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INTERACTING EFFECTS OF TEMPERATURE, PRESSURE AND CHOLESTEROL CONTENT UPON THE MOLECULAR ORDER OF DIOLEOYLPHOSPHATIDYLCHOLINE VESICLES

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Dioleoylphosphatidylcholine (DOPC) multilamellar vesicles containing varying amounts of cholesterol (0–50 mol%) were studied by measuring the polarisation of diphenylhexatriene fluorescence at 6, 23.5, and 35.5°C, and at hydrostatic pressures up to 1.5 kbar. Interactions between temperature and pressure were quantified as the temperature-pressure equivalence which was approximately 19–23 K · kbar $^{-1}$ for all binary mixtures of cholesterol and DOPC. Polarisation was linearly related to cholesterol/DOPC ratio, except at low temperature. In all cases pressure caused an increase in polarisation (i.e., an increase in molecular order) but did not alter the slope of the graph relating polarisation to cholesterol/DOPC ratio. The relative ordering effect of cholesterol and pressure was quantified by calculating the cholesterol-pressure equivalence. An increase in cholesterol/DOPC ratio of approximately 0.35–0.50 increased polarisation by an amount equivalent to an increase in pressure of 1 kbar. Cholesterol-pressure equivalence tended to decrease as temperature decreased and pressure increased; that is, as membrane order increased.

Introduction

Recent developments in pressure vessel design for fluorimetry [1] have provided means by which the effects of hydrostatic pressure upon biological and artificial membranes may be observed and quantified. For example, pressure-induced phase transitions (gel ⇋ liquid-crystalline) in pure dimyristoylphosphatidylcholine and dipalmitoylphosphatidylcholine vesicles have been observed using steady-state polarisation measurements [2]. In addition, steady state and differential polarized phase fluorimetry have demonstrated a progressively increasing membrane order with increasing hydrostatic pressure in a natural membrane [3]. Finally, the effects of hydrostatic pressure upon membrane order have been correlated with pressure-induced alterations in the activity of a membrane-bound enzyme, the $(Na^+ + K^+)$-ATPase of dog kidney [4].

Cholesterol is a major component of some biological membranes and a large body of experimental data on the structure of cholesterol/phospholipid membranes exists [5–7]. Cholesterol causes a general rigidification of liquid-crystalline membranes. Indeed, differences in the molecular order of natural membranes are closely correlated with differences in their cholesterol-phospholipid molar ratios [7,8]. Pressure also rigidifies membranes and it is clearly of interest to correlate the pressure-induced ordering (a physical modulator of membrane order) to the cholesterol-induced ordering (a chemical modulator).

We describe here the effect of cholesterol upon the pressure dependence of an index of membrane

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order, namely fluorescence polarisation of 1,6-diphenyl-1,3,5-hexatriene. Dioleoylphosphatidylcholine (DOPC) was chosen as phospholipid because its low phase transition temperature (−28°C) [9] provides a liquid-crystalline membrane at all temperatures employed in this study. Effects of cholesterol and pressure upon the phase transition of the bilayer were not therefore addressed in this work.

This study not only contributes to a comprehensive description of the physical structure and properties of binary mixtures under widely varying conditions of temperature and pressure, but also provides a reasonable prediction of the changes in cholesterol/phospholipid ratio required by abyssal organisms to offset the pressure-induced ordering of biological membranes.

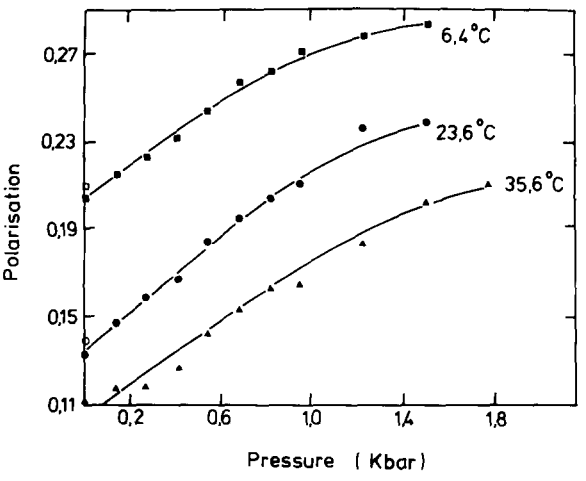

Fig. 1. A typical graph illustrating the effects of pressure upon the fluorescence polarisation of diphenylhexatriene in liposomes composed of cholesterol/DOPC (mole ratio = 0.121).

