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Partition of DDT and DDE into Membranes and Extracted Lipids of *Bacillus stearothermophilus*

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DDT (2,2-bis(*p*-chlorophenyl)- 1,1,1-trichloroethane) and its metabolite DDE (2,2-bis(*p*-chlorophenyl)-1,1-dichloroethylene) are very persistent contaminants and, consequently, widely distributed in the environment (Brooks 1974). Persistence associated to lipophilicity allows accumulation in tissues of man and members of ecological chains in general, with consequent undesirable toxic effects. Although the biochemical basis of DDT and DDE toxicities are still not clearly identified, the strong lipophilic character of the compounds suggests that biomembranes are potential target sites of acute and chronic actions. Previous experimentation and data indicate that these types of compounds exert their effects after incorporation into biomembranes (Antunes-Madeira and Madeira 1986; Videira et al. 1995), changing their physical properties (Antunes-Madeira and Madeira 1990, 1993), and altering membrane function (Antunes-Madeira et al. 1981; Antunes-Madeira and Madeira 1982: Lundholm and Mathson 1983). These membrane effects would gain increased significance if related with toxic effects at the organism level. Therefore, microorgamsms are often used as suitable models to couple the studies evaluated at the molecular with the organism level (Sikkema et al. 1995). The accumulation of lipophilic compounds will disturb membrane fluidity and function and, consequently, impair cell viability and growth (Calder and Lader 1976). Therefore, the toxic effects of lipophilic compounds on microorganisms can be easily monitored and may be helpful to understand basic mechanisms of action.

Bacillus stearothermophilus has been extensively used in our laboratory to study the toxic effects of several xenobiotics, namely, DDT and DDE. It has been shown that the physical perturbations induced by DDT (Donato et al. 1997) and DDE are similar to those described previously for several representative native membranes of eukaryotic cells (Antunes-Madeira and Madeira 1990, 1993). Furthermore, the effects of DDT and DDE in respiratory activity (one of the main functions of the bacterial plasma membrane) are similar to those observed for the respiratory activity of rat liver mitochondria (Moreno and Madeira 1991; Ferreira et al. 1997). Additionally, the increased inhibition of respiration enzymes by DDT as compared with DDE parallels its higher inhibitory effect on bacterial growth, indicating a higher level of toxicity of the former compound. These results induced us to study the partition of DDT and DDE into bacterial membranes, since it has been only determined in models of synthetic lipids and in native membranes of eukaryotic cells (Antunes-Madeira and Madeira 1986; Videira et al. 1995). Therefore, the partition of DDT and DDE into bacterial membranes would be relevant to relate membrane effects to the actual bilayer concentration. Additionally, partition data will provide valuable information for understanding the effects of the above xenobiotics on cell growth and activities relevant to their toxicities These studies

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demonstrated that the specific lipid composition of the bilayer, the temperature and the chemical nature of the compounds strongly influence partitioning.

MATERIALS AND METHODS

Liposomes were prepared from lipids of *Bacillus stearothermophilus* according to Donato et al. (1997). Aliquots from bacterial lipids in $CHCl_3$, containing 0.134 mg of lipid, were evaporated to dryness in a rotatory evaporator. The dry residues were hydrated under N_2 atmosphere at 50 °C by gentle shaking with 0.5 mL of 50 mM KCl, 10 mM Tris-maleate, pH 7.0. Then, the suspensions were vortexed for 1 min and stabilized overnight.

Native membranes were prepared from cells of *Bacillus stearothermophilus.* Cells were chilled on ice and harvested by low-speed centrifugation. The pellets were washed three times with buffer (40 mM Hepes-Tris, pH 7.5), resuspended in the same buffer, and stored at -20 °C. The cells, after equilibration at room temperature, were sonicated with a Vibracell Sonicator model VC-100 (Sonics & Materials Inc., Danbury, Connecticut, USA) for 10-15 min with pulses of 6 sec until the suspension turned translucent. The broken cells were then centrifuged at 16000 x g for 10 min, and the supernatant collected and centrifuged at 148000 x g for 90 min at 20 °C. The membranes were resuspended in the buffer. The lipid content was determined in lipid extracted by measuring the amount of phosphate (Bartlett 1959) after hydrolysis of the extract at 180 °C in 70 % $HClO_4$ (Böttcher et al. 1961).

