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PHENOTHIAZINE DERIVATIVE CAUSES PHASE SEPARATION IN PHOSPHATIDYLETHANOLAMINE MODEL MEMBRANES

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Phenothiazine derivatives are known as popular antipsychotic drugs and effective multidrug resistance modulators. The interactions of newly synthesised phenothiazine derivative: 2–trifluoromethyl–10–(4–[methylsulfonylamid]buthyl)–phenothiazine with dimyristoylphosphatidylethanolamine (DMPE) was studied by means of microcalorimetry. The drug caused decrease of phospholipid main phase transition temperature. In the range of 0.04–0.1 drug/lipid mole ratio the phase separation was observed. The transition enthalpy change was diminished in the presence of drug, however it stayed fairly constant in the concentration range in which phase separation occurred. The most likely reasons of phase separation in DMPE–phenothiazine derivative system are different spatial orientations that drug molecules might adopt inside the model membrane. It is apparently connected with different kinds of drug's interaction with the hydrogen bonds network crosslinking polar region of DMPE bilayer.

INTRODUCTION

Phenothiazine derivatives – apart from being widely used in psychiatry – are also known as effective multidrug resistance (MDR) modifiers (Ford, Prozialeck & Hait, 1989). The simultaneous resistance of cancer cells to many structurally diverse chemotherapeutic agents has now become a major obstacle to successful cancer treatment. Substances known as MDR modifiers or modulators are able to restore – at least partially – the cells' sensitivity. Proposed mechanism of their action is through the inhibition of multispecific transmembrane transporters (e.g. P-glycoprotein, MRP1), however drugs' interactions with lipid bilayers may also be of great importance. Being hydrophobic, often aromatic, cations is the only feature shared by the group of MDR modulators (Klopman, Shi & Ramu, 1997). The correlation found between drugs' lipophilicity and their anti–MDR effectivness (Wadkins & Houghton, 1993; Pajeva, Wiese, Cordes & Seydel, 1996; Castaing, Brouant, Loiseau, Santelli–Rouvier, Santelli, Alibert–Franco, Mahamoud & Barbe, 2000) additionally strengthens the hypothesis assuming that drug – membrane lipids interactions may be crucial for MDR modulators' action.

Our previous fluorescence spectroscopy and microcalorimetry studies on trifluoperazine (Hendrich, Wesołowska & Michalak, 2001) and newly synthesised phenothiazine derivatives have shown that this class of compounds interacts strongly with lipids and changes the properties of model membranes composed of phosphatidylcholine and phosphatidylserine. Phosphatidylethanolamine (PE) is one of main phospholipids that compose the membranes of eucariotic cells. The ability to form both bilayer and inverted hexagonal structures in polar media together with tightly hydrogen bonded polar headgroups are the main features of PE. The aim of this study was to investigate the interaction 2–trifluoromethyl–10–(4–[methylsulfonylamid]buthyl)–phenothiazine with DMPE by means of differential scanning calorimetry.

MATERIALS AND METHODS

1,2–Dimyristoyl–*sn*–glycero–3–phosphatidylethanolamine (DMPE) was purchased from Sigma (St. Louis, MO, USA). Lipid was used without further purification. 2–Trifluoromethyl–10–(4–[methylsulfonylamid]buthyl)–phenothiazine was newly synthesised. Its chemical structure is shown in Fig. 1. All other chemicals used in experiments were of analytical grade.

Phenothiazine derivative was dissolved in chloroform/methanol (1:1, v/v). The lipid/drug mixtures were obtained by dissolving DMPE in phenothiazine stock solution. The amounts of DMPE and drug were chosen to obtain the required drug/lipid mole ratios (0.02–0.12). Then the solvents were evaporated under nitrogen stream and the samples were placed in a vacuum desiccator for

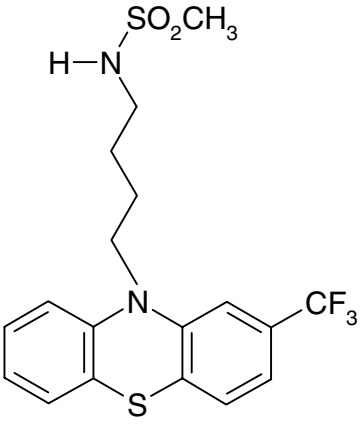

Fig. 1. Chemical structure of 2–trifluoromethyl–10–(4–[methylsulfonylamid]buthyl)–phenothiazine.

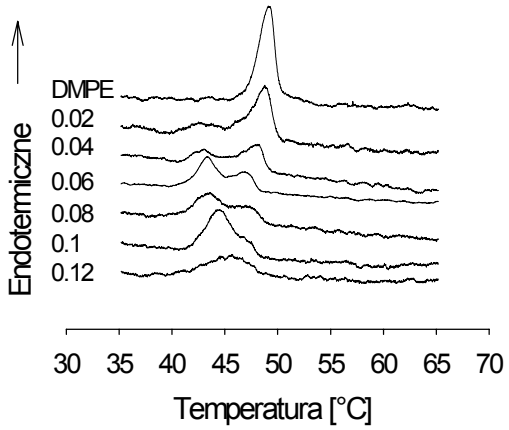

Fig. 2. Thermograms of DMPE (upper profile) and phenothiazine derivative – DMPE mixtures. Numbers on the figure represent drug to lipid mole ratios. The thermograms were normalised to equal amount of lipid for each profile.

minimum 4 hours. The dried samples (2 mg of lipid each) were hydrated in 20 µl of 20 mM Tris buffer (pH 7.4) containing 150 mM NaCl and 0.5 mM EDTA. Mixtures were heated to a temperature about 10 °C above the gel–liquid crystalline phase transition temperature of DMPE and were shaken in the thermostated mechanical shaker for several minutes. When the optical homogeneity was obtained, the mixtures were transferred into the aluminium pans and sealed. It should be emphasised that under experimental conditions used DMPE forms bilayer structures.