Materials and Methods

DOPC and cholesterol (Δ^5-cholesten-3-ol) were purchased from Sigma Chemical Co. DOPC and cholesterol, both dissolved in chloroform, were thoroughly mixed and then dried under vacuum. Vesicles were then prepared under argon gas as multilamellar vesicles by the method of Bangham et al. [10]. The phospholipid concentration was measured as inorganic phosphate by the method of Ames [11] and cholesterol was determined by the method of Trinder [12].

DPH polarisation measurements under pressure were made with a high pressure vessel mounted in a photon-counting polarisation fluorimeter, as described by Paladini and Weber [1]. The procedures for handling membrane samples for the high pressure vessel were described previously [2,3]. The depolarisation due to the pressure-induced birefringence from the pressure vessel windows was corrected according to the method of Paladini and Weber [1] and also Chong and Weber [2]. Temperature control and measurement have been described elsewhere [3]. Scattered light was found to comprise less than 0.5% of total emission. Corrections for this effect were not made.

Results and Discussions

The effect of hydrostatic pressure on diphenylhexatriene polarisation in cholesterol/DOPC vesicles is illustrated in Fig. 1. At all temperatures, polarisation increased with increased pressure in a similar way to DOPC vesicles which contain no cholesterol [2]. Similar experiments were carried out on vesicles containing cholesterol/DOPC ratios between 0 and 1.0. As before [2,3], there was no significant different between the values of polarisation recorded at any pressure during pressurisation and depressurisation.

From these isothermal curves the temperature-pressure equivalence (dT/dp) was calculated by determining the increase in temperature required to offset the ordering effects of 1 kbar pressure [3]. These values are presented in Table I where it is clear that there is little variation of dT/dp with cholesterol/DOPC ratio. The typical value of 19–23 $K \cdot kbar^{-1}$ is within the range observed using this and other techniques (Refs. 2–4, reviewed in Ref. 13).

The effect of cholesterol/DOPC ratio upon polarisation at different pressures is illustrated in Figs. 2(a–c). An increased cholesterol content resulted in higher polarisation values. In common with other studies [7,8,14] polarisation at 23.5°C and 35.5°C was linearly related to cholesterol/DOPC ratio and cholesterol/DOPC ratio rather than mol% phospholipid is the primary determinant of membrane order. At 6°C, however, the relationship was distinctly non-linear. The applica-

TABLE I

THE TEMPERATURE-PRESSURE EQUIVALENCE (dT/dp) FOR MULTILAMELLAR VESICLES COMPOSED OF DIOLEOYLPHOSPHATIDYLCHOLINE AND CHOLESTEROL

Values were calculated from graphs such as Fig. 1 by measuring the increase in pressure which produced identical values of polarisation over the stated interval of temperature. The temperature interval was then normalized to a 1 kbar pressure increase. n.d., not determined.

Cholesterol/DOPC (mole ratio)	dT/dp (K·kbar^{-1})	
	6–23.5°C	23.5–35.5°C
0.00	n.d.	22.8
0.12	20.2	21.1
0.30	22.3	21.4
0.58	17.1	18.9

TABLE II

THE CHOLESTEROL-PRESSURE EQUIVALENCE FOR MULTILAMELLAR VESICLES COMPOSED OF DIOLEOYLPHOSPHATIDYLCHOLINE-CHOLESTEROL MIXTURES AT 23.5°C AND 35.5°C

Values were calculated from Figs. 2(b,c) by measuring the change in cholesterol/DOPC mole ratio which produced identical values of polarisation over the stated interval of pressure. The ratios were then normalized for a 1 kbar interval of pressure.

