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SMALL-ANGLE X-RAY SCATTERING AND DIFFERENTIAL SCANNING CALORIMETRY STUDIES OF DPPC MULTILAMELLAR STRUCTURES CONTAINING MEMBRANOTROPIC AGENTS OF DIFFERENT CHEMICAL NATURE

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Multilamellar structures formed in DPPC/water/glycerol and DPPC/water/oxyethylated glycerol systems are studied by small-angle X-ray scattering (SAXS) and differential scanning calorimetry (DSC) methods. The effects of glycerol, oxyethylated glycerol, and other membranotropic agents (MTAs) on the lamellar repeat distance D are compared in gel, ripple, and high-temperature (L_α) liquid crystal phases of the hydrated phospholipids. It is noted that the introduction of MTAs could lead to different types of 'D vs. temperature' behavior in the L_α phase, which is correlated with changes in D caused by the introduction of these substances to the DPPC/water reference system.

K e y w o r d s: small-angle X-ray scattering, membranotropic agents, liquid crystal, dipalmitoylphosphatidylcholine.

1. Introduction

Multilamellar structures of hydrated phospholipids are generally considered as a convenient model system for biophysical studies of cell membranes. The model phospholipid membranes are multibilayer lamellar structures in the lyotropic liquid crystal (LC) state. The most commonly used phospholipid in studies of model membranes is dipalmitoylphosphatidylcholine (DPPC), and the sequence of LC phases in hydrated DPPC is $L_{\beta'} \rightarrow P_{\beta'} \rightarrow L_\alpha$, (often described as "gel → ripple phase → LC phase") with mesomorphic phase transitions at ∼36 °C (pretransition, T_p) and ∼42 °C (main phase transition, T_m) [1, 2]. From the viewpoint of molecular physics, the high-temperature L_α phase is structurally similar to the smectic-A phase in thermotropic liquid crystals, with translational ordering in one dimension and lamellar bilayers playing the role of smectic lay-

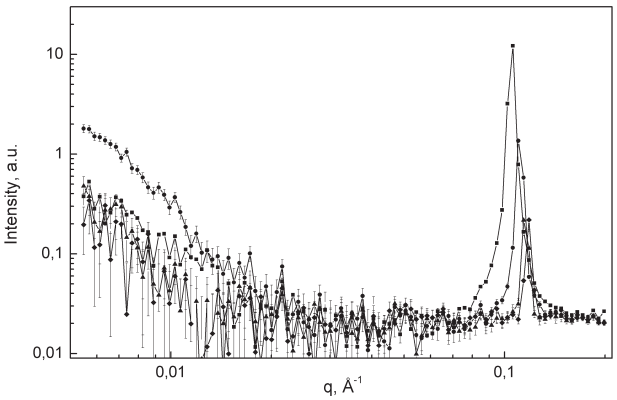

Fig. 1. Small-angle X-ray scattering profiles obtained for DPPC/water/OEG$_{n=5}$ system at different temperatures: 19.5 ° C (squares); 42.8°C (circles); 52.7°C (triangles), 63.5°C (diamonds)

ers. The low-temperature $L_{\beta'}$ is characterized by additional ordering within the bilayer (a certain analogy to smectic-B or smectic-H phases), and the "intermediary" $P_{\beta'}$ phase is marked by spatial undulations of the bilayer plane. With a particular reference to the well-known problem of drug-membrane interactions [3, 4], the lipid bilayers, alongside with DPPC and/or other compounds of similar chemical structures, can also include, as dopants, certain biologically relevant substances (drugs, steroids, cryoprotectants, *etc*). These substances can be designated as "membranotropic agents" (MTAs), and the main attention is paid to the effects of MTA dopants upon the characteristics of lipid bilayers. One of the most common methods used in such studies is differential scanning calorimetry (DSC), with MTA effects on the temperatures and enthalpies of phase transitions extensively discussed in detail [3–6]. Generally, the introduction of MTA is largely similar to the general picture of non-mesogenic dopants in liquid crystals, weakening (in most cases) the liquid crystalline ordering and decreasing the phase transition temperatures. However, certain cases of specific intermolecular interactions can change this picture and lead to a peculiar behavior of certain MTAs (several such cases were also considered in our earlier papers on DSC studies of model membranes [7–10]).