Microcalorimetric measurements were performed using Rigaku microcalorimeter equipped with measuring head constructed in our laboratory (scan speed 1.25°C/min). For each drug:lipid mole ratio at least two separate sample preparations were made, each sample was scanned at least four times. Samples were scanned immediately after preparation. Calorimetric data were computer analysed off–line using software developed in our laboratory. For thermograms in which two transition peaks were recorded the enthalpy change during the transition was calculated from the total area of both peaks.

RESULTS AND DISCUSSION

2–Trifluoromethyl–10–(4–[methylsulfonylamid] buthyl)–phenothiazine interacted strongly with DMPE model membranes. The thermograms of DMPE in absence and at increasing drug content are presented in Fig. 2. Even at the lowest drug/lipid mole ratio studied (0.02) the broadening of the gel–liquid crystalline transition peak and lowering of transition temperature (T_M) was observed. At mole ratio 0.04 the new peak appeared in the thermogram. Its T_M was lower than T_M of the main peak. Two separate peaks coexisted in the 0.04–0.1 mole ratio range. The appearance of separate peaks is usually explained to be the result of phase separation occurring in the sample. With the increase of phenothiazine derivative concentration the transition temperatures of both peaks con-

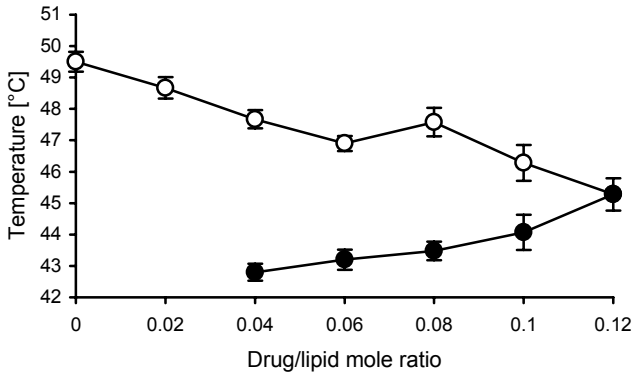

Fig. 3. The dependence of transition temperature on phenothiazine derivative/ DMPE mole ratio. Open symbols represent temperatures of main phase transition, filled symbols represent temperatures of additional transition appearing in thermograms.

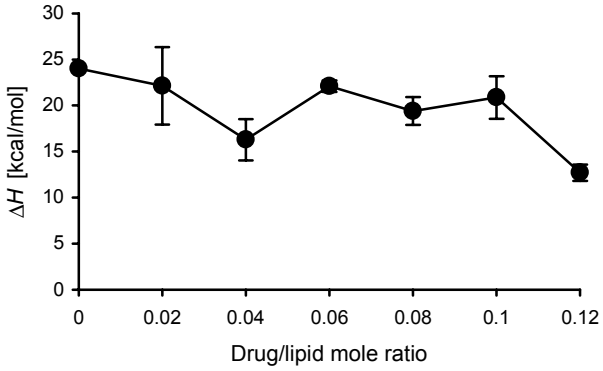

Fig. 4. The dependence of transition enthalpy change on phenothiazine derivative/DMPE mole ratio.

verged. At drug/lipid mole ratio 0.12 again only one peak was present in the thermogram. The transition temperatures versus the drug/lipid mole ratio are presented in Fig. 3. The transition enthalpy change (ΔH) measured in the presence of phenothiazine derivative was lower than for pure DMPE (Fig. 4). For the drug concentration range in which the two peaks coexist ΔH did not change. Only after the vanishing of phase separation ΔH distinctly dropped.

The above effects indicate the insertion of 2–trifluoromethyl–10–(4–[methylsulfonylamid] buthyl)–phenothiazine molecules into DMPE bilayer. The decrease of transition temperature and peak broadening suggest the drug's localisation at the polar/apolar interface of the membrane. Such position was previously proposed by Frenzel, Arnold and Nuhn (1978) and Nerdal, Gundersen, Thorsen, Hoiland and Holmsen (2000) for chlorpromazine in phosphatidylcholine systems.

In studied phenothiazine/DMPE systems phase separation was recorded in 0.04–0.1 drug/lipid mole ratio range. The reasons for its origin are not fully understood yet. It is likely that the two kinds of drug – lipid membrane interactions might exist. One of them dominates in lower drug concentration and becomes saturated as the phenothiazine derivative concentration is raised. Then the second kind of interaction becomes more and more pronuonced that results in the appearance of new peak in thermogram. The chemical structure of phenothiazine derivative molecule indicates that these two kinds of interactions are probably related to more than one different orientations that drug molecules may adopt inside the tight hydrogen–bond network that crosslinks the polar headgroup region of DMPE membranes.

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