Biochimica et Biophysica Acta 861 (1986) 159–164
Elsevier

BBA 73293

Partition of DDT in synthetic and native membranes

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(Received 11 June 1986)

Key words: DDT; Partition coefficient; Insecticide–membrane interaction; Cholesterol; Model membrane; Phase transition

Partition of DDT (2,2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane) was determined in artificial and native membranes. Partition in egg phosphatidylcholine of about 260 000 is independent of temperature over the range from 10 to 40°C, in which the lipid is in the liquid-crystalline state. Incorporation of 50 mol% cholesterol decreases DDT partition to about 120 000. First-order phase transitions of dimyristoyl-, dipalmitoyl- and distearoylphosphatidylcholines (DMPC, DPPC and DSPC) are accompanied by a sharp increase in DDT partitioning. Partition decreases symmetrically in the temperature ranges to both sides of the phase transition. The insecticide is preferentially accommodated in bilayers of short-aliphatic-chain lipids, since the partitions were 336 000, 180 000 and 88 000 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, myelin, brain microsomes and erythrocytes. This sequence is similar to that observed in related liposomes of total extracted lipids, although the absolute partitions showed decreased values. Partition of DDT in native membranes exhibits a negative temperature coefficient not apparent in related lipid dispersions. The effect of intrinsic membrane cholesterol on partition of DDT was also investigated.

Introduction

DDT, a persistent and lipophilic insecticide, has been of great benefit to man in the increase of agricultural production and control of vector-borne diseases, such as malaria. However, the persistence and lipophilicity of this insecticide induces progressive accumulation in tissues of man and other species. Therefore, toxic concentrations may be reached and consequent tremors, prostration and occasional convulsions often proceeding to death [1–5]. Additionally, resistance to DDT has developed in many species [4]. Therefore, this compound has been largely banned from agricultural use in developed countries, although it is still in use to combat insect vectors of disease in several countries. Consequently, it is of continuing interest to establish the extent of undesirable toxic effects in useful insects, other animals and man himself. Furthermore, experimental data might help in the development of analogs with reduced environmental persistence and with greater biological selectivity.

In the past two decades, great efforts to define the molecular basis of DDT toxicity have been relatively unsuccessful. However, its lipophilic

Abbreviations: DDT, 2,2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane; DMPC, dimyristoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; DSPC, distearoylphosphatidylcholine.

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character suggests its action at the membrane level, as indicated by several studies. DDT alters specific properties of ion channels in axon membranes responsible for delayed repolarization of the action potential and repetitive activity of the nerve [4–11]. Furthermore, DDT affects membrane ATPases associated with transport and energy transduction [12–22]. On the other hand, DDT alters the permeability of liposomes and native membranes to K^+ [23–25] as well as to other electrolytes and non-electrolytes [26–27]. It has been also shown that DDT affects the thermotropic properties of lipid bilayers [28–32]. The relationship of the mentioned effects to the neurotoxic action or overall toxic action of DDT in vivo is still unclear. However, the overall data suggest that biomembranes are good candidates for target sites of acute and delayed DDT action. Consequently, it is essential to relate the membrane effects to the actual bilayer concentration of DDT, i.e., to its membrane partition reported in this work.

Materials and Methods

Liposomes were prepared as described previously [28]. Lipid extraction and analysis were performed as previously described [33] and cholesterol was estimated relative to the phospholipid content. Native membranes were isolated as described elsewhere [33].

Partition coefficients of [^{14}C]DDT were determined as previously described for [^{14}C]parathion [33]. Incubations of membrane suspensions (1.3 μM in lipid) with DDT (0.065 μM) were carried out for 2 h. Data were analysed as previously [33] by means of an equation [34] relating the fraction of DDT retained in the membrane (p) and the amount of lipid (L, nmol) with the partition coefficient (K_p). In our conditions, the following equation applies:

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

Results and Discussion

Partitioning of DDT in liposomes

The organochlorinated insecticide, DDT, concentrates about 260 000-fold in egg phosphatidylcholine bilayers relative to the buffer phase, over the temperature range from 10 to 40 °C (Fig. 1A). Over this temperature range, egg phosphatidylcholine bilayers are in the liquid-crystalline state, since the phase transition is centered at −5 °C [35]. The partition decreases to about one-half of the control value when 50 mol% cholesterol is incorporated in bilayers (Fig. 1). This observation prompted us to study the dependence of DDT partitioning on sterol concentration. A linear inverse relationship between the partition of