In many papers, DSC studies were accompanied by other experimental methods, such as FTIR or NMR spectra. This naturally allowed getting a deeper insight into the MTA behavior in a lipid bilayer. In this respect, an interesting possibility is offered by small-angle X-ray scattering (SAXS), where the MTA-induced change in the lamellar repeat distance D of the multilamellar structure could be used as a relatively simple parameter for the preliminary assessment of the emerging picture of a supramolecular structure. In our previous paper [11], just the D vs. temperature data obtained for the hydrated DPPC and DPPC/POPC (palmitoyl-oleoyl-phosphatidylcholine) systems allowed a conclusion about a full miscibility of two lipids in the L_α phase and the strong phase separation in the gel state, with no ripple phase in the mixed system. So, it seemed promising to expand this approach also to DPPC/MTA systems.

The first step in this direction was made in our recently published work [12], with silver nitrate salt and urocanic acid used as MTAs. Since the data obtained in [12] were rather scarce, certain obvious implications were not considered. In this paper, we report the SAXS data obtained for DPPC/glycerol and DPPC/oxyethylated glycerol systems in a broad range of temperatures and concentrations of the components.

2. Materials and Methods

DPPC was obtained from Alexis Biochemicals, Switzerland, and used without further purification. The lyotropic phases of DPPC/MTA systems were obtained by mixing DPPC with double-distilled water or the corresponding MTA/water solution with the DPPC-water ratio of 1:9 w/w (for SAXS experiments) or 2:3 w/w (for DSC measurements). In these conditions, the formation of multibilayer vesicles in excess water is known, with a conventional smectic-like multilamellar structure. Among other MTAs, we used glycerol and OEG$_{n=5}$ as an example of oxyethylated glycerols of the general formula $CH_2O[(CH_2CH_2O)_kH]$–CHOH–$CH_2O[(CH_2CH_2O)_{n-k}H]$ ($k \leqslant n$), with OEG$_{n=5}$ corresponding to $n = 5$, and glycerol – to $n = 0$. These substances, as examples of cryoprotectants, were obtained from the Institute for Problems of Cryobiology and Cryomedicine, NAS of Ukraine, Kharkiv.

DSC thermograms of the systems studied were obtained by using a Mettler DSC 1 calorimeter (Mettler Toledo, Switzerland), a sample mass of ∼20 mg, and a scanning rate of 2 K/min. Parameters of the phase transitions were determined by using the original Mettler DSC 1 STAR^e software. The experimen-

