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Biochimica et Biophysica Acta 1238 (1995) 22–28

BBA Biochimica et Biophysica Acta

Partition of DDE in synthetic and native membranes determined by ultraviolet derivative spectroscopy

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Received 28 December 1994; revised 27 March 1995; accepted 30 March 1995

Abstract

Partition coefficients of DDE (2,2-bis(*p*-chlorophenyl)-1,1-dichloroethylene) were determined, in model and native membranes, as a function of temperature, lipid chain length, cholesterol content and DDE concentration, by means of second derivative ultraviolet spectrophotometry. DDE incorporation increases with the temperature, since the partition values in dimyristoylphosphatidylcholine (DMPC), at 24, 30 and 37°C, are 5722 ± 138, 10356 ± 763 and 14006 ± 740, respectively. The insecticide incorporates better into bilayers of DMPC as compared with DPPC (dipalmitoylphosphatidylcholine). The partition decreases from 10355 ± 763 in DMPC to 6432 ± 613 in DPPC, at temperatures 5–7°C above the midpoint of their transitions. The addition of cholesterol to fluid membranes of DMPC depresses the partition of DDE. In agreement with the results in models of synthetic lipids, the partition of DDE into native membranes increases with the temperature and decreases with the intrinsic cholesterol. It is concluded that a fluid membrane favors the accumulation of DDE.

Keywords: DDE; Partition coefficient; Model membrane; Native membrane; Fluidity

1. Introduction

DDE, the major metabolite of DDT, is also very persistent and, consequently, widely distributed in the environment [1,2]. Its persistence associated with its lipophilicity and low water solubility [3], render it amenable to the progressive accumulation in tissues of man and members of ecological chains in general. Evidence of the environmental problems associated with DDT and some of its metabolites, namely, DDE, led the Environmental Protection Agency to ban the use of DDT [4]. However it is still under usage in several Third World countries [5]. Therefore, the studies involving DDT or its metabolites are of continuing interest.

DDE, in spite to its structural similarity to DDT [2], has little insecticidal activity in parallel with a small effect on the nerve potential [2,6,7]. Therefore, DDE effects are not readily comparable with its insecticide power. DDE has been increasingly implicated in the eggshell thinning in sensitive species of birds and, consequently, in the decline of several species, namely, predators and fish-eating birds [4]. In this respect DDE has being more active than its parental insecticide DDT [4]. DDE is also more efficient than DDT in the inhibition of the ATP-dependent Ca^{2+}-binding and the Ca^{2+}/Mg^{2+}-activated ATPase of avian eggshell gland mucosa cells [8,9]. Therefore, these biochemical mechanisms are putatively involved in the eggshell thinning. Furthermore, the ability of DDE to affect other ATPase enzymes, namely, trout brain Mg^{2+}-ATPase [10], rat brain $(Na^+ + K^+)$-ATPase [11] and the Ca^{2+}-ATPase of brush-border membranes from human placenta [12] is clearly established. Additionally, DDE depresses the respiratory transport chain [13–15] and the oxidative phosphorylation [14] in mitochondria. Moreover, DDE has antihemolytic action in human erythrocytes [16],

Abbreviations: DDE, 2,2-bis(*p*-chlorophenyl)-1,1-dichloroethylene; DDT, 2,2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane; DMPC, dimyristoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; DPH, 1,6-diphenyl-1,3,5-hexatriene; Py(3)Py, 1,3-di-(1-pyrenyl)propane.

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SSDI 0005-2736(95)00102-6

increases the permeability properties of lipid membranes [17] and induces changes in the organization of model and native membranes [18,19]. The effects of DDE in these biochemical and biophysical mechanisms are, in most cases, similar to those observed for its parental compound DDT.

The above results generally indicate that biomembranes are important target sites of DDE action. Consequently, it is important to relate biochemical and biophysical effects to the partition coefficients, to provide a consistent basis to evaluate the molecular interaction of DDE with membrane constituents.