Temperature (°C)	Cholesterol-pressure equivalence		
	0.001–0.5 kbar	0.5–1.0 kbar	1.0–1.5 kbar
23.5	0.40	0.36	0.35
35.5	0.50	0.44	0.36

tion of hydrostatic pressures up to 1.5 kbar at all temperatures had no significant effect upon the slope of the curves; they were simply translated to higher polarisation values.

The relationship between cholesterol-induced ordering and pressure-induced ordering of DOPC membranes may be conveniently expressed as the cholesterol-pressure equivalence. This expresses the change in cholesterol/DOPC ratio which is equivalent in its effects to an increase in pressure of 1 kbar. The linear relationships illustrated in Figs. 2(b) and 2(c) facilitate this exercise since a single value describes the equivalence over the entire range of cholesterol/DOPC ratios. The equivalence was calculated by measuring the decrease in cholesterol/DOPC ratio required to maintain a constant polarisation despite an increase in pressure of 0.5 kBar. Values were calculated from Figs.

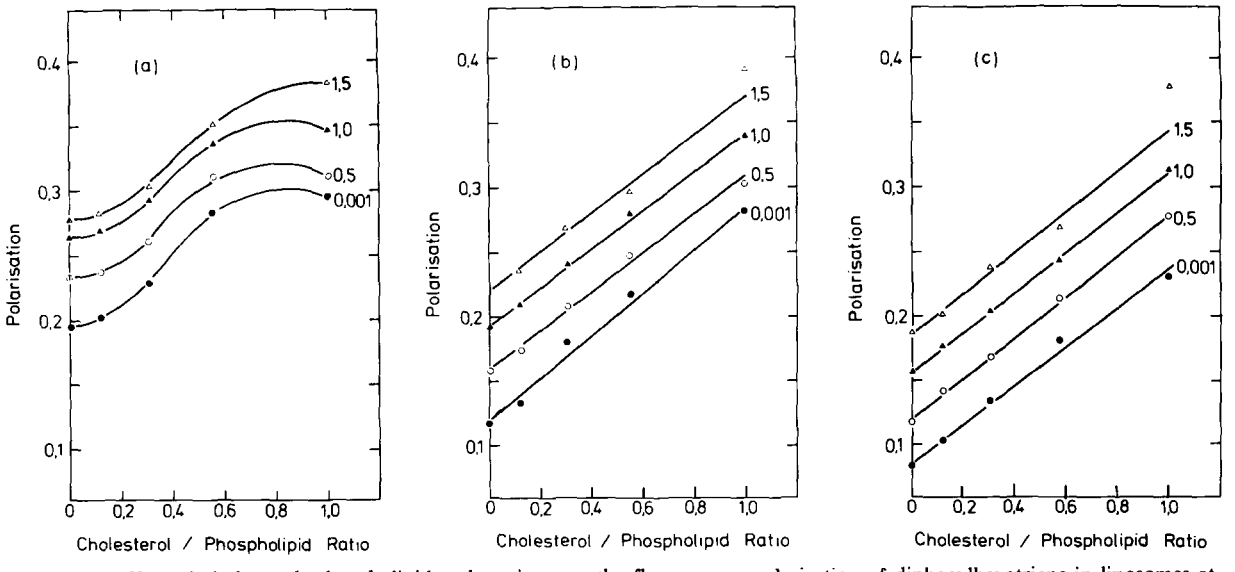

Fig. 2. The effect of cholesterol/phospholipid mole ratio upon the fluorescence polarisation of diphenylhexatriene in liposomes at different hydrostatic pressures. Results are shown at 6°C (a), 23.5°C (b), and 35.5°C (c). The numbers adjacent to each graph represent the hydrostatic pressure in kbar.

