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Partition of malathion in synthetic and native membranes

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Partition coefficients of [^{14}C]malathion in model and native membranes are affected by temperature, cholesterol content, and lipid chain length. Partition in egg phosphatidylcholine bilayers decreases linearly with temperature, over a range (10–40 °C) at which the lipid is in the liquid-crystalline state. Addition of 50 mol% cholesterol severely decreases partition and practically abolishes the temperature dependence. First-order phase transitions of dimyristoyl-, dipalmitoyl- and distearoylphosphatidylcholines (DMPC, DPPC and DSPC) are accompanied by a sharp increase in malathion partition. Apparently, the insecticide is easily accommodated in bilayers of short-aliphatic-chain lipids, since the partitions were 225, 135 and 48 in DMPC, DPPC and DSPC, respectively, at temperatures 10 Cdeg below the midpoint of their transitions. Partition values in native membranes decrease sequentially as follows: sarcoplasmic reticulum, mitochondria, brain microsomes, myelin and erythrocytes. This dependence parallels the relative content of cholesterol and is similar in liposomes of total extracted lipids, although the absolute partitions showed decreased values.

Introduction

Organophosphorus insecticides or their metabolites are powerful inhibitors of acetylcholinesterase [1–4]. This inhibition results in accumulation of acetylcholine in neuromuscular junctions, producing hyperexcitability, convulsions, muscular paralysis and respiratory failure, events which precede death in poisoned animals [1,3–6]. In mammals, other markers of toxicity unrelated to acetylcholinesterase inhibition may chronically develop, namely: memory and visual disturbances, schizophrenia, depression, ataxia and alterations in immune system [1,4,6–8]. Malathion, owing to its generally good insecticidal properties and its low mammalian toxicity related to the rapid detoxication in mammals compared to insects, is probably the most widely used organophosphorus insecticide [1,9]. Its wide and repeated utilization leads to situations where man and animals are heavily exposed, with consequent occurrence of poisoning [10]. Therefore, it is of continuing interest to know exactly its precise biochemical mechanisms, not yet completely understood.

As most insecticides, malathion is a lipophilic compound and, therefore, it is likely to accumulate in membrane lipid moieties en route to its target sites. Its effects in well-defined membrane mechanisms have been a matter of study in our laboratory in the past few years [11–14] in an effort to define the molecular basis of toxicity. The results suggest that malathion may exert certain of its biochemical toxic effects by altering the

Abbreviations: Malathion, *O,O*-dimethyl *S*-(1,2-dicarboxyethyl)phosphorodithionate; DMPC, dimyristoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; DSPC, distearoylphosphatidylcholine; T_c, midpoint temperature of thermotropic transition.

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structure, function and activity of biological membranes and membrane-associated enzymes.

To characterize further the nature of malathion interaction, we now report its partitioning in several model and native membranes, since no systematic information is available, and most studies requring partition data have inadequately relied on partition coefficients in solvents.

Materials and Methods

Biological material

Liposomes were prepared by established procedures [12] except that buffer contained 50 mM KCl and 10 mM Tris-maleate (pH 7.0). Cholesterol-containing liposomes were obtained by supplementing original lipid solutions with appropriate amounts of cholesterol up to 50 mol%. Liposomes of mitochondrial lipid extracts were prepared at pH 8.5, vortexing five times for 30 s each time, at room temperature, as indicated elsewhere [15]. Preparation of liposomes with lipids from erythrocyte ghosts is also facilitated by using this methodology [15]. Native membranes were obtained as described before [16].

Determination of partition coefficients

The partition coefficients of [^{14}C]malathion were determined as previously described for [^{14}C]parathion [16]. Incubations of membrane suspensions (528 μM in lipid) with malathion (10^{-6} M) were carried out for 1 h. Data were analysed as described previously [16], by means of Connors' equation [17], which in our conditions can be rewritten as:

$$K_p = \frac{p}{1.22L(1-p)} \cdot 10^6$$

This equation relates the fraction of malathion retained in the membrane (p) and the amount of lipid (L, nmol) with the partition coefficient (K_p).

Reagents

Egg phosphatidylcholine (type V-E), cholesterol, DMPC, DPPC and DSPC, at least 98% pure, were obtained from Sigma. [^{14}C]Malathion (15 mCi/mmol) was obtained from Amersham International, U.K.

