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An X-Ray and Vapour Pressure Study on Lecithin—Cholesterol—Water Interactions

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The molecular interactions in the lamellar mesomorphous phase of the lecithin-cholesterol-water system have been examined by X-ray diffraction and vapour pressure measurements. Cholesterol is found to have a condensing effect on the molecules of egg lecithin. This effect is accompanied by an increase in the thickness of the lipid layer and a decrease in the thickness of the water layer. The water binding capacity of egg lecithin is only slightly affected by cholesterol. The effect of cholesterol on hydrated egg lecithin seems mainly to be restricted to the hydrocarbon region.

Cholesterol is a prominent constituent in biological structures, *e.g.*, membranes and may also play a part in the development of pathological structures as atherosclerotic lesions and gall-stones. Cholesterol by itself insoluble in water can be solubilised in the mesomorphous phase of the lecithin-water system.[1]

A significant feature of the molecular interactions between cholesterol and lecithins is the condensing effect of cholesterol in mixed monolayers with most lecithins above their chain melting temperature.[2] This reduction in the mean molecular area was also found to be valid for bulk systems from calculations of X-ray data.[1]

Cholesterol has on the contrary been shown to cause a considerable increase in the surface area of lecithin in sonicated vesicles.[3]

Ladbrooke *et al.* applied differential scanning colorimetry (DSC) to cholesterol-lecithin suspensions and found that cholesterol inhibits the chain motion of lecithin above the gel-liquid crystal transition temperature and concluded that the transition vanishes at a 1:1 mol ratio.[4] The deuteron NMR [5] and ESR spin label method [6] have provided more details of the molecular interaction. It has been found that the restriction of the lecithin chain motion is smaller at the free nonpolar ends of the chains than in the region near the glycerol backbone. The lecithin phosphate group and cholesterol hydroxyl appear to be juxtaposed and may be hydrogen bonded.[7]

Regarding the interaction between mixtures of lecithin-cholesterol and water, DSC measurements indicate that the bound water is a maximum at 50 mol % cholesterol.[8] Other authors, on the contrary, have found decreasing affinity for water with increasing cholesterol concentration.[9] No vapour pressure measurements on lecithin-cholesterol mixtures seem to have been reported.

MATERIALS AND METHODS

The cholesterol used (Merck *p.a.*) was recrystallized three times from 1,2-dichlorethane. It was found to be chromatographically pure by TLC and GLC. The egg lecithin was isolated in this laboratory.[10] It was proved to be chromatographically and spectroscopically pure by TLC and IR-spectroscopy. The water was distilled twice in an apparatus of silica.

The lipid mixtures were prepared by dissolving cholesterol and lecithin in ampouls and the solvent was then removed in a stream of nitrogen. The last traces of solvent were removed *in vacuo* and then an appropriate amount of water was added. The ampouls were sealed under nitrogen and placed in a thermostated water bath to equilibrate over a period of ten days. After the X-ray diffraction measurements the water contents were checked with a Karl Fisher titration.

The X-ray diffraction measurements were conducted with an apparatus previously de-

scribed.[11] The Cu$K\alpha$ radiation used was Ni-filtered and the incident X-ray beam was collimated by a slit system. The scattering angle was measured with a Rigaku-Denki low-angle goniometer. No slit corrections were done.

The adsorption of water vapour by egg lecithin-cholesterol mixtures was measured by keeping the lipid mixtures in weighing bottles over standard saturated salt solutions in vacuum. All measurements were performed at a temperature of (25 ± 1) °C.

RESULTS

The X-ray diffraction measurements were limited to the region of the lamellar mesophase in the egg lecithin-cholesterol-water system. To make sure that the samples belonged to this phase they were examined by a polarizing microscope after the X-ray measurements. In order to make the results as surveyable as possible the cholesterol concentration at three constant lecithin/water ratios (9:1, 7.5:2.5, 6:4) was varied. The variations in long spacings, D, with cholesterol for the three different lecithin to water ratios are shown in Fig. 1. For the lecithin/water ratio of 9:1 the maximum solubilising capacity is reached at about 20 % cholesterol concentration then the curve stops.