2(b) and 2(c) over three pressure intervals and were normalized to give the equivalence for a 1 kbar pressure step (Table II). In general, the cholesterol-pressure equivalence decreased as the absolute pressure increased, and as temperature decreased; that is, as membrane order increased. The complex non-linear curves at 5.6°C (Fig. 2a) means that the equivalence was not constant but progressively decreased with an increase in cholesterol/DOPC mole ratio; values have not been included in Table II. It should also be noted that as the curves were not equally spaced, the pressure-dependence of polarisation for each binary mixture was not linear.

Despite the convenience of linear relationships such as those shown in Figs. 2(b,c), the cholesterol/DOPC ratio may not be the most meaningful way of expressing their relative concentrations. Transformation of the data into graphs of polarisation versus mol% phospholipid yields curved lines with progressively increasing slopes as the mol% phospholipid decreases (graphs not shown). As before, the lines obtained at increasing hydrostatic pressure were parallel but shifted upwards to higher values of polarisation. The non-linearity of these graphs means that the cholesterol-pressure equivalence calculated from these graphs also decreased progressively as the mol% phospholipid decreased and the overall order within the membrane increased.

In view of the possibility that bilayer compressibility decreases with an increase in molecular order, it is perhaps not surprising that the cholesterol-pressure equivalence gradually decreases with an increase in pressure and a decrease in temperature (see Table II). In fact, Lis et al. [15] have shown that melting generally increases the lateral compressibility of lipid bilayers. They found that the lateral compressibility of egg phosphatidylcholine was higher than that of an egg phosphatidylcholine/cholesterol mixture (1 : 1) by a factor of 2.39 at 25°C and that the molecular surface area of DOPC tended to approach a lower limiting value when the lateral pressure was quite high (Fig 3a in Ref. 15). Although no information of this sort was given regarding the cholesterol effect on DOPC, it is conceivable that the effect of cholesterol upon the compressibility of DOPC resembles that of egg phosphatidylcholine. In other words, the density of the bilayer may approach a maximal value when the vesicle contains a high cholesterol content (such as 50 mol%) and at the same time is subject to high pressure (such as 1.5 kbar). Indeed, it is noticeable from (Figs. 2(a–c) that polarisation under these conditions is essentially temperature independent and approaches a value which Van Blitterswijk et al. [8] suggest may be an upper limit to fluorescence polarisation of diphenylhexatriene in membranes.

The cholesterol-pressure equivalence enables a prediction of the magnitude of the change in membrane cholesterol content in abyssal organisms, which may occur to offset the ordering effects of their hyperbaric, cold environment. The hypothesis underlying this prediction is that of 'homeoviscous adaptation'; that is, organisms have the ability to modulate the order of their cellular membranes to compensate for the perturbing effects of environmental factors such as temperature and pressure. Whilst such putative adaptations have been observed in microorganisms, protozoans and fish with respect to variations in temperature [16,17], no information exists with respect to hyperbaric environments. However, if a change in the cholesterol content of membranes is an important strategy of cellular adaptation to the abyssal environment, then a change in cholesterol/phospholipid ratio of 0.035–0.05 would be required to preserve a constant membrane order for each 0.1 kbar pressure (approx. 1000 m depth). This is probably an underestimate because of the substantial temperature difference of 10–20°C which also exists between surface and abyssal waters. According to the temperature-pressure equivalence calculated (Table I), this reduction in temperature in the ocean depths is equivalent in its effects to an additional pressure of 0.5–1.0 kbar. Thus an organism at 4000 m (0.4 kbar) and 4°C would have a cholesterol/phospholipid ratio of between 0.3 and 0.7 below that of a surface-dwelling species in order to possess a similar membrane order in each habitat. This prediction is obviously subject to other biochemical alterations which may also contribute to the overall physical state of the constituent membranes of abyssal organisms, and applies only to those cellular membranes which contain appreciable quantities of cholesterol.

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