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THE INFLUENCE OF CHOLESTEROL ON HEAD GROUP MOBILITY IN PHOSPHOLIPID MEMBRANES

JOHN C.W. SHEPHERD and GEORG BÜLDT

Department of Biophysical Chemistry, Biocentre of the University of Basel, Klingelbergstrasse 70, CH-4056 Basel (Switzerland)

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Summary

The dielectric dispersion in the MHz range of the zwitterionic dipolar phosphocholine head groups has been measured from 0—70°C for various mixtures of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and cholesterol. The abrupt change in the derived relaxation frequency f_2 observed for pure DPPC at the gel-to-liquid crystalline phase transition at 42°C reduces to a more gradual increase of frequency with temperature as the cholesterol content is increased. In general the presence of cholesterol increases the DPPC head group mobility due to its spacing effect. Below 42°C no sudden changes in f_2 are found at 20 or 33 mol% cholesterol, where phase boundaries have been suggested from other methods. Above 42°C, however, a decrease in f_2 at cholesterol contents up to 20—30 mol% is found. This is thought to be partly due to an additional restricting effect of the cholesterol on the number of hydrocarbon chain conformations and consequently on the area occupied by the DPPC molecules.

Introduction

The conformational and motional spectrum of bilayers containing only one kind of lipid has been well characterized by different methods in those thermodyanmic phases which are important for the understanding of biological membranes [1—6]. In the next step towards more complexity in membrane model systems, binary mixtures of lipids have been investigated [7—10]. The phase diagrams showed homogeneous phases and regions where phase separation

Abbreviations: DPPC, dipalmitoyl phosphatidylcholine.

occurred, in particular between one lipid in the gel state and the other in the liquid crystalline state. It has further been shown that these domains have drastic effects on the protein distribution in the plane of the bilayer [11].

Since cholesterol is an important component of membranes from animal cells, many studies have been concerned with binary systems of cholesterol and other lipids. It appears in all these cases, however, that it has not been possible to establish a reliable phase diagram which could explain all the experimental data. It therefore seems important to look for methods that could elucidate some aspects of this problem. For example, for 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) and cholesterol mixtures, as studied in this paper, Rubenstein et al. [12] measured the lateral diffusion of a fluorescent-labelled phospholipid and found sharp changes at 20 mol% cholesterol at temperatures below the main transition of pure DPPC (42°C).

In the present work the rotational motion of the zwitterionic head group of DPPC is investigated in the presence of various amounts of cholesterol and 25% water in the temperature range 0—70°C. Neutron diffraction experiments [6] show that in pure DPPC bilayers at this water content the average dipole orientation is nearly parallel to the bilayer plane. In addition there is strong evidence from other methods (^{2}H and ^{31}P NMR, [13,14]) that the rotational motion of the dipole occurs predominantly in the plane of the bilayer. This is also consistent with the results of previous dielectric measurements on pure DPPC bilayers. In this earlier investigation [15] an increase of relaxation frequency was obtained when more area per lipid molecule in the plane of the bilayer became available at higher temperatures. From neutron diffraction experiments [16] it has been found that the oxygen in the head group of cholesterol is positioned near to the glyceryl-fatty acid ester bonds of the phospholipid and thus well below the plane of rotation of the phosphocholine group. A cholesterol molecule inserted into the bilayer might therefore be expected to increase the area available for the rotational motion of the neighbouring head groups. On these grounds the motion of the head group as seen by a dielectric method could be an indicator of the distribution of cholesterol in the bilayers and of its interactions with the neighbouring phospholipids.

Materials and Methods

The DPPC and cholesterol were purchased from Fluka, Switzerland and used without further purification. No impurities had been detected in earlier tests. Five samples with different molar percentages (0, 9, 19, 33 and 50) of cholesterol were weighed into a glass tube having two parts separated by a narrow constriction. A chloroform/methanol solvent was added in order to dissolve this mixture. The solvent was then removed by blowing a stream of nitrogen over the samples, followed by 24 h under high vacuum. In order to ensure proper incorporation of the cholesterol into the bilayer this procedure was repeated at least once with each sample. Then the precise amount (25%) of bidistilled water was added. After sealing the tube, the material was centrifuged eight times at about 44°C to and fro through the constriction, thus ensuring a homogeneous mixture.

The dielectric measuring technique has been previously described [15]. In

the present experiments circular stainless steel condenser plates separated by a perspex ring of 0.1 cm thickness and an internal diameter of 1.0 cm were used. The complete filling of this circular cavity was achieved by injecting the sample material (~80 mg) through a small hole (diameter 0.5 mm) in the plate, which was then sealed together with a small hole of the same diameter offset in the opposite plate.