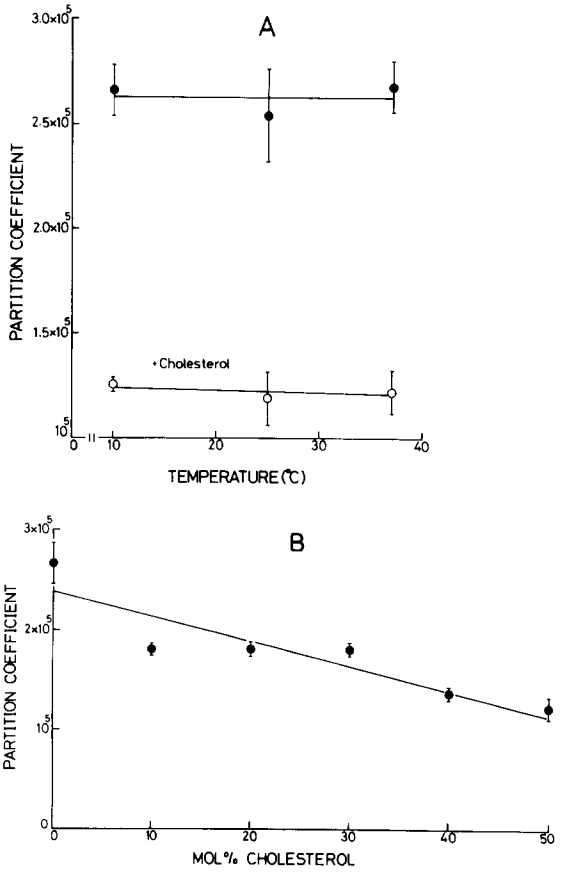

Fig. 1. Temperature dependence of DDT partition coefficients in egg phosphatidylcholine bilayers. These bilayers remain in the liquid-crystalline state over the temperature range under study. Partition of DDT decreases to about 46% when 50 mol% cholesterol is incorporated in bilayers (open symbols). No dependence on temperature was observed in both types of membranes, since the partition profiles remain almost flat over the entire temperature range (10–40 °C). Part B shows the effect of increasing concentrations of cholesterol at 37 °C.

DDT and the molar ratio of cholesterol was detected (Fig. 1B). The slope of this linear function is −2593 and the correlation coefficient −0.91. Apparently, cholesterol excludes DDT from membranes with an efficiency lower than that observed for other insecticides, namely, parathion and lindane. These are completely excluded when the molar ratio of cholesterol reaches 50 mol%, i.e., 1 : 1 stoichiometry relative to phospholipid [33,36]. DDT would be completely excluded at a theoretical cholesterol concentration of about 94 mol%, i.e., if cholesterol were the major component.

The data in Fig. 2 compare the partition coefficients of DDT into membranes of DMPC, DPPC and DSPC, i.e., lipids with identical head groups, but differing in the chain length of their fatty acids. Aqueous dispersions of these phospholipids undergo structural changes at discrete temperature ranges, i.e., the aliphatic chains of the phospholipids change from a gel crystalline phase to a liquid crystalline phase [37–38]. In all synthetic phosphatidylcholines under study, DDT incorporates maximally within the range of the phase transition temperature. The maximal partitions for DMPC, DPPC and DSPC were 412000, 234000 and 170000, respectively, as illustrated in Fig. 2. The thermal reorganization of phospholipids at the phase transition and the consequent structural oscillations in discrete domains (coexistence of gel and liquid-crystalline phases) creates packing defects in the membrane which presumably favour the incorporation of DDT. Below an above the phase transition temperature, where the bilayer structure is either more ordered or disordered, a sharp decrease in partition is observed (Fig. 2). Irrespectively of the temperature, DDT incorporates to a greater extent in bilayers of short-aliphatic-chain lipids. As illustrated in Fig. 2C, an increase in chain length by two carbon atoms results in a partition quenching of about 50%. A good correlation (correlation coefficient about −0.99) between the partition of DDT and the chain length of phospholipids has been observed at temperatures 10 Cdeg below the midpoint of their phase transitions. Since short-chain lipids produce membranes with higher fluidity relatively to those formed by long-chain species [39], it can be concluded that membrane fluidity determines to some extent the partitioning of DDT.

Also interesting is the fact that the rate of partition dependence on temperature significantly increases with the length of the 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 $T_r = (T - T_c)/T_c$.