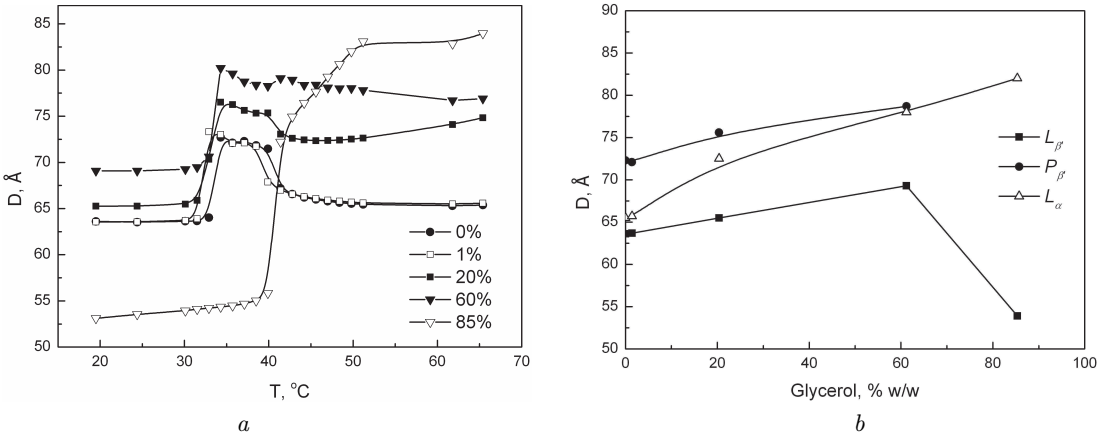

Fig. 2. Lamellar repeat period D as a function of the temperature (a) and the glycerol concentration in a water/glycerol solution (b) for the DPPC/water/glycerol system

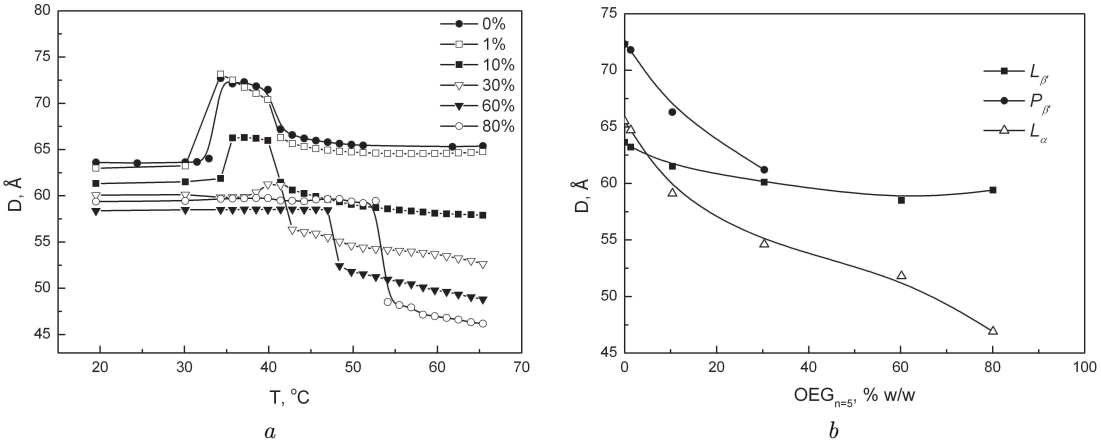

Fig. 3. Lamellar repeat period D as a function of the temperature (a) and $OEG_{n=5}$ concentration in a water/$OEG_{n=5}$ solution (b) for DPPC/water/$OEG_{n=5}$ system

tal errors for T_m and ΔH_m were, respectively, ± 0.1°C and ± 1.5 J/g.

SAXS experiments were carried out on a Rigaku X-ray instrument with a high-speed Cu rotating anode SMAXS-3000 Point SAXS system. The experimental procedures are essentially similar to those whose detailed description was presented in [12]. The samples studied were placed in borosilicon capillaries 1.5 mm in diameter fixed on a special holder ensuring the temperature control within ± 0.5°C. The measurements were carried out in the temperature interval (28–66)°C. A typical example of the obtained SAXS profiles (for the DPPC/water/$OEG_{n=5}$ system with 30% $OEG_{n=5}$ in the water-$OEG_{n=5}$ subphase) is shown in Fig. 1.

The lamellar repeat distance D of the lipid bilayer was calculated as $D = 2\pi/nq$, where q is the peak location, and n is the order of reflection. Upon heating, the location of the diffraction peak is changed, suggesting changes in the phase state of the lipid system.

3. Results and Discussion

The obtained experimental SAXS data are presented in Fig. 2 (DPPC/glycerol) and Fig. 3 (DPPC/$OEG_{n=5}$ systems). The most physically relevant features of the obtained results can be summarized as follows.