Partitionings of [^{14}C]DDT and [^{14}C]DDE were determined as previously described for [^{14}C]parathion (Antunes-Madeira and Madeira 1984). Incubations of membrane suspensions (1.3 µM lipid) with DDT or DDE (0.065 µM) were carried out for 2 h. Aliquots of 0.5 mL were rapidly filtered through Whatman GF/B filters (Whatman International Ltd., Maidstone, UK), under vacuum, and the filters transferred into vials containing 8 mL Universol scintillation fluid (ICN Research Products Division, Costa Mesa, California, USA). After several hours of equilibration, the radioactivity was counted in a Packard 2000 spectrometer (Packard Instrument Company Inc., Downers Grove, Illinois, USA) programmed with dpm correction. Data were analyzed as previously described (Antunes-Madeira and Madeira 1984) by means of an equation (Connors 1967) relating the fraction of DDT or DDE retained in the membranes (p) and the amount of lipid (L, nmol) with the partition coefficient (Kp). In our conditions the following equation applies:

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

The average of at least six independent measurements was estimated and standard deviations calculated.

RESULTS AND DISCUSSION

The incorporation of DDT and DDE into polar (Fig. 1A) and total (Fig. 1B) lipid dispersions of *Bacillus stearothermophilus* was studied at the phase transition temperature of bacterial lipids (at 27 °C for polar lipids and at 29 °C for total lipids), below the phase transition (at 15 °C) and above it (at 40 °C). DDT and DDE incorporated maximally at the phase transition temperature (Fig. 1). Maximal partitions for DDT into polar lipids and total lipids were 7.7 x 10^5 and 6.9 x 10^5, respectively. For DDE, maximal partitionings were 5.4 x 10^5 and 4.7 x $l0^5$ into polar lipids and total lipids, respectively. The thermal reorganization of

phospholipids in the range of the phase transition creates gel and fluid domains that oscillate at a high rate (Mouritsen and Jorgensen 1994), originating packing defects in the membrane, which presumably favour the incorporation of lipophilic compounds. Studies previously carried out in membranes of synthetic lipids (dimirystoyl, dipalmitoyl and distearoylphosphatidylcholine) also established that DDT and other insecticides incorporate maximally at the phase transition temperature (Antunes-Madeira and Madeira 1989). Below and above the phase transition temperature (Figs. 1A and B), we observed a decrease in DDT and DDE partitioning. Independently of temperature, DDT and DDE incorporated in higher yields into bilayers of polar lipids compared to bilayers of the total lipid fraction. These observations confirm previous studies (Antunes-Madeira and Madeira 1989: Videira et al. 1995) indicating that the specific lipid composition of the bilayer influences partitioning to some extent. Therefore, incorporation of DDT and DDE (and presumably other xenobiotics) is optimized at a certain degree of lipid order, dependent on the temperature and lipid composition. Furthermore, the total lipid fraction contains carotenoids and/or hopanoids that, similarly to cholesterol in other membranes (Antunes-Madeira and Madeira 1986; Videira et al. 1995), may change the membrane organization in such a way that the free volume for xenobiotic incorporation and interaction decreases.

Partitioning studies were extended to the intact native membranes of *Bacillus stearothermophilus* (Fig. 1C). In the range of temperature under study (from 15 °C to 40 °C), a negative temperature coefficient for DDT and DDE partitionings has been observed. A similar phenomenon has been observed and described for DDT partitioning into several native membranes of eukaryotic cells (Antunes-Madeira and Madeira 1986). Therefore, this DDT effect probably mirrors the negative temperature coefficient of its insecticidal activity (Brooks 1974). We also observed a higher partition of DDT relative to DDE (Fig. 1C). Partitions of DDT varied from 7.9×10^5 to 3.8×10^5 and those of DDE from 5.9×10^5 to 2.6×10^5. Preferential incorporation of DDT also occurred in the native membranes, as described above for lipid membranes. Higher partitionings of DDT relative to DDE have also been described previously for other membrane types (Antunes-Madeira and Madeira 1986; Videira et al. 1995). Partition coefficients of DDT into membranes of mitochondria and sarcoplasmic reticulum at 15 °C are 8×10^5 and 11×10^5, respectively, as compared with 9.6×10^4 and 4.6×10^4 for DDE. By comparing the data of native membranes and total lipids (Fig. 1C and Fig. 1B) it is clear, for temperatures below and above the phase transition of total lipids, that membrane proteins together with surrounding lipids determine to a large extent the partitionings of DDT and DDE. Native membranes of eukaryotic cells incorporate an excess of DDT and other insecticides over related lipid dispersions (Antunes-Madeira and Madeira 1989). Since the activities of most membrane enzymes depend on the physico-chemical properties of boundary domains (Lee 1991), the incorporation of strange compounds in these discrete regions will certainly affect protein function.

