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Janusz Błasiak

PARTITION COEFFICIENT OF ORGANOPHOSPHORUS INSECTICIDES EVALUATED BY FLUORESCENCE QUENCHING

The partition coefficient of two organophosphorus insecticides: malathion and methylparathion was evaluated in membranes of various cholesterol content. Fluorescence quenching studies revealed that methylparathion bonded strongly (in a dose-dependent manner) to liposomes formed from phosphatidylcholine. The binding of the insecticide was weaker when the vesicles were prepared from equimolar phosphatidylcholine:cholesterol mixtures. Quenching was more pronounced when a fluorescence probe localized in deep core of the phospholipid (perylene) was used than when a probe binding to more outer region of the membrane (ANS) was employed. The results indicate that binding of some insecticides to membrane can be modulated by cholesterol content.

1. INTRODUCTION

Organophosphorus insecticides are known as powerful inhibitors of acetylcholinesterase [1]. The consequences of this effect are increased excitability, convulsions and muscular paralysis which take place before death of poisoned animals [2–6]. However, there are some symptoms of organophosphorus insecticide toxicity, such as memory and visual disturbances, the immune system alternation which cannot be related to the inhibition of acetylcholinesterase [2, 6, 7]. The exact molecular mechanisms of insecticide action are poorly understood in general.

Due to their lipophilic character insecticides can accumulate in the lipid rich biomembranes which can be taken into account as possible target and sites of immediate and chronic insecticide action.

To further understand the membrane mechanisms affected by the insecticides, it is essential to relate the membrane effects to the actual membrane concentrations, i.e. to the partion coefficient. Insecticide partitioning is affected by multiple parameters, namely temperature, cholesterol content, fluidity, membrane geometry and the intrinsic molecular properties of the compounds [8–12].

In the present work the partition coefficient of malathion (0,0-dimethyl S– (1,2-dicarboxyethyl) phosphorodithioate) and methylparathion (0,0-dimethyl 0– (p-nitrophenyl) phosphorothioate) has been evaluated for membranes of different cholesterol content.

2. MATERIALS AND METHODS

Fragmented pig erythrocyte membranes were obtained according to the method of A u [13]. Liposomes were formed either of pure phosphatidylocholine or from equimoral amounts of phosphatidylcholine and cholesterol. A thin film of desired lipid was hydrated with 50 mM KCl, 10 mM Tris-maleate buffer (pH 8.0) and dispersed under a nitrogen atmosphere shaking by hand at a room temperature. The liposomes was dispersed by vortexing and sonication. The final concentration of lipid was 300 μM in each kind of preparation.

Protein was estimated by the protein-dye binding method of B r a d f o r d [14] with bovine serum albumin as the standard.

Steady-state fluorescence measurements were made with a Perkin-Elmer LS-5B luminescence spectrometer. The theory of the quenching of fluorescence was applied to determine qualitatively the affinity of organophosphorus insecticides to membranes. The quenching of an excited fluorophore in free solution is expected to follow the Stern-Volmer equation

$$\frac{I_o}{I} - 1 = K_{SV}[Q] \tag{1}$$

where I and I_o are the fluorescence intensities in the presence and absence of quencher, respectively, K_{SV} is the Stern-Volmer quenching constant and [Q] is the concentration of quencher. The distribution of a quenching molecule between aqueous (A) and lipid (L) phase may be described by a partition coefficient $K_p = [Q_L]/[Q_A]$ [15]. Thus the Stern-Volmer equation becomes

$$\frac{I_o}{I} - 1 = K_{SV}K_p[Q_A] \tag{2}$$

Since

$$V_T[Q_T] = V_A[Q_A] + L_L[Q_L] \tag{3}$$

where V denotes volume and subscript T referees to either total volume or total concentration, equation (2) becomes

