Perturbation of Lipid Membranes by Organic Pollutants

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Abstract. The ability of a range of organic pollutants—hexachlorobenzene, mirex(1,1a,2,2,3,3a,4,5,5,5a,5b,6-dodecachlorooctahydro-1,3,4-methyleno-1H cyclobuta(cd) pentalene), 1,3,5-trichlorobenzene, 2,4,6-trichlorophenol, p-nitrophenol, p-chlorophenol, DDT, and pentachlorophenol—to perturb liposomes of dipalmitoyl phosphatidylcholine (DPPC) has been measured by differential scanning calorimetry. The degree of perturbation was measured by the increase in breadth of the main DPPC phase transition in both heating and cooling scans. DDT and the phenol derivatives were effective perturbers of phospholipid, broadening the transition by as much as 12-fold. Hexachlorobenzene and mirex did not perturb at all when mixed with DPPC at concentrations as high as 20 mol%, although 1,3,5-trichlorobenzene caused slight broadening of the main transition at this concentration. Perturbation is facilitated by the presence of a hydroxyl group on the benzene ring and hindered by increasing degrees of chloride substitution. An apparent correlation exists between the extent of phospholipid perturbation measured by differential scanning calorimetry and LD₅₀ values for these compounds taken from the literature. This suggests the possibility of formulating an "index of perturbation" which could be used to screen certain classes of organic compounds for potential biological toxicity on a routine basis.

Phospholipid, dried in a thin film, will form multilamellar structures known as liposomes when taken up in aqueous media. Each lamella consists of a lipid bilayer of phospholipid analogous to that found in natural membranes, which is separated from neighboring bilayers by water spaces (Bangham 1972). Multilamellar liposomes will trap electrolytes in their water spaces which are released only slowly over time, suggesting that each bilayer is a closed phospholipid vesicle and can serve as a selectively permeable barrier. Thus, liposomes are, in effect, lipid membranes, and have proved useful as models of natural membranes for elucidating the molecular details of membrane function.

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They will also interact with cells in vitro (Grant and McConnell 1973) and can be injected i.v. into animals where they are rapidly cleared from the blood and taken up by tissues (Juliano and Stamp 1975). These observations have raised the prospect of using liposomes in pharmacological and toxicological applications. For example, chelating agents trapped in liposomes are capable of removing heavy metals from contaminated liver tissue (Rahman et al. 1973). There is some suggestion from both in vitro and in vivo experimental strategies that liposomes could be used to deliver enzymes to tissues where the products of enzyme-deficient storage diseases accumulate (Colley and Ryman 1976; Ryman et al. 1978). Antitumor drugs have also been trapped successfully in liposomes (Gregoriadis 1976) and the efficacy of the liposome as a vehicle for tumor-directed delivery of drugs in vivo has been explored (Kimelberg and Atchison 1978; Tyrrell et al. 1976).

In this study, liposomes of dipalmitoyl phosphatidylcholine (DPPC) were used as a model system to gauge the relative affinities of selected pollutants for natural membranes. Many organic pollutants are soluble in the lipid matrices of cell membranes by reason of their hydrophobic character; they behave as impurities in the lipid bilayer, perturbing its molecular structure and altering the phase properties of the lipid. For pure phospholipids these phase changes can be quantified by differential scanning calorimetry (DSC) of liposomes. This technique establishes a perturbation index for a limited number of organic pollutants which correlates with corresponding LD_{50} values obtained from the literature.

**Materials and Methods**

Reagent grade mirex, hexachlorobenzene, pentachlorophenol, p-chlorophenol, 2,4,6-trichlorophenol, 1,3,5-trichlorobenzene, DDT, and p-nitrophenol were obtained from Analabs, Inc., North Haven, CT and Chem Service Inc., West Chester, PA.

Liposomes were prepared from DPPC in the presence and absence of pollutants as described by Demel et al. 1977. Five ~\mu{} moles of either pure DPPC or DPPC mixed with pollutant at concentrations ranging from 5 to 20 mol% were dissolved in 10 ml of chloroform and then dried under N\textsubscript{2} as a thin film to the inside surface of a 5-ml reaction vial. Residual solvent was removed in vacuo and liposomes were formed by suspending the lipid in 30 ~\mu{}l of 40 mM MES buffer (in 100 mM NaCl), pH 7.0. The suspension was allowed to equilibrate at 60\degree C under N\textsubscript{2} for 15 min before DSC.

A 15 ~\mu{}l aliquot of the liposome suspension was transferred to a DSC volatile sample pan (Perkin-Elmer). The sample was scanned in a Perkin-Elmer DSC-2 at a scanning rate of 5\degree/min and a range of 2 mcal/s in both the heating and cooling modes. The reference pan contained 15 ~\mu{}l of 40 mM MES buffer (in 100 mM NaCl), pH 7.0. The suspension was allowed to equilibrate at 60\degree C under N\textsubscript{2} for 15 min before DSC.

Results

In the absence of the selected pollutants, DPPC undergoes a sharp endothermic transition at 41\degree C, which reflects melting of the fatty acid side chains from a gel