It can be concluded that the incorporation of DDT and DDE into membranes of *Bacillus stearothermophilus* is very strong as also verified in eukaryotic membranes. The present and previous studies indicate that partitioning of xenobiotics into membranes is affected by several parameters, including the nature of the membrane itself. Therefore, partitioning studies in particular membranes are needed for quantitative estimations of real concentrations of xenobiotics that may come into contact with membrane components. The different partitionings of DDT and DDE, either in prokaryotic or eukaryotic membranes, are modulated by the physico-chemical properties of the membrane and by the chemical nature and structure of the compound. These parameters condition the distribution of the

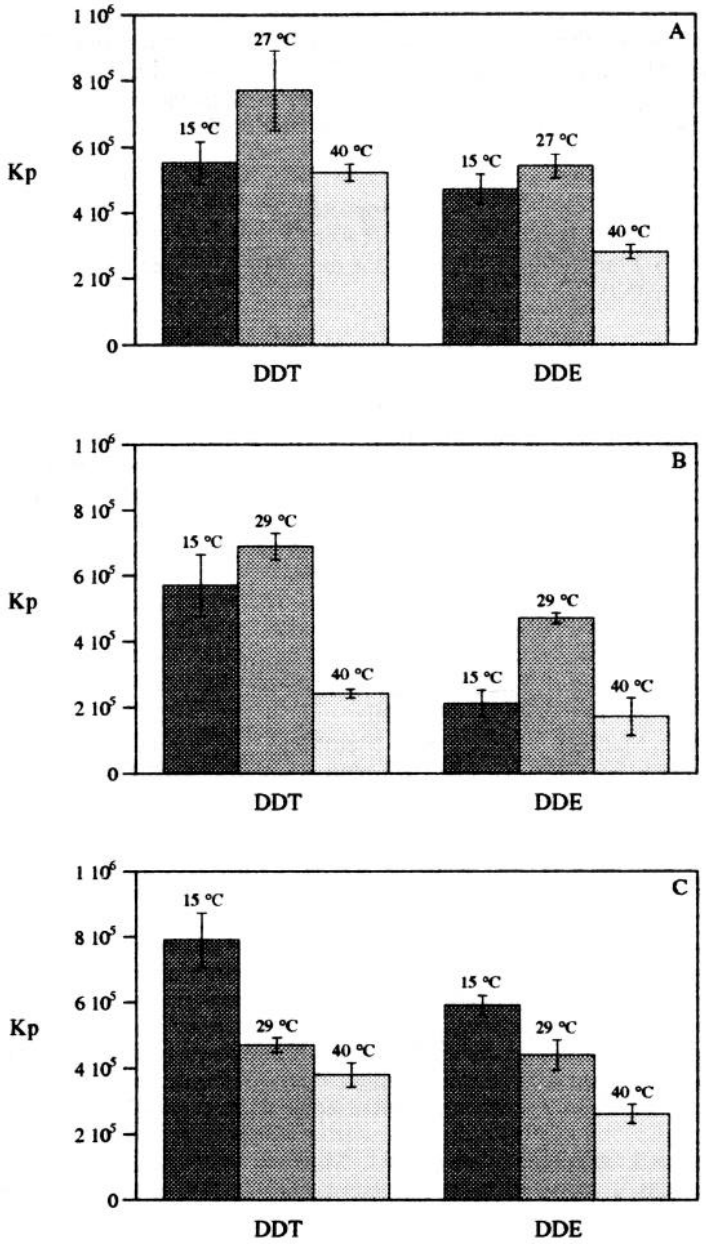

Figure 1 Mean (±SD) partition coefficients (Kp) of DDT and DDE into polar lipid dispersions (A), total lipid dispersions (B), and native membranes (C) of *Bacillus stearothermophilus*, as a function of temperature. Transition temperatures were 27 °C and 29 °C for polar lipid and total lipid bilayers, respectively.

xenobiotic in the bilayer and, consequently, their effects on physical (Antunes-Madeira and Madeira 1990, 1993: Donato et al. 1997) and functional properties of membranes (Antunes-Madeira and Madeira 1982). The similarity of DDT and DDE partitioning into membranes of *Bacillus stearothermophilus* with those observed in eukaryotic membranes suggests the reliability of our bacteria1 model system for toxicity assessment.

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