$$\frac{I_o}{I} - 1 = \frac{K_{SV}K_pV_T[Q_T]}{V_A + V_LK_p} \quad (4)$$

The first derivative of I_o/I as a function of K_p is always positive, so the higher value of K_p, the higher coefficient I_o/I. Thus, the value of I_o/I may be considered as a measure of the affinity of a quencher to membrane. The insecticides malathion and methylparathion were assumed to be quenchers. Two fluorescent probes were used, perylene for very hydrophobic region of membrane and ANS (8-anilino-1-napthalene sulfonate) for more hydrophilic domains. Perylene, at a final concentration of 2.5 μM, was excited at 410 nm and emission was measured at 468 nm. For ANS at 25 μM, those parameters were 366 and 480 nm, respectively. Both excitation and emission slits were 5 nm in either case. One-way analysis of variance was used to analyze obtained results. The differences between means were compared by means of the Scheffe's multiple comparison test [16].

3. RESULTS AND DISCUSSION

Three kinds of preparation were used: fragments of the pig erythrocyte membrane, liposomes formed from phosphatidylcholine and liposomes from phosphatidylcholine and cholesterol in equimolar ratio. These preparations were labeled with two fluorescent probes: perylene, which binds to the hydrophobic core of lipids and ANS, which localized in more hydrophilic region of the membrane.

Malathion did not change the ratio I_o/I of any preparation, when either perylene or ANS was used (Figs. 1A and B).

Methylparation increased the value of I_o/I for liposomes of phosphatidylcholine (PC) and erythrocyte membrane as measured with the fluorescent probe perylene (Fig. 2A). The changes caused in liposomes from PC and cholesterol (PC + ChE) were not significant. An increase in the ratio was also observed with ANS-labeled PC liposomes and erythrocyte membranes (Fig. 2B). There were no significant changes for PC + ChE liposomes. For each probe methylparathion increased the value I_o/I in a dose-dependent manner. The changes observed for PC liposomes were more pronounced than for erythrocyte membranes.

In general, it follows from the results that methylparathion has an ability to quench the fluorescence of used probes, whereas malathion either has no such ability or does not bind to membrane at all. The latter statement does not seem to be true in the light of the results of Antunes-Madeira and Madeira [8], who showed that malathion did bind to liposomes prepared from PC.