For DPPC/water/glycerol systems in the low-temperature $L_{\beta'}$ and$P_{\beta'}$ phases, the repeat distance is gradually increasing with the glycerol concentration up to ∼60%. At higher concentrations, the D

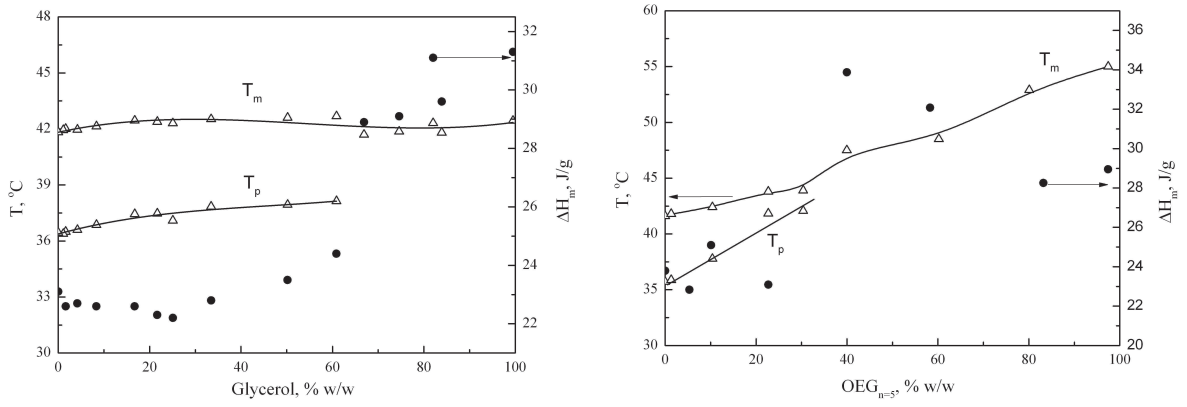

Fig. 4. Phase transition temperatures T_m, T_p (open triangles) and the melting enthalpy ΔH_m (filled circles) for the DPPC/water/glycerol (*a*) and DPPC/water/$OEG_{n=5}$ (*b*) systems

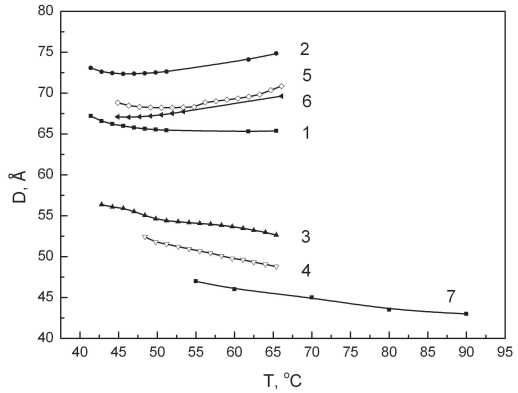

Fig. 5. Lamellar repeat period D *vs.* temperature in the L_α phase of systems: DPPC/water (*1*), DPPC/water/glycerol (*2*), DPPC/water/$OEG_{n=5}$ (30%) (*3*), DPPC/water/$OEG_{n=5}$ (60%) (*4*), DPPC/water/urocanic acid (*5*) [12], DPPC/water/silver nitrate (*6*) [12], DPPC/water/DMSO (*7*) [17]

values are dramatically falling (by ∼15 Å), reflecting the fundamental changes in the phase state, such as the formation of the interdigitated gel phase $L_{\beta I}$ [13] accompanied by the disappearance of the $P_{\beta'}$ phase and an increase in the melting enthalpy ΔH_m evidenced by DSC data. However, in the L_α phase, the general tendency toward larger repeat distances persists practically up to the full substitution of glycerol for water. Possible interpretations of this increase in D can be made in terms of "swelling" [14] or "unbinding" [15] of lipid bilayers due to a change in the balance between the attractive and repulsive interactions of the bilayers [16].

The picture becomes completely different with DPPC/water/$OEG_{n=5}$ systems (Fig. 3). The repeat period in all the phases is decreasing with $OEG_{n=5}$ concentration. Unlike the DPPC/glycerol systems, no concentration intervals, where D would increase with the temperature, were noticed.