Results

Measurements of dielectric permittivity ϵ' and loss ϵ'' were made on each mixture in the frequency range from 1 to 50 MHz and at temperatures between 0 and 70°C. Each mixture had been pre-heated to 80°C and then cooled in order to stabilize the orientation of the individual multilayer stacks, and during the measurements adequate time was allowed at each temperature for complete equilibration. Fig. 1 shows typical measured values for ϵ' and ϵ'' with various cholesterol contents and at a temperature of approx. 38°C, well below the main phase transition for DPPC. As in the previous paper [15], these points have been fitted by assuming two Debye dispersions

$$\epsilon' = \frac{A_1}{1 + (f/f_1)^2} + \frac{A_2}{1 + (f/f_2)^2} + \epsilon_\infty \tag{1}$$

and

$$\epsilon'' = \frac{A_1(f/f_1)}{1 + (f/f_1)^2} + \frac{A_2(f/f_2)}{1 + (f/f_2)^2} \tag{2}$$

The low frequency dispersion (relaxation frequency f_1) is adequately approximated by the first terms in these equations and the second terms have been shown to derive from the relaxation of the zwitterionic phosphocholine dipoles, f_2 being their relaxation frequency. A_1 and A_2 are the respective amplitudes and ϵ_∞, the permittivity of the specimen at frequencies considerably higher than f_2, has been included as a parameter to be fitted. In the case taken in Fig. 1, it is directly seen from the dispersion curves that the relaxation fre-

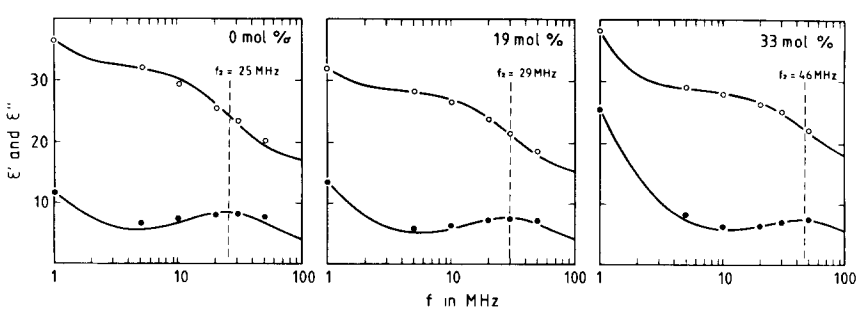

Fig. 1. Dielectric permittivity, ϵ' (○) and loss, ϵ'' (●), measured at 38°C for various cholesterol/DPPC mixtures with 25 weight % water. The curves represent the fit obtained from Eqns. 1 and 2. The derived relaxation frequency f_2 for the dipolar head groups is indicated.

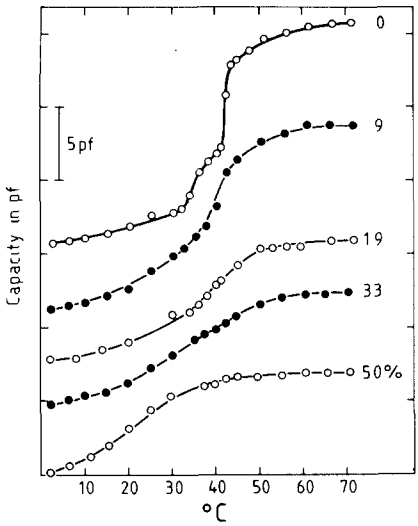

Fig. 2. The phase transitions of DPPC/cholesterol mixtures are followed by means of the directly observable capacity changes at 50 MHz.

quency f_2 of the phosphocholine group shows little change between 0 and 19 mol% cholesterol but is more increased at 33 mol% (see Discussion).

The smearing out of the very sharply defined phase transitions for pure DPPC caused by the addition of cholesterol can also be directly observed from the experimental data. This is illustrated in Fig. 2 where the measured capacity of the various samples at 50 MHz is plotted against temperature. After deriving the relaxation frequency f_2 from the least squares fit, a similar effect can be seen by following the changes of f_2 with temperature (Fig. 3). Although the frequency f_2 at the phase change for pure DPPC is not significantly different from

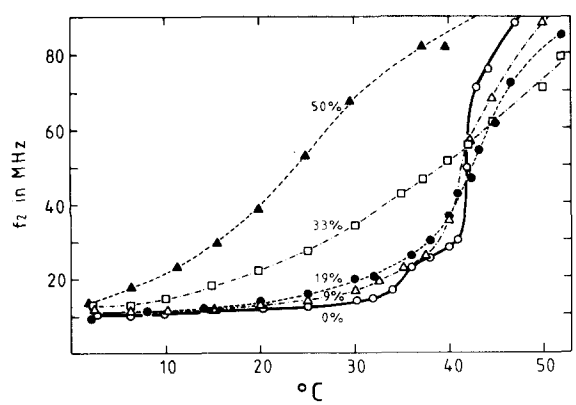

Fig. 3. The temperature dependence of the relaxation frequency f_2 is shown for various mixtures of DPPC and cholesterol. (The f_2 values derived above 90 MHs are more inaccurate, since the relaxation frequency is then too far above the measuring range, and have not been shown in this figure.)

the value previously found [15], yet the f_2 values at lower temperatures are considerably smaller. The improved cells and measuring technique and the fact that a significant ϵ_∞ value could now be deduced (it was previously fixed at an estimated value) may well account for these differences. The ϵ_∞ values ranged from about 11 to 16 and give an indication that part of the 25 weight % water still behaves as free water. This would correlate with estimates made by means of ^{2}H-NMR [17].