2. Materials and methods

2.1. Materials

DDE (chromatographic grade) was obtained from Chemservice (West'Chester, UK). Dimyristoylphosphatidylcholine (DMPC), dipalmitoylphosphatidylcholine (DPPC), cholesterol and 4-(2-hydroxyethyl)-1-piperizineethanesulfonic acid (Hepes) were obtained from Sigma.

2.2. Methods

Preparation of liposomes and native membranes

Liposomes from synthetic lipids were prepared by established procedures [20] except that buffer contained 50 mM NaCl and 10 mM Hepes (pH 7.4). Hepes was chosen since it does not absorb in the UV range of interest, where DDE absorbs. Several native membranes, namely, mitochondria, sarcoplasmic reticulum and brain microsomes were prepared as described elsewhere [21]. The protein contents were determined by the biuret method [22], calibrated with serum albumin. Lipid extraction was performed as previously described [23,24]. Phospholipids were quantitated by measuring inorganic phosphate [25] released after hydrolysis of extracts at 180°C in 70% $HClO_4$ [26]. Cholesterol in lipid extracts was assayed by the Lieberman-Bürchard method [27] and expressed relatively to the phospholipid content. Model and native membranes were briefly sonicated to disperse aggregates and get homogeneous suspensions with a turbidity equivalent to 0.22 A, measured in a Spectronic 21 spectrophotometer at 600 nm, 1 cm light path.

Model and native vesicles with the required lipid concentrations were prepared by dilution of stock and let to equilibrate overnight, before use.

Determination of partition coefficients

The partition coefficients of DDE were determined by means of derivative spectrophotometry as previously described for the anticancer drugs tamoxifen and hydroxytamoxifen [28], with appropriate modifications. The period of equilibration of DDE with membrane suspensions in buffer was 30 min, at required temperatures, before recording of spectra. The concentration of DDE was 10 μM, except when the effect of various DDE concentrations on the partition were tested (Fig. 6).

The present method explores a blue shift in the absorption spectra of DDE when it is removed from the buffer to the lipid phase. The characteristic absorbance peaks in buffer and in the lipid are 256 nm and 248 nm, respectively. To determine DDE incorporated in the lipid phase, the second derivative spectra were recorded (Fig. 1). The overall change in absorbance (A), after addition of DDE,

