Small-angle X-ray scattering technique has been used to study multilayer lipid membranes of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and the 3:1-mixture DPPC/1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) in excess water. The temperature dependences of the repetition period for lipid bilayers in the temperature range 20–55 °C are obtained. A comparative analysis of the scattering curves obtained for multilayer membranes showed that, below a temperature of 40 °C, there emerges an additional ordering with a repetition period of 66 Å in the lipid mixture, which we associate with the lipid phase separation. A disappearance of the so-called ripple (wave-like) phase of DPPC lipid in the mixture is also observed.

1. Introduction

The study of physical and physico-chemical aspects of phase transitions in membranes has been lasting for many years [1–3]. Such an interest is associated, on the one hand, with the biological importance of membranes and the prospects of their use, e.g., as sensors, transducers, or filters and, on the other hand, with fundamental problems of physics concerning phase transitions.

In 1975, a theoretical model of lipid bilayer was proposed [4]. Taking advantage of statistical methods, the phase diagram of a lipid monolayer was constructed in the coordinates “the area occupied by a lipid molecule” versus “the surface pressure corresponding to this area”. In work [5], the same authors suggested a model of lipid bilayer, which made allowance for weak interactions between lipid headgroups. It was shown that even weak interactions between lipid headgroups have an effect of surface pressure on the bilayer, which affects, in turn, the phase transition temperature.

A considerable number of publications were devoted to studying the influence of various admixtures on the phase transitions in lipids. For instance, using the wide-angle diffraction of X-radiation for a mixture of two lipids with cholesterol, the authors of work [6] discovered two different types of a lipid molecule arrangement at low temperatures. However, the issue whether the microphase separation of lipids takes place or a new phase is formed, which is specific of the studied mixture, still remained obscured. In work [7], a model was proposed to explain the phase transition “cylindrical micelles–bilayer vesicles” in a mixture of phospholipids and surfactants. The model predicts that it is a transition of the first order, with the transition temperature depending on the ratio between the mixture components. According to the authors’ opinion, the suggested model is in qualitative agreement with experimental data. Work [8] was devoted to studying the influence of electrolytes on lipid solutions with the use of small-angle neutron and X-ray scattering techniques and differential scanning calorimetry. The influence of cholesterol, anesthetics, and polypeptides on the temperature of the main phase transition from the ripple phase into the liquid-crystal one was studied within the nuclear magnetic resonance...
2. Experimental Materials and Methods

Synthesized 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) were kindly provided by Avanti (Birmingham, UK). In our work, those chemicals were used without further purification.

The procedure of preparation of multilamellar vesicles was as follows. DPPC and the 3:1-mixture DPPC/POPC were dissolved in water. A shaker was used to mix the lipids. Homogenization of the solution was attained, by using cyclic freezing–thawing procedures. Afterward, the solution was introduced into a borosilicate capillary 1.5 mm in diameter with a wall thickness of 0.01 mm (W. Muller, Berlin, Germany). The total concentration of lipids in the mixture was 100 mg/ml.

Small-angle X-ray scattering researches were carried out in a pinhole camera of a Molecular Metrology SAXS System spectrometer at the Institute of Macromolecular Chemistry of the Academy of Sciences of the Czech Republic (Prague, the Czech Republic). In this spectrometer, a microfocused X-ray beam generator Osmic MicroMax 002 operating at a voltage of 45 kV and a current of 0.66 mA was used. The diameter of a radiation beam was 0.3 mm. The spectrometer was equipped with a gas-filled position-sensitive detector 20 cm in diameter. The absolute values of scattering vectors accessible to our measurements fell within the range \( q = 0.0045 \pm 0.2 \text{ Å}^{-1} \).

3. Experimental Results

3.1. DPPC membranes

In Fig. 1, the experimental curves of small-angle X-ray scattering measured for an aqueous solution of DPPC under normal pressure conditions are exhibited. We were interested only in the positions of curve maxima; therefore, the curves were not normalized with respect to the scattering intensity. As the temperature was elevated above 34.5 °C, the diffraction peak positions become shifted, which evidences a change on the phase state of the lipid.

In Fig. 2, the small-angle X-ray scattering curves measured for a DPPC solution at temperatures of 35.2 and 36 °C are shown. In this temperature range, the lipid transforms from the gel phase into the so-called ripple (wave-like) one [14]. The diffraction maxima are smeared, which testifies to the solution inhomogeneity. This fact is connected with the presence of two lipid phases. Each of them is characterized by its own char-
The characteristic repetition period, which is evidenced by two maxima in each curve.