Starting with the D values and the corresponding lecithin, cholesterol, and water percentages one can calculate a number of parameters in the system studied. In the calculations the following considerations were accounted for. From the molecular volumes and volume fractions of this egg lecithin [10] and cholesterol the mean molecular volumes for

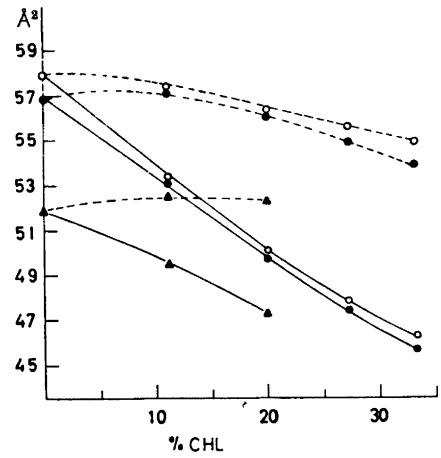

Fig. 2. The mean molecular surface area (S_M), continuous lines, and partial molecular area of lecithin (S_L), broken lines, as a function of the dry weight percentage of cholesterol. Each curve is relative to a fixed lecithin to water ratio of 9:1 (▲), 7.5:2.5 (●), and 6:4 (○), respectively.

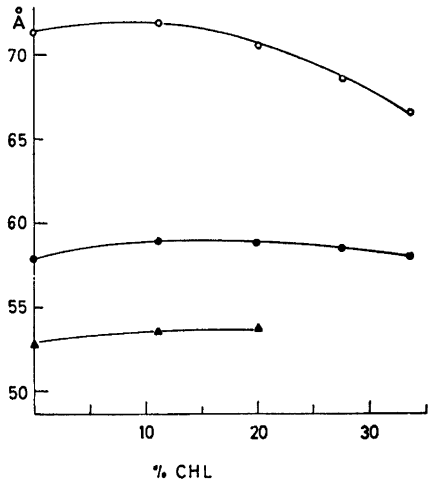

Fig. 1. The long spacings (D) as a function of a dry weight percentage of cholesterol. Each curve is relative to a fixed lecithin to water ratio of 9:1 (▲), 7.5:2.5 (●), and 6:4 (○), respectively.

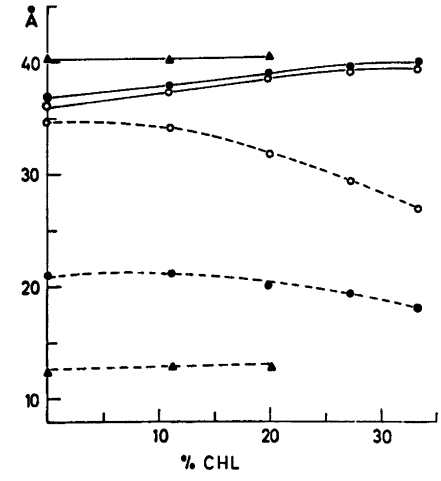

Fig. 3. The thickness of the lipid layer (d_L), continuous lines, and the thickness of the water layer (d_w), broken lines, as a function of the dry weight percentage of cholesterol. The curves represent lecithin to water ratios of 9:1 (▲), 7.5:2.5 (●), and 6:4 (○), respectively.

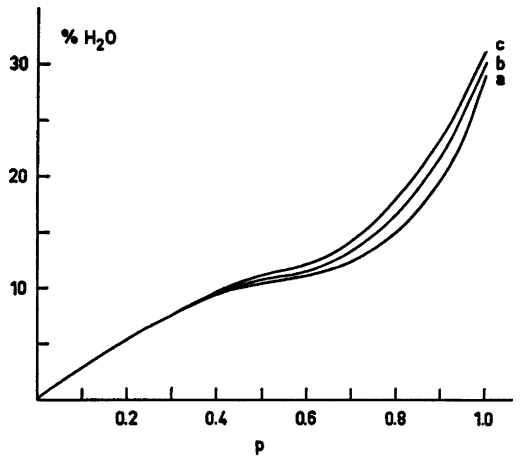

Fig. 4. Adsorption of water vapour by egg lecithin in mixtures with cholesterol. The weight percentage of adsorbed water based on water and lecithin weight only is plotted against the relative vapour pressure (p). Curve a 0 % cholesterol; b 15 % cholesterol, and c 30 % cholesterol.