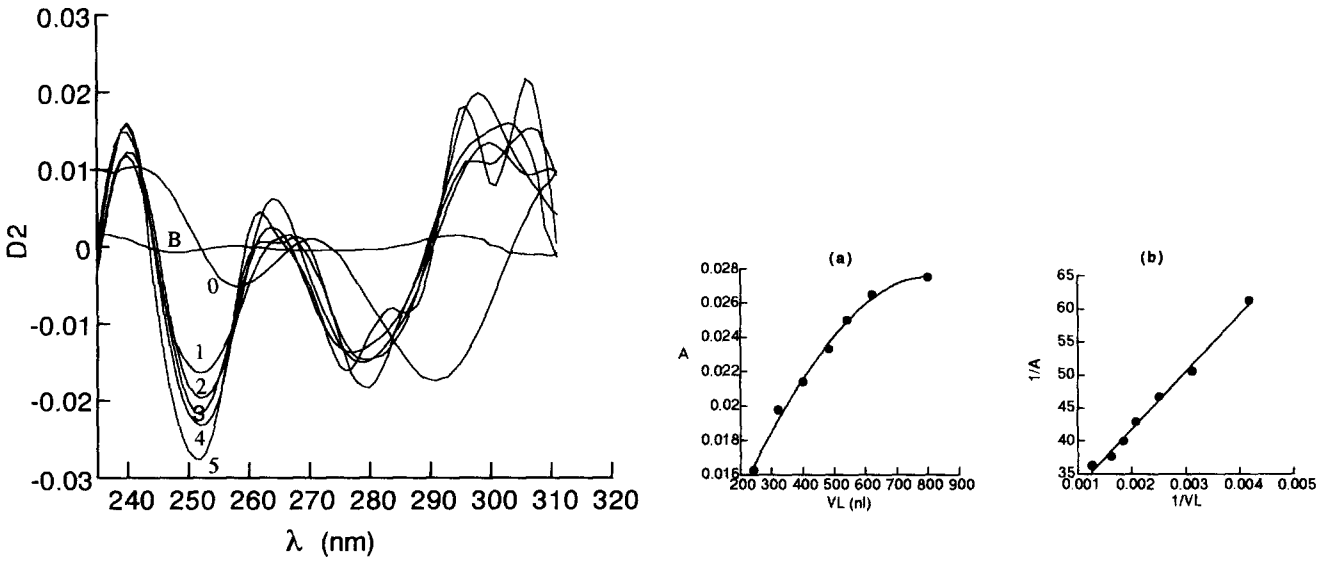

Fig. 1. Second derivative absorption spectra of 10 μM DDE in DMPC membranes at different lipid concentrations. The nominal concentrations of DMPC in suspensions was 0 (curve 0), 180 (1), 240 (2), 300 (3), 360 (4) and 600 (5). Inset (a) depicts the relationship between the second derivative absorbance (A) and the membrane lipid volume (V_L). The double-reciprocal plot of these data is represented in the inset (b).

was measured as the vertical distance between the baseline and the minimum value of the peak of the second derivative spectrum. The amplitude of the peak and, consequently, the second derivative absorbance (A) increases with the increase in the lipid concentration. Thus, at high lipid concentrations, A approaches its maximum (A_{max}) which corresponds to a complete incorporation of DDE. A plot of A versus lipid volume (V_L), assuming the lipid specific volume as 0.984 μl/mg [29], yields a curve (Fig. 1, inset a). A_{max} was determined from a double-reciprocal plot (Fig. 1, inset b). The calculation of the amount of DDE in the membrane correspondent to each lipid concentration is $Q_L = (A/A_{max})$. Q_T, where Q_T is the total amount of added DDE.

The partition coefficient is expressed by $K_p = C_L/C_W$ [30], where C_L and C_W are molar concentrations of DDE in the lipid and aqueous phases, respectively. The total amount of added DDE is $V_T\ C_T = V_L\ C_L + V_W\ C_W$ or $Q_T = Q_W + Q_L$, were V, C and Q state for volume, concentration and DDE amount, respectively, and the subscripts T, L and W state for total, lipid phase and aqueous phase, respectively.

Rearrangements give $K_p = (Q_L/V_L)/(Q_W/V_W)$. Replacing $Q_W = Q_T - Q_L$, it follows that $[K_p(Q_T - Q_L)]/V_W = Q_L/V_L$ or $1/V_L = [(K_p\ Q_T)/V_W]\,(1/Q_L) - (K_p/V_W)$ which represents a linear function of $1/V_L$ versus $1/Q_L$ with an ordinate intercept (b) given by $-K_p/V_w$ and, therefore, K_p may be calculated as: $K_p = -b\ V_w$.

The normal and second derivative spectra were recorded in a Perkin-Elmer Lambda 6 UV-visible spectrophotometer and the automatic base-line correction mode was used for all spectra recordings.

3. Results and discussion

3.1. Partition coefficients into pure phospholipid bilayers

As illustrated in Fig. 2, the partition of DDE was studied in pure DMPC membranes in a temperature range from 24 to 37°C, i.e., in the liquid-crystalline state [31]. Partition at temperatures below the phase transition temperature were also theoretically estimated. However, we are not confident about these results since instability of the spectrometric signal was noticed, probably as consequence of water condensation at cuvette surfaces. The partitions at 24, 30 and 37°C are 5722 ± 138, 10356 ± 630 and 14006 ± 740, respectively. Although over this range DMPC membranes are in the fluid state [31], the relative degree of fluidity increases with the temperature either in the bilayer inner core [32] or in the outer regions of the bilayer [33]. Thus, the temperature and, consequently, the fluidity modulates the incorporation of DDE. This conclusion is extensive to DPPC bilayers since the partitions of DDE at 30 and 46°C are 5115 ± 718 and 6432 ± 613, respectively.