Results and Discussion

Partition of malathion into liposomes

The partition of malathion into egg phosphatidylcholine bilayers decreases from 120 at 10 °C to about 70 at 40 °C, as reported in Fig. 1A. Egg phosphatidylcholine bilayers remain in the fluid state over the temperature range under study (10–40 °C), since the phase transition is centered at −5 °C [18]. The highest partition is reduced to about 10% in bilayers supplemented with 50 mol% cholesterol (Fig. 1) and temperature dependence is abolished. This observation prompted us to study the effect of the relative concentrations of cholesterol in malathion partitioning. Data of Fig. 1B clearly indicate that the partition is maximal in the absence of cholesterol and is gradually depressed as the cholesterol content is increased. An apparent inverse linear relationship between the partition of malathion and the molar ratio of cholesterol was detected. This linear dependence is statistically significant (correlation coefficient −0.98) and the slope of this linear function is −2.184. Extrapolation of the theoretical curve to abscissa predicts a zero partition at about 50 mol% cholesterol. This effect of cholesterol assumes a general character, since it has been observed previously for parathion [16], lindane [19–21], DDT [22] and other drugs [23–30], whose partition coefficients also decrease with the cholesterol content. The effects of cholesterol on membrane fluidity and geometry [31] allow us to predict the withdrawal of malathion (and other drugs) from the membrane. Data also suggest that the membrane composition and the structural order of phospholipids modulated by intrinsic and extrinsic parameters determines malathion incorporation. In order to understand better the influence of these parameters on malathion partitioning, we studied its incorporation further in model membranes of DMPC, DPPC and DSPC, i.e., lipids with the same headgroup, but differing in aliphatic chain length. Additionally, the effect of temperature was also investigated. In all cases, malathion incorporated maximally within the temperature range of cooperative phase transition. Maximal partitions were 270, 244 and 174 for DMPC, DPPC and DSPC, respectively (Fig. 2A). The increased disorder and coexistence of do-

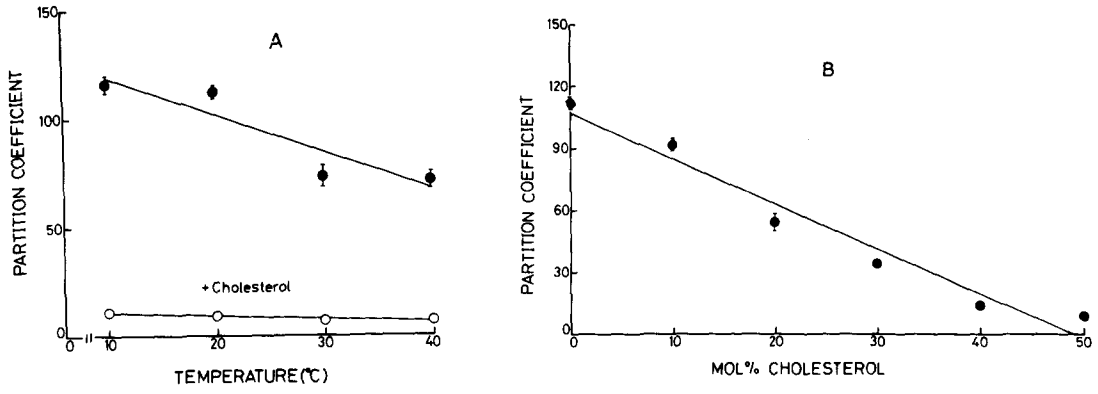
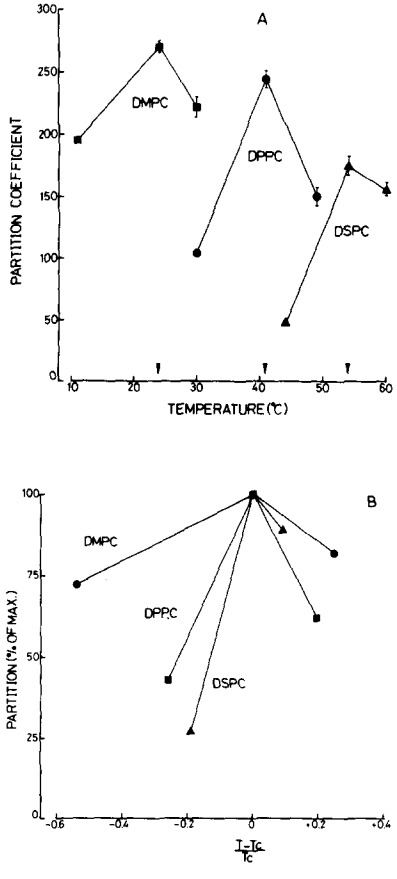