Discussion

As mentioned in the introduction, one main factor which influences the mobility of the phosphocholine groups is thought to be their increased separation in the presence of cholesterol. The present results are well illustrated in Fig. 4, where the relaxation frequencies f_2 are plotted against cholesterol content for various temperatures below and above the phase transition for pure DPPC at 42°C. Below this temperature the spacing effect of cholesterol is clearly demonstrated by a gradual increase of f_2 with increasing cholesterol content, although it is observed that at 20°C and below, f_2 stays constant up to 20 mol% cholesterol. In this context it is interesting to mention some results that have been obtained by other methods in the region of the phase diagram below 42°C. From spin label data Shimshick and McConnell [18] have suggested the existence of two solid phases up to 20 mol% cholesterol. This interpretation has been supported by freeze fracture experiments with an ATPase as a marker [11] and by the observation of the lateral diffusion of a fluorescent-labelled phospholipid [12]. A reinvestigation with high sensitivity scanning calorimetry [19] showed a sharp peak that vanishes also at 20 mol% cholesterol. This peak was attributed to domains of pure DPPC passing through the gel liquid crystalline phase transition. This observation seems to be in disagreement with an early investigation of Engelman and Rothman [20]. They observed the

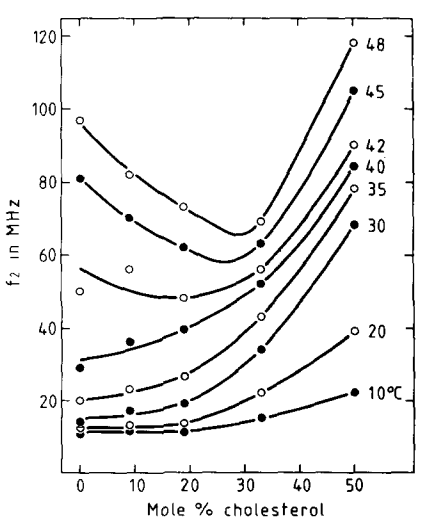

Fig. 4. A direct view of the dependence of the relaxation frequency f_2 on cholesterol content.

gel phase X-ray diffraction line (at 4.15 Å) of pure DPPC up to 33 mol% cholesterol. One explanation would be that the 4.15 Å reflection is more sensitive to small domains of pure DPPC which may still exist above 20 mol% cholesterol. With regard to the relaxation frequencies f_2 now observed for the head group dipoles, no breaks are seen below 42°C either at 20 or 33 mol% cholesterol, although below 20°C and 20 mol% cholesterol f_2 is almost constant. Moreover, the deuterium quadrupole splitting of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine labelled as N-C^2H_3 in the choline head group also showed only gradual changes with increasing cholesterol content below the phase transition temperature of the pure phospholipid [21].

At temperatures higher than 42°C, an interesting new effect is seen in Fig. 4, in that the relaxation frequency f_2 decreases at low cholesterol content, reaching a minimum value between 20 and 30 mol% cholesterol, and then increases as for temperatures below 42°C. From present knowledge of the changes introduced in the bilayer by the presence of cholesterol it is not possible to give a complete explanation of this effect. One contribution that leads to this behaviour is the fact that cholesterol restricts the number of possible lipid chain conformations above the phase transition of the pure lipid. Experimentally this is seen from the increase in the order parameters of the hydrocarbon chains (determined from deuterium magnetic resonance [21]), as well as from the increase in bilayer thickness (observed from X-ray profiles [22]). Both observations indicate a decrease in the area per lipid molecule. It is easily estimated, however, that this effect alone cannot overcompensate the spacing effect of cholesterol and reduce the head group relaxation frequency. For instance at 48°C, the minimum value of f_2 is attained at a lipid to cholesterol ratio of approx. 2 : 1 and the maximum decrease in area of two lipid molecules (~10 $Å^2$ per lipid as estimated from the difference between the gel and liquid crystalline phases) could not be greater than the increase of area due to one cholesterol molecule (~38 $Å^2$). Hence there must also be other reasons for the observed decrease in f_2 above 42°C, and one possibility would be that changes dependent on the cholesterol content may have occurred in the water structure in the neighbourhood of the head groups, thus altering their rotational coefficient.

In conclusion the present method's advantage lies in the fact that it observes the rotation of the whole zwitterionic head group without introducing any disturbing probe. Thus the changes in the interactions between the head groups in the plane of the bilayer can be directly followed as temperature and cholesterol content are varied.

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