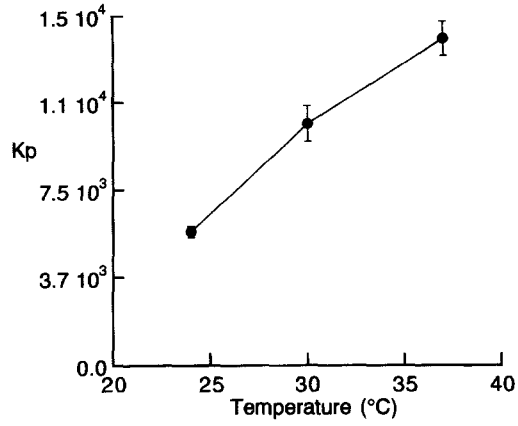

Fig. 2. Partition of DDE into DMPC bilayers, as a function of temperature. Over the temperature range under study, these bilayers are in the liquid-crystalline state, since the phase transition is centered at 23–23.5°C. As in subsequent figures, vertical bars indicate standard deviations of the mean for three separate determinations..

This temperature dependence has been also observed by Chefurka et al. [3], by the use of discontinuous sucrose gradient centrifugation.

As shown in Fig. 3, the increase in chain length of membrane lipids, which decreases the fluidity [34], results in a partition quenching. For example, the partition decreases from 10356 ± 763 in DMPC to 6432 ± 613 in DPPC, at temperatures 5–7°C above the midpoint of their transitions. Previous studies involving fluorescence quenching of carbazoles [35] also indicate that DDE [36] as well as lindane [37] interact preferentially with short-chain as compared with long-chain lipid species. Furthermore, it has been shown by the use of radioactive labeling, that DDT, lindane, parathion and malathion partitioning decreases with the increase in chain-lipid species [21].

The above results indicate that membrane fluidity, as modulated by the temperature and lipid chain length, significantly determines the incorporation of DDE. As revealed by fluorescent studies [19], the distribution of the compound across the bilayer thickness is modulated by the

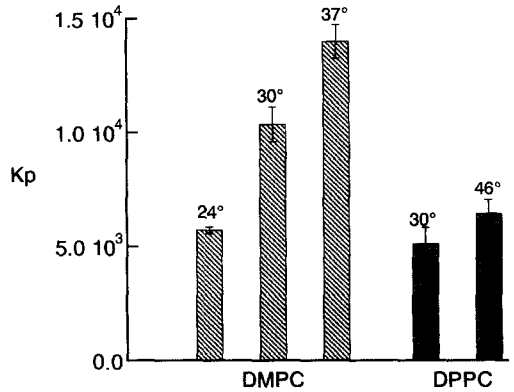

Fig. 3. Partition of DDE into DMPC and DPPC bilayers, as affected by temperature. An increase in chain length by two carbon atoms (bilayers of DPPC) results in significant partition quenching.