Fig. 1. Partition coefficients of malathion in egg phosphatidylcholine membranes as affected by temperature. The partition decreases to about 10% when 50 mol% cholesterol is incorporated in bilayers (open symbols). Note that partition of malathion has a negative dependence of temperature which is much more apparent for membranes without cholesterol. Part B describes the effect of increasing concentrations of cholesterol at 20 °C. The partition would theoretically approach zero for about 50 mol% cholesterol (abscissa intersection). Vertical traces indicate the range of S.D.



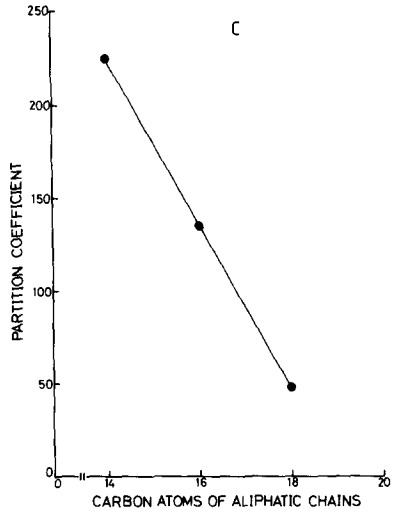


Fig. 2. Partition coefficients of malathion into bilayers of DMPC (■), DPPC (●) and DSPC (▲), as a function of temperature. Malathion incorporates maximally at the midpoint temperatures of thermotropic phase transitions (T_c values for DMPC, DPPC and DSPC are 24, 41 and 54 °C, respectively, as indicated by the arrow-heads). Malathion partitioning decreases sharply above and below the range of phase transition. Normalized data are replotted in part B at a 'reduced temperature scale'. Partition of malathion is a function of the aliphatic chain length (C) at temperatures 10 Cdeg below the midpoint of the transitions.

mains in distinct physical states at the phase transition create conditions which facilitate incorporation of malathion. Identical results have been observed previously for other insecticides and other drugs [16,21,22,30,32].

The data of Fig. 2A also show that malathion incorporation decreases below and above the midpoint temperature of the phase transition. Additionally, it appears that malathion is preferentially accommodated in the fluid phase, since partitions are significantly higher at temperatures above than below the midpoint of phase transition. The gel phase, owing to the molecular packing of individual lipids with increased chain-chain interactions, excludes malathion from the hydrophobic membrane moiety. Above the phase-transition temperature, the disordered bilayer structure [33] permits better accommodation of malathion. However, the molecular shapes of individual lipids and malathion may also modulate malathion incorporation as a consequence of complementary relationships.

Independently of temperature, malathion incorporates better in bilayers of short-aliphatic-chain lipids, as illustrated in Fig. 2C. A good correlation (correlation coefficient about -0.99) between the partition of malathion and the chain length of phospholipids has been observed at temperatures 10 Cdeg below the midpoint of their phase transitions. These findings again suggest that malathion preferentially interacts with fluid phases, since short-aliphatic-chain lipids produce membranes with higher fluidity relatively to those formed by long-chain species [33]. This conclusion is substantiated by the fact that the rate of partition dependence on temperature significantly increases with the length of acyl chains, since the slopes of normalized curves are highest for DSPC and lowest for DMPC, as concluded from Fig. 2B with data plotted on a 'reduced temperature scale', where the reduced temperature is defined as $T_r = (T - T_c)/T_c$.

Partition coefficients of malathion in native membranes and their lipid dispersions

The partitioning of malathion was also studied in a variety of biomembranes, namely: mitochondria, sarcoplasmic reticulum, brain microsomes, erythrocyte ghosts, myelin and liposomes obtained from their extracted lipids. This study is of obvious interest, since the conclusions derived from models of synthetic lipids cannot be readily extrapolated to real membranes, although they may facilitate the interpretation of results.