Apparently, the structural order of lipids mod-

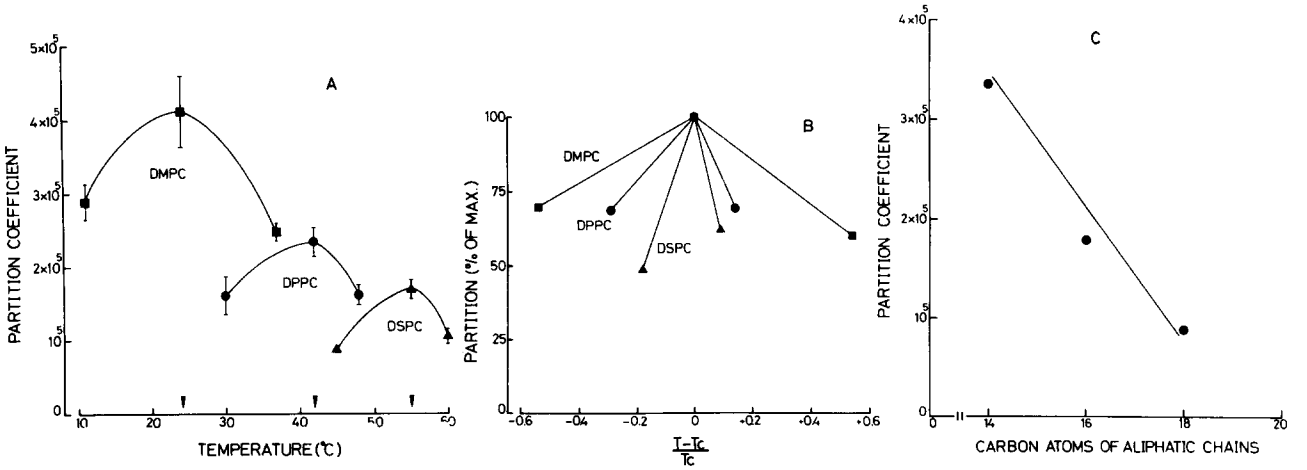

Fig. 2. Partition coefficients of DDT into bilayers differing in aliphatic chain lengths, DMPC (■), DPPC (●) and DSPC (▲), as a function of temperature. Partition reaches maximal values at the midpoint temperatures of thermotropic phase transitions, 24, 42 and 54°C for DMPC, DPPC and DSPC, respectively, as indicated by the arrowheads. Partition profiles are approximately symmetric on both sides of the phase transition. Normalized data are replotted in part B at a 'reduced temperature scale'. Partition of DDT is a function of the aliphatic chain length (C) at temperatures 10 Cdeg below the midpoint of the transitions.

ulated by temperature does not directly affect DDT incorporation, since identical values of K_p were found in gel and fluid states of lipids. Therefore, molecular geometrical factors and the molecular structure of lipids may determine to some extent the interaction of DDT with membranes.

Partition coefficients of DDT in native membranes and their lipid dispersions

Partition of DDT was also studied in native membranes and liposomes of their total lipid extracts. Several representative membranes were deliberately chosen, namely, sarcoplasmic reticulum, mitochondria, brain microsomes, myelin and erythrocytes, since they can be obtained in a pure state, avoiding contaminants which would lead to misinterpretations. Therefore, these well-defined systems may be taken as general prototypes of biomembranes.

Data presented in Fig. 3 indicate that DDT is incorporated to a greater extent in native membranes than in respective lipid dispersions. 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 > myelin > microsomes > erythrocytes. Interesting also is the fact that a negative dependence on temperature over the range from 10 to 37°C was observed for native membranes, but not for their relative lipid dispersions. DDT partition decreases with temperature, an effect more marked for sarcoplasmic reticulum membranes. The negative temperature coefficient for partitioning is consistent with a predominantly hydrophobic interaction [40] and mirrors the negative temperature coefficient of insecticidal potency [4,10]. However, no dependence on temperature was observed in lipid dispersions, since the partition profiles remain almost flat over the entire temperature range (Fig. 3). Therefore, lipid-protein interphases are presumably related to the negative temperature coefficient of DDT poisoning.