bilayer fluidity gradient, which is larger in the gel than in the fluid state [38]. Thus, in the gel condition, DDE distributes, preferentially, in the fluid interior of the bilayer, inducing an increase in fluidity, as reported by DPH, also located in the bilayer core [39]. Py(3)Py excimerization is silent to this modification owing the probe localization in the outer regions of the bilayer [40], not reached by DDE in the gel phase. A preferential distribution in the bilayer center, in the gel phase, has been also previously postulated for its parental compound DDT [41]. In the fluid phase of the lipid, a fluidity gradient is still apparent with the inner core more fluid relatively to domains closer to the surface [38]. In this condition, DDE has a wider distribution across the bilayer thickness, and induces no apparent effect in the high fluid bilayer core but orders the outer regions due to an increase in packing density [19]. Similar effects have been previously described for DDT [41]. Thus, DDE, as well as DDT, populates the fluid domains of the bilayer, i.e., the inner core in the gel phase and the entire width in the fluid phase. Therefore, the fluidity of the bilayer modulates the distribution and accumulation of DDE. However, recent work on the free volume theory [42] indicates that in fluid membranes, the free volume increases with the temperature and decreases with the addition of cholesterol. Thus, the free volume reflected by fluidity accounts for the distribution and accumulation of DDE.

3.2. Partition of DDE into phospholipid-cholesterol bilayers

The partitions of DDE into fluid bilayers of DMPC, as affected by cholesterol, are represented in Fig. 4. An inverse relationship between the partition of DDE and the molar ratio of cholesterol was noticed. This conclusion, in opposition to that of Omann and Glaser [36], agrees with data reported for other insecticides [43–46] and drugs in general [47–54]. As indicated in Fig. 4, the partition values of DDE for 0, 10 and 30 mol% of cholesterol are 5722 ± 138, 1583 ± 180 and 804 ± 122, respectively. However, the decrease in K_p is steeper when the molar ratio of cholesterol increases from 0 to 10 mol% than when it changes from 10 to 30 mol%. Fluorescence data [19] indicate that the physical effects of DDE in membranes with low cholesterol are identical to those observed for pure phospholipid bilayers. Therefore, it has been suggested the occurrence of DMPC-DDE rich domains separated from those containing cholesterol. However, this does not explain the dramatic decrease of partition in membranes with low cholesterol concentrations. It has been postulated, previously, that fluid phospholipid membranes can exist in a liquid-disordered phase, at low cholesterol concentrations, a liquid-ordered phase, at high cholesterol concentrations, or these two phases for intermediate cholesterol concentrations [55]. It has also been postulated that in liquid-disordered phase, cholesterol molecules have a wider distribution across the bilayer thickness [56] and its primary effect is related to occupancy of the free volume [42]. Therefore, the occupancy of free volume opposes the distribution of DDE and, consequently, the partition decreases sharply (Fig. 4). The liquid ordered phase induced by the packing of cholesterol with lipids in the cooperativity region [57] allows to a regular distribution of DDE and, consequently, to a monotonic decrease in K_p (Fig. 4), at high cholesterol concentrations. Also interesting is the fact that very high cholesterol concentrations ($\geq$ 30 mol%), at which the entire bilayer is in a liquid-ordered condition [55], do not prevent DDE incorporation. Additionally, the inner bilayer core and the outer regions of the bilayer are fluidized by DDE, as revealed by fluorescence studies [19]. It has been postulated that DDE increases the intermolecular distances of phospholipids in order to be accommodated with cholesterol in the cooperativity region. Similar results have also been reported for DDT [41]. Unlike DDE and DDT, lindane [32,33], parathion [58] and malathion [59] fail to affect cholesterol-rich membranes. Partition data concur well with these observations, since a complete exclusion of these insecticides is observed for cholesterol concentrations of about 50 mol% [21]. Therefore, a localization identical to that of cholesterol has been suggested for these compounds [32,33,58,59]. Conversely, highly apolar molecules, e.g., DDT and DDE are accommodated in the hydrophobic inner core of the membrane, thus explaining the significant partitions even at high cholesterol which populates the outer bilayer regions.