The calorimetry data of the systems are presented in Fig. 4; they are in agreement with SAXS data. The main phase transition peak of DPPC multilamellar structures persists up to the full substitution of glycerol and $OEG_{n=5}$ for water with preservation of periodic multilamellar structure as evidenced by SAXS data. Both glycerol and $OEG_{n=5}$ increase the temperatures of the pretransition (T_p) and the main phase transition (T_m) reflecting an increase of lipid-lipid interactions. This effect is more pronounced for $OEG_{n=5}$. The concentration region of a sharp decrease of the repeat period for the DPPC/water/glycerol system in the gel phase corresponds to an increase of the melting enthalpy reflecting the formation of the interdigitated phase. But this is not the case for DPPC/water/$OEG_{n=5}$, where increasing ΔH_m is not accompanied by an abrupt fall of D.

To further generalize the observed features, it is rather instructive to consider the D *vs.* temperature dependences in the L_α phase in various DPPC/water/MTA systems for membranotropic agents of different chemical nature (Fig. 5).

The statistical base may seem not very convincing (just five MTA dopants to the hydrated DPPC systems – though these dopants can be considered as representatives of essentially different chemical

classes). However, certain fundamental physical conclusions can be (at least tentatively) expressed:

• All MTAs added as dopants to the model membranes based on hydrated DPPC can be subdivided into two groups according to the character of the dependence of D on the temperature T in the L_α phase: $dD/dT > 0$ (glycerol, silver nitrate, urocanic acid) or $dD/dT < 0$ ($OEG_{n=5}$, DMSO).

• This behavior clearly correlates with changes in D induced by the introduction of the respective dopants. If we denote the concentration of the introduced MTA as c, then the positive or negative value of dD/dT corresponds to the respective value of dD/dc.

• This behavior appears to be strikingly similar to the dependences of the helical twisting p^{-1} in cholesteric systems with non-mesogenic dopants – the character of the p^{-1} dependences on the temperature and the dopant concentration is the same [18].

4. Conclusions

It has been demonstrated by SAXS and DSC data that, under the substitution of oxyethylated glycerol $OEG_{n=5}$ for water in DPPC vesicles, the multilamellar structure is retained in all the $OEG_{n=5}$ concentration range. The repeat period D is gradually decreasing in all phases, and the mesomorphic phase transition temperatures are increasing with $OEG_{n=5}$ concentration. The ripple phase disappears above ∼30%. Unlike similar systems with glycerol, no clear evidence of the interdigitated $L_{\beta I}$ phase formation could be noted from SAXS data.

It has been shown that membranotropic agents of different chemical classes introduced into DPPC systems can lead to different types of the $D(T)$ behavior in the L_α phase. The sign of an MTA-induced shift of the repeat distance correlates with the sign of dD/dT at temperatures above the melting transition.

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МАЛОКУТОВЕ РОЗСІЮВАННЯ Х-ПРОМЕНІВ ТА ДИФЕРЕНЦІАЛЬНА СКАНУЮЧА КАЛОРИМЕТРІЯ В МУЛЬТИЛАМЕЛЯРНИХ СТРУКТУРАХ ДПФХ, ЩО МІСТЯТЬ МЕМБРАНОТРОПНІ АГЕНТИ РІЗНОЇ ХІМІЧНОЇ ПРИРОДИ

Р е з ю м е

Мультиламелярні структури, утворені в системах ДПФХ/вода/гліцерин та ДПФХ/вода/оксиетилований гліцерин, були досліджені методами малокутового розсіювання Х-променів та диференціальної скануючої калориметрії. Проведено порівняння впливу гліцерину, оксиетилованого гліцерину та інших мембранотропних агентів (МТА) на період повторювання в гель-фазі, ріпл-фазі та високотемпературній (L_α) рідкокристалічній фазі гідратованих фосфоліпідів. Відзначено, що внесення МТА може приводити до різних типів залежності D від температури в L_α-фазі, що корелює зі змінами D, спричиненими внесенням цих речовин до референтної системи ДПФХ–вода.