The data processing was carried out as follows. The scattering curves (Fig. 1) were used to determine the coordinates of \( q \)-maxima. Those coordinates were used to calculate the repetition period of lipid bilayers, \( D \), by the formula

\[
D = \frac{2\pi}{q}.
\]

The repetition period \( D \) was calculated for every studied temperature. Then the temperature dependence of the lipid bilayer repetition period was plotted (Fig. 3). A drastic increase of the lipid bilayer repetition period at the temperature elevation from 35.2 to 36 \( ^\circ \)C corresponds to the transition of the system under investigation from the gel phase into the ripple one. Figure 3 also demonstrates that, when the temperature grows further, a reduction of the lipid layer repetition period is observed in a vicinity of 41 \( ^\circ \)C, which is associated with the so-called main phase transition in the lipid.

3.2. DPPC/POPC membranes

While processing the small-angle scattering curves obtained for a mixture of DPPC and POPC lipids, we took advantage of the same approaches, as were applied to the solution of DPPC. The corresponding fabricated system also had a multilayered structure, which was confirmed by the presence of a peak on the scattering curve (see Fig. 4).

One can see that, for the lipid mixture, the peaks of small-angle X-ray scattering change their characteristic dependence on the temperature. For instance, they are unary (without additional maxima) at above 34.5 \( ^\circ \)C. In the temperature range from 20.3 to 34.5 \( ^\circ \)C, the peaks become wider (Fig. 5), and an additional separate peak can be resolved in the spectra, which testifies to the existence of one more order in the lipid bilayer arrangement.

In Fig. 6, the temperature dependence of the repetition period is depicted for the 3:1-mixture of lipids DPPC and POPC. The repetition periods for additional peaks are denoted by circles, and those for main peaks by squares. We explain the simultaneous presence of two repetition periods in the mixture as a result of the fact that, up to a certain temperature, the lipids do not mix together and do not form a homogeneous solution. The
temperature, at which the additional structural ordering disappears, corresponds to that of the main phase transition in DPPC (see Fig. 3). Below this temperature, DPPC exists in the so-called gel phase characterized by a dense packing of lipid molecules, so that the lipids do not mix. Above the main phase transition temperature, the lipid bilayer transforms into a liquid crystal phase and becomes similar to a two-dimensional liquid. Lipid molecules can move freely enough in the bilayer, and the mixing of lipids becomes possible. At temperatures below 20.3 °C and above 47.8 °C, the repetition periods for the POPC lipid bilayer (triangles) and the DPPC/POPC lipid mixture (squares) coincide (Fig. 7). This fact testifies that it is DPPC that is responsible for the main peaks in the small-angle scattering curve obtained for the mixture DPPC/POPC. This conclusion is also confirmed by the comparison between the intensities of main and additional peaks in Fig. 5, where the main peak is associated with DPPC, whose content in the mixture is higher than that of POPC. Therefore, the intensity of scattering from the DPPC lipid has to be larger than that from POPC, whose content in the mixture is lower. The repetition period of about 66 Å associated with the positions of small peaks (the circles in Fig. 7) corresponds to that of POPC lipid bilayer in
the liquid crystal phase. In the examined mixture, it is the POPC lipid that is in the liquid crystal phase, because the phase transition temperature for this lipid is $-2^\circ$C. While comparing the temperature dependences of the repetition periods for the lipid mixture and the pure lipid (see Fig. 7), besides an additional ordering, we also can observe the disappearance of the intermediate ripple phase of pure DPPC lipid.

4. Conclusions

The 3:1-mixture of lipids DPPC/POPC is shown to be inhomogeneous at temperatures lower than 36 $^\circ$C under normal pressure. Under those conditions, the indicated lipids are statistically independent, which is confirmed by the presence of two structural peaks in the spectrum of small-angle X-ray scattering. At temperatures higher than 36 $^\circ$C, this spectrum contains a single maximum; however, the small-angle X-ray scattering technique does not allow us to distinguish whether the lipids are statistically dependent, so that the lipid bilayers have a unique repetition period, or they are statistically independent, but the lipid bilayers have the repetition periods, which are so close by magnitude that the experimental technique does not allow them to be resolved. It was found that, if the POPC lipid is added to DPPC, the phase transition temperature of the latter does not change, but the intermediate ripple phase of DPPC with a repetition period close to 73 Å disappears.


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