mixtures of them (V_{LC}) are calculated. The mean molecular volume (V_L) of the lipidic layer exclusive the phosphoryl choline group is calculated from the molecular volumes of the glycerohydrocarbon part [10] and cholesterol and the volume fractions of them. The cholesterol molecules are assumed to occupy a practically constant area in the mixed layers of the lamellar phase whatever the molar fraction is. From monolayer measurements the surface area of cholesterol has been calculated to 37.5 Å² per molecule.[14] Starting with the related values, the following parameters can be estimated.

1. The mean molecular surface area, S_M, in Å² per molecule. $S_M = V_{LC}/(D/2 \times \phi_{LC})$, where $D/2$ is one half of the long spacing and ϕ_{LC} equals volume fraction of lecithin and cholesterol.

2. The surface area, S_L, per lecithin molecule in Å² per molecule. $S_L = S_M - (X_c \times 37.5)/X_L$, where X_C and X_L are mol fractions of cholesterol and lecithin, respectively.

3. The thickness, d_L, in Å of the lipid layer: $d_L = 2V_L/S$.

4. The thickness in Å of the water layer, d_w, including the phosphoryl choline group: $d_w = D - d_L$.

The experimental D values and the calculated parameters are summarized in Figs. 1, 2, and 3.

The results from the vapour pressure measurements are presented in Fig. 4. To make the curves more commensurable the water percentages are calculated on water and lecithin weight only. A slight increase in water adsorption with increasing cholesterol concentration is noted.

DISCUSSION

The most striking changes when cholesterol is incorporated into the hydrated lecithin bilayers are the reduction in the surface area of lecithin (see Fig. 2) and the corresponding increase in lipid layer thickness (see Fig. 3). It is still under discussion whether these cholesterol effects are caused by inhibition of chain motions or by orientation of their long axes more nearly perpendicular to the plane of the bilayer or by both these effects. NMR studies have shown that cholesterol severely restricts the lecithin chain motions.[5] Spin label studies indicate that lecithin which makes an angle of about 30°, with the normal to the lamellar phase, is made more nearly perpendicular by the addition of cholesterol.[15] It may be that the reduction in surface area and increase in lipid layer thickness is due to both the effects mentioned.

Although the reduction in surface area of lecithin by cholesterol is significant it is not of the same order of magnitude as in mixed films. For a 1:1 mixed monolayer the surface area of lecithin is at the collapse point *ca.* 44 Å²/molecule compared with *ca.* 55 for the same molar proportions at 6:4 hydration (see Fig. 2) in the bulk system. This discrepancy may be explained by the fact that the conditions are not identical. In a monolayer the paraffinic ends are in contact with air, whereas in a bulk system they face other paraffinic ends.

The vapour pressure measurements show that the water binding capacity of lecithin is not much altered by the incorporation of cholesterol. This indicates that the configuration of the lecithin phosphoryl choline group is not much altered by the interaction with the cholesterol hydroxyl. NMR studies have indicated a reduction in the $N(CH_3)_3$ mobility. The proportion of bulky hydrophilic end groups

decreases, however, when cholesterol is incorporated and therefore there is more space for water and greater freedom for hydrogen bondings.

The results from the vapour pressure measurements favour the opinion that the lecithin-cholesterol interaction is mainly hydrophobic. The most noticeable apolar effect is the reduction in the molecular area. In the light of the present study this condensing effect mainly found in membrane model studies with monolayers may have been overstressed. The situation with paraffinic ends facing each other is the same for both lamellar bulk systems and biomembranes. Therefore the much smaller condensing effect calculated from X-ray measurements may be relevant for membranes too. The main effect of cholesterol in natural structures seems more likely to be the chain melting effect thus regulating the physical state of the hydrocarbon region.

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