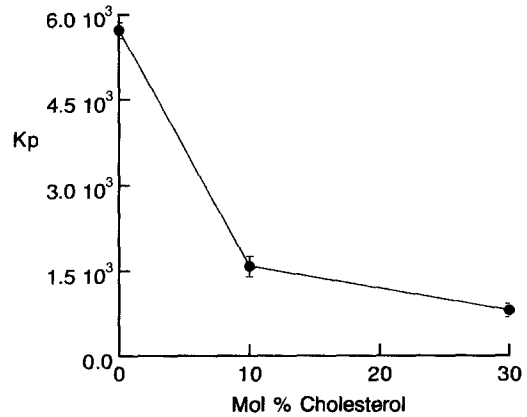

Fig. 4. Partition of DDE into DMPC bilayers enriched with cholesterol, at 24°C. The decreasing in K_p is stronger when the molar ratio of cholesterol increases from 0 to 10 mol% than when it changes from 10 to 30 mol%.

3.3. Partition into native membranes

Data illustrated in Fig. 5 indicate that partition of DDE into native membranes depends on the membrane type and composition, since the partition values are higher in mitochondria than in sarcoplasmic reticulum. For example, at a

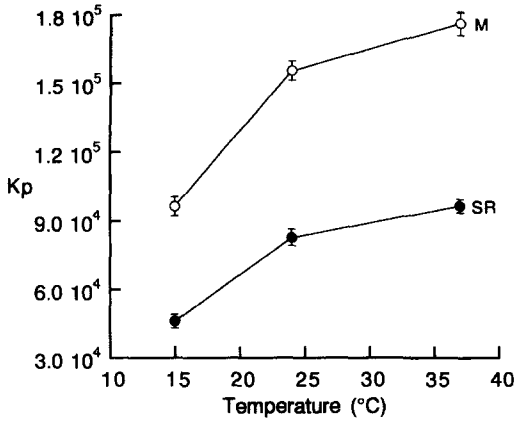

Fig. 5. Partition of DDE into fluid native membranes of mitochondria (○) and sarcoplasmic reticulum (●), as a function of temperature. As in models of synthetic lipids, the partition increases with the temperature.

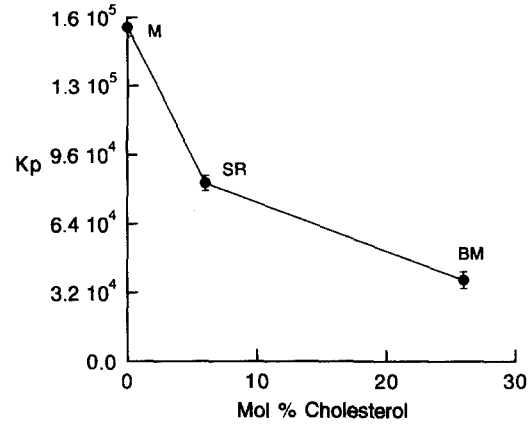

Fig. 7. Dependence of DDE partitioning on the intrinsic cholesterol content of native membranes, at 24°C. Cholesterol/phospholipid molar ratios for mitochondria (M), sarcoplasmic reticulum (SR) and brain microsomes (BM) are 0, 6 and 25 mol%, respectively.

temperature of 15°C, the partitions in mitochondria and sarcoplasmic reticulum are 96440 ± 4150 and 46280 ± 3015, respectively. Fig. 5 also illustrates that the incorporation of DDE increases with the temperature. Although, in the range of the temperature studied (from 15 to 37°C), native membranes are in the fluid state [60], the relative degree of fluidity increases with the temperature [32]. Thus, as in model of synthetic lipids (Fig. 2 and Fig. 3), the incorporation in native membranes increases with the temperature and, consequently, with the fluidity.

The effect of DDE concentration (total concentration of added DDE) on the partition was also studied in sarcoplasmic reticulum, at 24°C (Fig. 6). The partition increases up to a concentration of 10 μM, whereas it decreases sharply to higher values, suggesting saturation of the membrane with DDE. Indeed, membrane saturation occurs when the molar ratio $[DDE]_{total}/[lipid]$ is about 0.2. Saturation was also noticed for model membranes of DMPC (not shown).