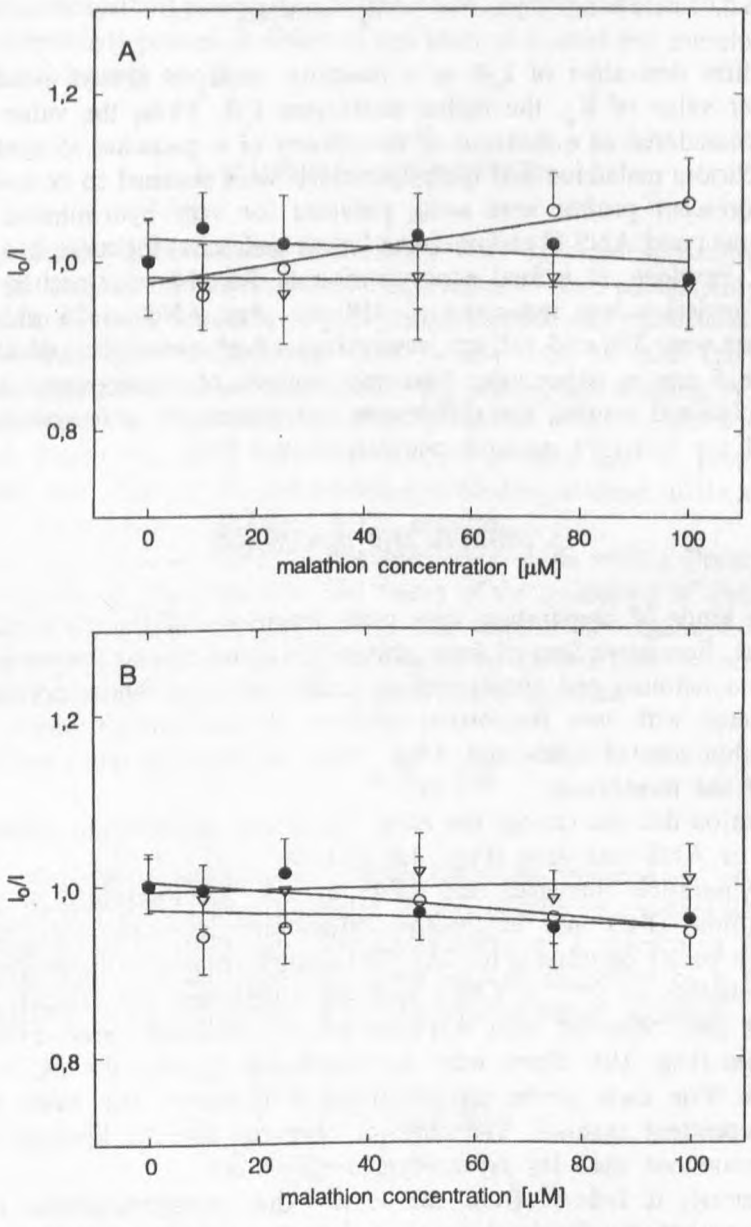

Fig. 1. Quenching of fluorescence of perylene (A) and ANS (B) in the presence of malathion. I_o denotes the fluorescence in the absence of the insecticide, whereas I – in the presence. (○) – erythrocyte membranes, (●) – liposomes of phosphatidylcholine, (▽) – liposomes from phosphatidylcholine and cholesterol mixed in equimolar ratio. Each point represents the mean of five replications. Error bars represent SE

