

Changes in the fluidity of model lipid membranes evoked by the organophosphorus insecticide methylbromfenvinfos

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The ability of many insecticides to bind to the lipids form a basis for considering biological membranes as a primary target of interaction of these agents with living organisms [1 - 6]. However, the ability of insecticides to bind to the membrane which, generally, is not correlated with their toxicity, is dependent on many factors, such as temperature, cholesterol content, physical-chemical profiles and fluidity of the membrane, and the nature of the insecticide itself [7 - 12]. The latter two seem to be the main factors determining the ability of insecticides to bind to the membrane [13].

In the present work the fluidity of liposomes made of synthetic phosphatidylcholine has been investigated in the presence of organophosphorus insecticide methylbromfenvinfos (2-bromo-1-(2,4-dichlorophenyl)-vinyl dimethyl phosphate).

Although the results obtained on simple artificial membranes cannot be directly extrapolated to the complex natural ones, the well defined subject of such a study allows one to interpret the results obtained in more precise physical-chemical terms.

Liposomes were formed of distearoylphosphatidylcholine (DSPC)¹ by shaking, sonication and centrifugation. All fluorescence spectra were measured in a Perkin-Elmer luminescence spectrometer, model LS-50 equipped with a thermostated sample cell holder. Interaction of methylbromfenvinfos with membranes was monitored by fluorescence anisotropy of 1,6-diphenyl-1,3,5-hexatriene (DPH), a

probe located at the bilayer center, and by intramolecular excimerization of 1,3-bis-(1-pyrene)propane (Py(3)Py), a probe located in the outer regions of the bilayer. Steady-state fluorescence anisotropy was measured as described in the accompanying communication [14] perpendicularly to the exciting beam according to [15, 16].

All the measurements were corrected for parasite light scattering [17]. DPH was injected from stock solution into the membrane suspension to give a final lipid:DPH molar ratio of 300. The excitation was set at 336 nm and the emission observed at 450 nm.

Intramolecular excimer formation, a phenomenon characteristic of the other fluorescence probe used, Py(3)Py, is caused by association of an excited and an unexcited aromatic group incorporated in the same molecule and can be described by the excimer to monomer fluorescence quantum yield ratio, Φ'/Φ , which is proportional to the fluorescence intensity ratio, I'/I [18, 19]. The latter ratio for Py(3)Py was shown to increase with fluidity in highly viscous media [19 - 21]. The technique is therefore suitable for determination of fluidity of the probe environment in such media as lipid bilayers. Aliquots of the stock solution of Py(3)Py (0.1 mM in ethanol) were added to the membrane suspension to give a final probe:lipid ratio of 1:900. The excitation was set at 342 nm, emission of monomer, depending on temperature, either at 377 or 378 nm, emission of excimer at 490 nm. After a period of incubation

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¹Abbreviations: DPH, 1,6-diphenyl-1,3,5-hexatriene; DSPC, distearoylphosphatidylcholine; Py(3)Py, 1,3-bis-(1-pyrene)propane

with the probes, methylbromfeninfos was added from stock (50 mM) ethanolic solution to give a final concentration of 50 μ M. The samples were equilibrated for the period of 1 h 10°C above the temperature of the main phase transition.

No changes were observed in fluorescence anisotropy of DPH either in the fluid or solid phase of DSPC vesicles in the presence of 50 mM methylbromfeninfos (Fig. 1A). The phase transition profile of the insecticide treated liposomes was somewhat broadened compared to the control. The insecticide evoked a shift in the middle temperature of phase transition (T_m) towards the lower temperature.

The presence of the insecticide caused a significant lowering of the ratio I'/I in the fluid phase as indicated by the probe Py(3)Py (Fig. 1B). However, there were no changes in the T_m of the main transition. In the control vesicles a pretransition was detected, which also was abolished by the insecticide. No changes were observed in the solid phase.

The use of two fluorescent probes distributed in different regions of the bilayer permits to conclude that methylbromfeninfos perturbs selectively the fluidity of the membrane: a more distinct effect was observed when information came from the highly cooperative region of the membrane structure. Thus, the insecticide

binds probably to the membrane in this region. The decrease of T_m and broadening of the transition profile support this hypothesis because such phenomena are characteristic of compounds known to be localized in the cooperativity region [22]. Localization of methylbromfeninfos in this membrane region may help to explain the different effects of the insecticide on the membrane in the fluid phase demonstrated with the use of DPH and Py(3)Py. The insecticide would increase the ordering (solidification) of lipids by increasing their packing density in the region where it is accumulated, but would not affect this ordering measurably in the region where it accumulates in much lower concentration.

Lowering of T_m of the transition indicated by DPH, and lack of this effect in the case of Py(3)Py allows to conclude that methylbromfeninfos evokes phase separation.

In addition to the main thermotropic phase transition, Py(3)Py indicated also a pretransition in DSPC (Fig. 1B). Since this pretransition occurs in the cooperativity region [23] and is abolished by the presence of 50 μ M methylbromfeninfos, thus provides further evidence that the insecticide is located in this region of the membrane.

This study was performed at the Department of Zoology, University of Coimbra, Portugal.

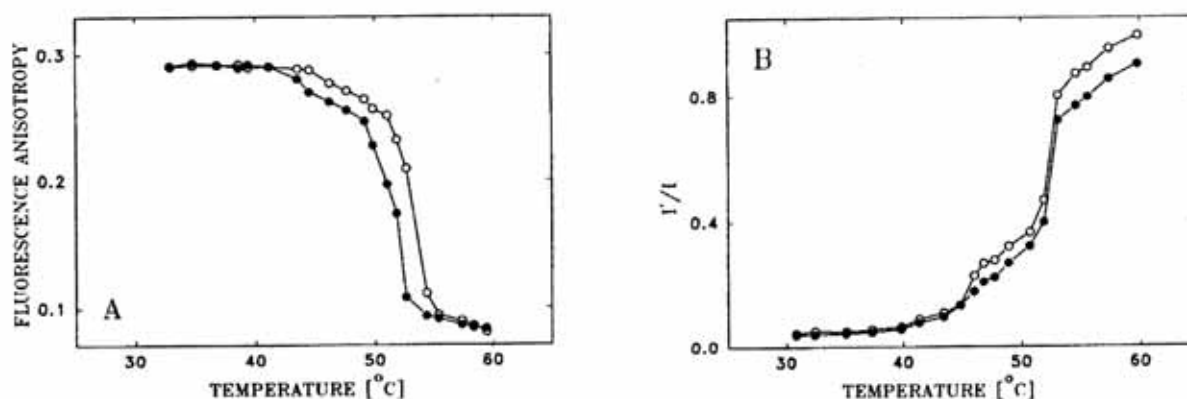


Fig. 1. Fluorescence anisotropy of DPH (A) and intramolecular excimerization of Py(3)Py (B) of distearoylphosphatidylcholine liposomes in the absence (○) and in the presence (●) of 50 μ M methylbromfeninfos. Each experimental point is the mean of three measurements. Error bars were omitted to increase the clarity of the picture

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