The effect of native cholesterol on the partition of DDE was also investigated in mitochondria, sarcoplasmic reticulum and brain microsomes, where cholesterol accounts for 0, 6 and 25 mol%, respectively. As in models of synthetic lipids (Fig. 4), DDE partitioning decreases with the increase of the relative content of cholesterol (Fig. 7), i.e., with the decrease in fluidity [32]. Interesting also is the fact that partition decreases sharply when the molar ratio of cholesterol increases from 0 to 6 mol% than when it changes from 6 to 25 mol% (Fig. 7), similarly to the observations in model membranes (Fig. 4).

Also interesting is the fact that partition values are higher in native membranes than in models of synthetic lipids. Partition values are expected to be higher in native membranes than in respective lipid dispersions as previously shown for parathion [43], lindane [44], DDT [45] and malathion [46]. The presence of proteins in native membrane favors the incorporation of insecticides [43–46]. The extra incorporation presumably occur in the lipid-protein boundaries relatively scarce in cholesterol [61].

The partition studies in native membranes associated with previous fluorescence data [19] clearly indicate a distribution of DDE across the bilayer thickness identical

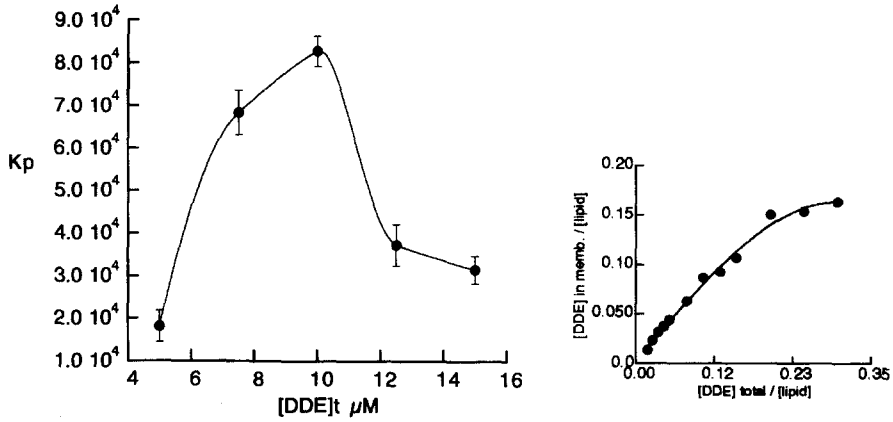

Fig. 6. Partition coefficients of DDE into native membranes of sarcoplasmic reticulum, as a function of various DDE concentrations added to the medium. The inset represents the molar fraction of DDE in membrane versus the ratio $[DDE]_{total}/[lipid]$.

to that proposed for models of synthetic lipids. Thus, DDE populates the hydrophobic inner core of fluid native membranes and extends to the cooperativity region. An identical distribution has been proposed for DDT [62].

4. General conclusions

The results in model and native membranes indicate that DDE preferentially accumulates into fluid membranes. Similar results have been obtained for DDT [45], parathion [43], lindane [44] and malathion [46], although DDE and DDT partitionings are significantly higher according to the following order: DDE ≈ DDT ≫ lindane > parathion > malathion.

As revealed by fluorescence data [19] in association with the present partition studies, the distribution of DDE across the bilayer thickness is also modulated by the fluidity, or, more precisely, by the bilayer fluidity gradient. Also, an important parameter modulating DDE distribution is its structure itself. Identical conclusions have been obtained for the other insecticides [21,32,33,41,58,59,62].

The proposed distribution of DDE suggest that it preferentially affects the lipid–lipid and lipid–protein interactions occurring in the hydrophobic inner core and extending to the cooperativity region, as previously suggested for DDT [41,62]. Therefore, the differences in effects of DDE and DDT on biochemical mechanisms and, consequently, the toxicologic behavior, is certainly related to the small molecular differences of these compounds.

Acknowledgements

This work was supported by grants of JNICT.

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