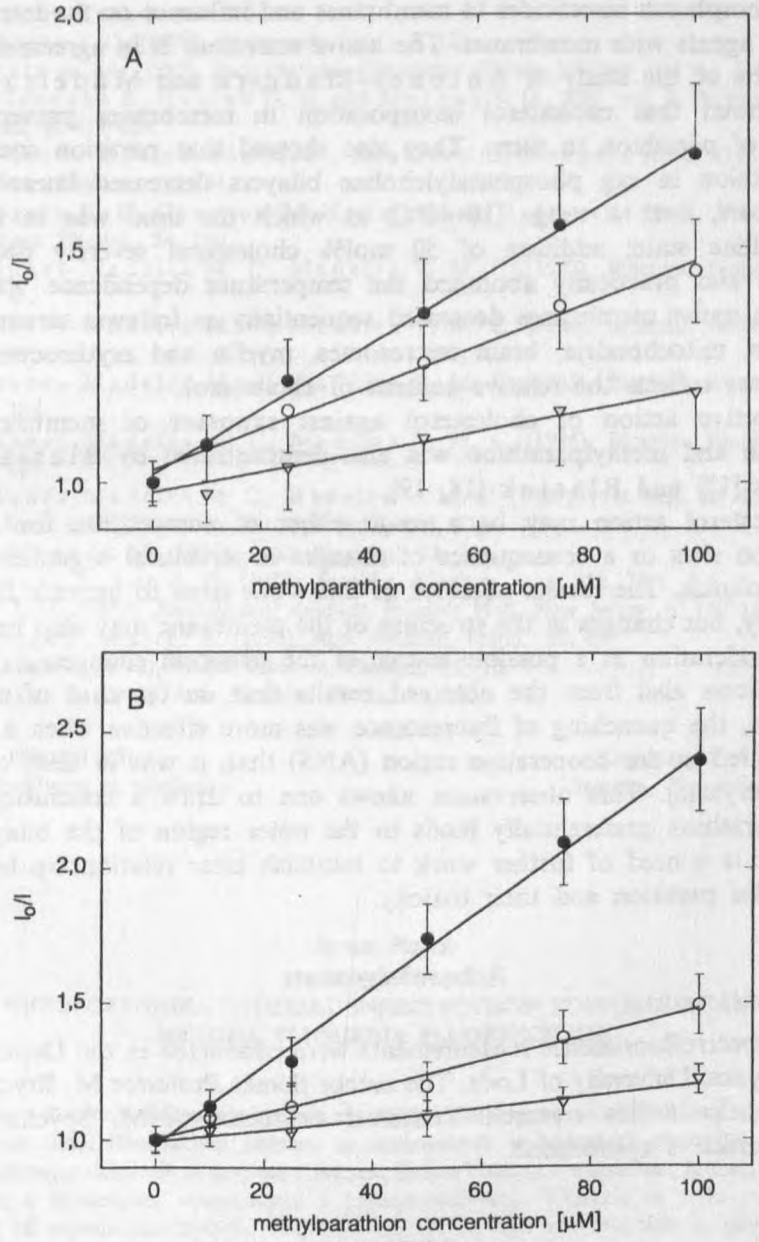

Fig. 2. Quenching of fluorescence of perylene (A) and ANS (B) in the presence of methylparathion. I_0 denotes the fluorescence in the absence of the insecticide, whereas I – in the presence. (○) – erythrocyte membranes, (●) – liposomes of phosphatidylcholine, (▽) – liposomes from phosphatidylcholine and cholesterol mixed in equimolar ratio. Each point represents the mean of five replications. Error bars represent SE

Obtained results indicate that cholesterol can modify the binding of organophosphorus insecticides to membranes and influence on the interaction of these agents with membranes. The above statement is in agreement with the results of the study of Antunes-Madeira and Madeira [8, 9], who showed that cholesterol incorporation in membranes prevents the binding of parathion to them. They also showed that partition coefficient of malathion in egg phosphatidylcholine bilayers decreased linearly with temperature, over a range (10–40°C) at which the lipid was in a liquid-crystalline state; addition of 50 mol% cholesterol severely decreased partition and practically abolished the temperature dependence. Partition values in native membranes decreased sequentially as follows: sarcoplasmic reticulum, mitochondria, brain microsomes, myelin and erythrocytes; this dependence reflects the relative content of cholesterol.

Protective action of cholesterol against exposure of membranes to malathion and methylparathion was also demonstrated by Blasiak and Walter [17] and Blasiak [18, 19].

Cholesterol action may be a result either of competition for similar interaction sites or a consequence of changes in structural organization of phopspholipids. The results obtained in this work seem to indicate the first possibility, but changes in the structure of the membrane may also be taken into consideration as a possible source of the observed changes.

It follows also from the obtained results that, in the case of methylparathion, the quenching of fluorescence was more effective when a probe was situated in the cooperative region (ANS) than it was in deep core of lipids (perylene). This observation allows one to draw a conclusion that methylparathion preferentially binds to the outer region of the bilayer.

There is a need of further work to establish clear relationship between insecticides partition and their toxicity.

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Department of Molecular
Genetics, University of Łódź
Poland

Janusz Błasiak

OCENA WSPÓŁCZYNNIKA PODZIAŁU INSEKTYCYDÓW FOSFOROORGANICZNYCH METODĄ TŁUMIENIA FLUORESCENCJI

Badając tłumienie fluorescencji przez malation i metyloparation stwierdzono, że pierwszy związek nie tłumi fluorescencji żadnego ze stosowanych w badaniach znaczników (perylen i ANS). Opisując tłumienie za pomocą równania Sterna-Volmera stwierdzono, że metyloparation wiązał się z liposomami utworzonymi z fasfatydylocholiny. Wiązanie to wykazywało silną zależność od stężenia insektycydu. Wiązanie insektycydu było znacznie słabsze, gdy liposomy tworzone były z różnych ilości molowych fosfatydylocholiny i cholesterolu. Tłumienie fluorescencji było wyraźniejsze, gdy stosowano znacznik wiążący się z rdzeniem dwuwarstwy lipidowej (perylen), niż wówczas, gdy stosowano znacznik wiążący się z bardziej zewnętrznym obszarem dwuwarstwy (ANS). Uzyskane wyniki wskazują, że wiązanie niektórych insektycydów fosforoorganicznych może być zmieniane przez cholesterol.