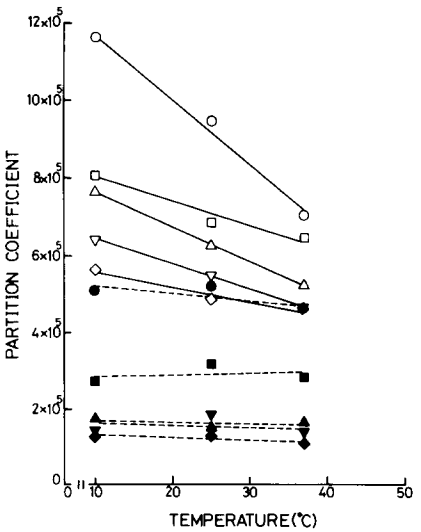

Fig. 3. Partition of DDT into native membranes (open symbols) and representative lipid dispersions (solid symbols) as a function of temperature. ○, sarcoplasmic reticulum; □, mitochondria; △, myelin; ▽, brain microsomes; ◇, erythrocytes. DDT partitioning into native membranes decreases with increasing temperature; this negative temperature coefficient observed in native membranes is not apparent in dispersions of total extracted lipids.

Comparing the results obtained in native membranes with those of total lipid dispersions, it is clear that membrane proteins together with surrounding lipids determine to a large extent the partitioning of DDT. Partition of DDT is also apparently dependent on the amount of membrane intrinsic cholesterol (Fig. 4), but a linear correlation was not observed as in the case of parathion and lindane [33,36]. However, a linear correlation is apparently approached at 37°C in native membranes. Native sarcoplasmic reticulum and, to a smaller extent, myelin deviate significantly from the apparent linear correlation existing for mitochondria, brain microsomes and erythrocytes (Fig. 4). These deviations are mainly determined by the protein compositions, but lipids of sarcoplasmic reticulum also have a significant contribution.

Despite the observed deviations, cholesterol is presumably a main parameter controlling distribution of DDT. Cholesterol effectively excludes DDT from membranes, although to a lesser extent than parathion and lindane [33,36]. These two compounds are completely excluded when membrane cholesterol approaches 50 mol%, i.e., when the stoichiometry cholesterol : phospholipid is 1 : 1. Previously, we have interpreted this effect in terms of strong interactions between the sterol and phospholipid preventing void volumes for insecticide

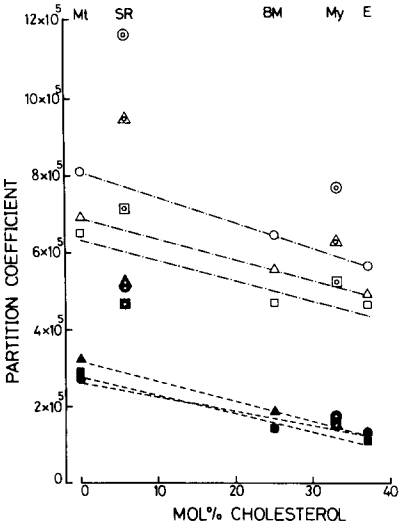

Fig. 4. Dependence of DDT partitioning on the intrinsic cholesterol content of native membranes (open symbols) and representative lipid dispersions (solid symbols). Cholesterol/phospholipid molar ratios for mitochondria (Mt), sarcoplasmic reticulum (SR), brain microsomes (BM), myelin (My) and erythrocytes (E) are 0, 5.8, 25, 33 and 37 mol%, respectively. ○, results obtained at 10°C; △, at 25°C, and □, at 37°C. Symbols with an internal small circle denote membranes (sarcoplasmic reticulum and myelin) not fitting the linear correlation.

incorporation. The main effect of cholesterol is presumably mediated by the overall structure of the bilayer. Therefore, the mutual interaction of DDT and membrane lipids differs from other insecticide compounds. It is tentatively suggested that the DDT planar molecule may align and coexist with the planar moiety of cholesterol in the region of membrane cooperativity.

According to the measured partitions, DDT would preferentially accumulate in functional membranes of organelles. Similar results have been obtained for parathion [33] and lindane [36]. However, partition of DDT is much higher: DDT ≫ lindane > parathion. Nevertheless, perturbations induced by parathion are dramatically extensive as compared with those induced by DDT and lindane [26–28,41], in a straight correlation with the higher toxicity of parathion [1]. Therefore, partition coefficients, although important, are not readily related to toxic potency.

Acknowledgements

This work was supported by I.N.I.C. and the Calouste Gulbenkian Foundation.

