindane Indeed, lindane partitioning, previously studied in egg-PC membranes at 24°C, decreases linearly with the increase of cholesterol content, and a complete exclusion of lindane is observed for cholesterol concentrations of about 50 mol% (Fig 2 of Ref. 1). Therefore, in native membranes, lindane influence would be greater in organelles and regions with low cholesterol contents, i e , highly functional biomembrane systems such as mitochondria and endoplasmic reticulum

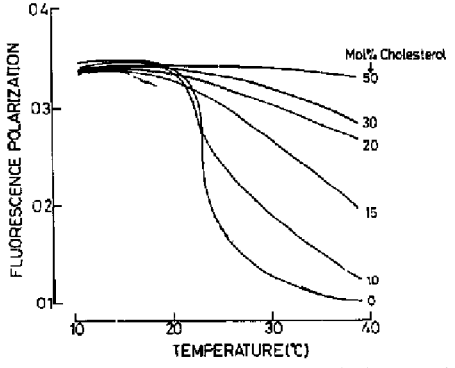

Fig 3 Fluorescence polarization of DPH in DMPC bilayers enriched with cholesterol in the absence (solid lines) and in the presence (dotted lines) of 50 μM lindane Each curve was drawn across 16–20 experimental points For the sake of clarity, the experimental points were removed The numbers adjacent to the curves represent mol% of incorporated cholesterol

Fluidity of native membranes

Fluorescence polarization of DPH was studied in several representative native membranes, namely, erythrocytes, brain microsomes, myelin, sarcoplasmic reticulum and mitochondria, over the temperature range from 10 to 40 °C Data illustrated in Fig. 4 clearly indicate that, independently of temperature, membrane fluidity depends considerably on the membrane type and composition Cholesterol is an intrinsic modulator of native membrane fluidity, since it decreases linearly (correlation coefficient = 0 975) with the increase of 'native' cholesterol content (Fig 4B) Temperature also modulates the general membrane fluidity since it increases linearly with temperature (Fig 4A)

Lindane has no apparent disordering effects into fluid native membranes (Fig 4, dotted lines), in close agreement with the results obtained in model bilayers in the fluid state However, a significant incorporation was detected into the above membranes, over the temperture range under study, i e , from 10 to 40 °C (Fig 4 of Ref 1) Therefore, lindane accommodates into the mem-

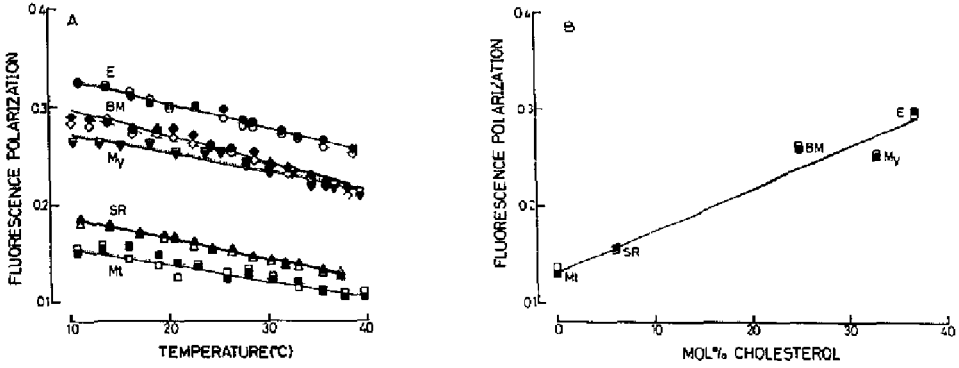

Fig 4 Fluorescence polarization of DPH in native membranes as a function of temperature (A) and mitin ic cholesterol content (B) in the absence (solid symbols) and in the presence (open symbols) of 50 μM lindane Data of B were taken from A (fluorescence polarization data at 24°C) Regression lines were calculated by the least-squares method Correlation coefficients of −0 991 −0 973 −0 96 −0 993 and −0 96 were calculated for erythrocytes (E) brain microsomes (BM) myelin (My) sarcoplasmic reticulum (SR) and mitochondria (Mt) respectively Cholestero/phospholipid molar ratios for mitochondria sarcoplasmic reticulum brain microsomes myelin and erythrocytes are 0 6 25 33 and 37 mol% respectively

branes without causing perturbation of the general membrane fluidity However, this finding does not rule out that discrete membrane domains cannot be perturbed by lindane Especially ordered domains surrounding integral proteins ('annular lipids') have been described [36–38] These relatively ordered domains may be differently affected by lindane and consequently the activities of integral proteins dependent on the physico-chemical characteristics of boundary domains [39], may be affected by the insecticide Accordingly, it has been inferred that the perturbation of the Ca^{2+}-pump activity of sarcoplasmic reticulum induced by lindane may reflect the insecticide interaction with the boundary lipid of the pump [13,40]

Acknowledgements

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