Malathion incorporates poorly in native membranes as well as in dispersions of total lipid extracts (Fig. 3). Furthermore, the incorporation, either in native membranes or their lipid dispersions, depends considerably on the membrane type and composition and follows the sequence: sarcoplasmic reticulum > mitochondria > microsomes > myelin ≥ erythrocytes (Fig. 3). Temperature controls malathion incorporation, but no consistent action was detected among the membrane types under study. Thus, mixed negative and positive profiles were found in native membranes, over the temperature range from 10–37 °C. On the other hand, a negative dependence on temperature was consistently observed for bilayers prepared with lipids extracted from sarcoplasmic reticulum, mitochondria, brain microsomes and myelin, as in

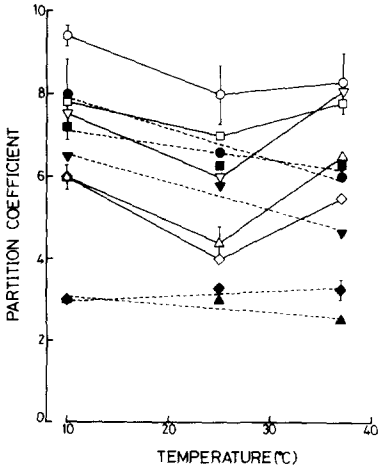


Fig. 3. Partition coefficients of malathion into native membranes (open symbols) and representative lipid dispersions (solid symbols) as a function of temperature. ○, sarcoplasmic reticulum; □, mitochondria; ▽, brain microsomes; △, myelin; ◇, erythrocytes. Elsewhere within the temperature range, malathion partitioning into native membranes changes from a negative to a positive slope. The partition in dispersions of total extracted lipids decreases with temperature, an effect more marked for sarcoplasmic reticulum and brain microsomes and not observed in erythrocytes. As for the other figures, vertical traces indicate the range of S.D. For some points, the range of S.D. is encompassed in the size of symbols.

the case of egg phosphatidylchline liposomes, and a slightly positive temperature coefficient was observed in lipid dispersions of erythrocyte ghosts.

A linear decrease of insecticide partition is observed with the increase of cholesterol molar concentration, either in intact membranes or their lipid representatives (Fig. 4), i.e., a behaviour similar to the observed for egg phosphatidylcholine bilayers, where a complete exclusion of malathion was theoretically extrapolated at 50 mol% cholesterol. Theoretically, native membranes and respective lipid dispersions show complete exclusion of malathion only at 83 and at 66 mol% cholesterol, respectively. These results may reflect the heterogeneous lipid composition of the biomembranes and interaction of cholesterol with specific phospholipids [34], inducing lateral phase separations. Some of the microdomains may be specially sensitive to malathion incorporation. Moreover, native membranes incorporate an excess of malathion over the related lipid dispersions. The presence of proteins in native membranes favours malathion incorporation presumably occurring in lipid-protein boundaries relatively scarce in cholesterol [35]. Therefore, besides cholesterol as modulator of malathion partitioning, other parameters, namely, temperature, lipid-protein composition and geometrical factors may also have significant contributions.

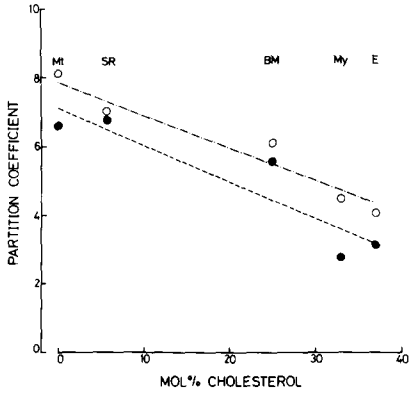


Fig. 4. Dependence of malathion partitioning on the intrinsic cholesterol content of native membranes (open symbols) and representative lipid dispersions (solid symbols). These data were taken from Fig. 3 (partitions at 24°C). Cholesterol/phospholipid molar ratios for mitochondria (Mt), sarcoplasmic reticulum (SR), brain microsomes (BM), myelin (My) and erythrocytes (E) are 0, 6, 25, 33 and 37 mol%, respectively.

The present in vitro investigations provide evidence to suggest that the insecticide malathion preferentially accumulates in highly functional membranes, namely, mitochondria and sarcoplasmic reticulum. Similar results have been obtained for parathion [16], lindane [21] and DDT [22].

Therefore, the accumulation of malathion and other insecticides in biological structures cannot be estimated on the basis of classical octanol/water partitioning.

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