References

1 Metcalf, R.L. (1971) in Pesticides in the Environment (White-Stevens, R., ed.), Vol. I, Part I, pp. 67–144, Marcel Dekker, New York
2 Portman, J.E. (1975) Proc. R. Soc. London, Ser. B 189, 291–304
3 Ware, G.W. (1978) The Pesticide Book, pp. 27–52, W.H. Freeman, San Francisco
4 Brooks, G.T. (1974) in Chlorinated Insecticides (Zweig, G., ed.), Vol. II, pp. 130–144, CRC Press, Cleveland, OH
5 O'Brien, R.D. (1978) in Biochemistry of Insects (Rockstein, M., ed.), pp. 515–539, Academic Press, New York
6 Narahashi, T. and Haas, H.G. (1967) Science 157, 1438–1440
7 Narahashi, T. and Haas, H.G. (1968) J. Gen. Physiol. 51, 177–198
8 Doherty, J.D. (1979) Pharmacol. Ther. 7, 123–151
9 van den Bercken, J. and Vijverberg, H.P.M. (1980) in Insect Neurobiology and Pesticide Action, pp. 79–85, Society of Chemical Industry, London
10 Beeman, R.W. (1982) Annu. Rev. Entomol. 27, 253–281
11 Narahashi, T. (1983) in Pesticide Chemistry: Human Welfare and the Environment, Vol. 3 (Matsunaka, S., Hutson, D.H. and Murphy, S.D., eds.), pp. 109–114, Pergamon Press, Oxford
12 Jackson, D.A. and Gardner, D.R. (1978) Pestic. Biochem. Physiol. 8, 123–128
13 Matsumura, F. and Patil, K.C. (1969) Science 166, 121–122
14 Schneider, R.P. (1975) Biochem. Pharmacol. 24, 939–946
15 Ghiasuddin, S.M. and Matsumura, F. (1979) Pestic. Biochem. Physiol. 10, 151–161
16 Ghiasuddin, S.M. and Matsumura, F. (1981) Biochem. Biophys. Res. Commun. 103, 31–37
17 Patil, T.N. and Koch, R.B. (1979) Pestic. Biochem. Physiol. 12, 205–215
18 Huddart, H., Greenwood, M. and Williams, A.J. (1974) J. Comp. Physiol. 93, 139–150
19 Huddart, H. (1977) Comp. Biochem. Physiol. 58C, 91–95
20 Price, N.R. (1976) Comp. Biochem. Physiol. 55C, 91–94
21 Ohyama, T., Takahashi, T. and Ogawa, H. (1982) Biochem. Pharmacol. 31, 397–404
22 Khan, H.M. and Cutkomp, L.K. (1982) Bull. Environ. Contam. Toxicol. 29, 577–585
23 Hilton, B.H. and O'Brien, R.D. (1970) Science 168, 841–843
24 Chefurka, W., Zahradka, P. and Bajura, S.T. (1980) Biochim. Biophys. Acta 601, 349–357
25 Ahmad, P. and Chefurka, W. (1982) Biochim. Biophys. Acta 689, 135–142
26 Antunes-Madeira, M.C. and Madeira, V.M.C. (1979) Biochim. Biophys. Acta 550, 384–392
27 Antunes-Madeira, M.C., Carvalho, A.P. and Madeira, V.M.C. (1981) Pestic. Biochem. Physiol. 15, 79–89

28 Antunes-Madeira, M.C., Carvalho, A.P. and Madeira, V.M.C. (1980) Pestic. Biochem. Physiol. 14, 161–169
29 Buff, K. and Berndt, J. (1981) Biochim. Biophys. Acta 643, 205–212
30 Buff, K., Bründl, A. and Berndt, J. (1982) Biochim. Biophys. Acta 688, 93–100
31 Bach, D. and Sela, B. (1981) Biochem. Pharmacol. 30, 1777–1780
32 Bach, D. and Sela, B.-A. (1984) Biochem. Pharmacol. 33, 2227–2230
33 Antunes-Madeira, M.C. and Madeira, V.M.C. (1984) Biochim. Biophys. Acta 778, 49–56
34 Connors, K.A. (1967) in A Textbook of Pharmaceutical Analysis, pp. 277–294, J. Wiley & Sons, New York
35 Bittman, R. and Blau, L. (1976) J. Chem. Educ. 53, 259–261
36 Antunes-Madeira, M.C. and Madeira, V.M.C. (1985) Biochim. Biophys. Acta 820, 165–172
37 Chapman, D. (1974) in Biomembranes – Lipids, Proteins and Receptors (Burton, R.M. and Packer, L., eds.), pp. 65–74, Science Publications Division, Webster Groves, MO
38 Lee, A.G. (1977) Biochim. Biophys. Acta 472, 237–281
39 Lenaz, G. (1979) Subcell. Biochem. 6, 233–343
40 Tanford, C. (1980) The Hydrophobic Effect, 2nd. Edn., p. 233, Wiley, New York
41 Antunes-Madeira, M.C. and Madeira, V.M.C. (1982) Pestic. Biochem. Physiol. 17, 185–190