An X-Ray and Vapour Pressure Study of the Monoolein—Cholesterol—Water System

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The structural characteristics and molecular interactions in the monoolein — water system and the effect of cholesterol on these are studied with X-ray diffraction and vapour pressure measurements.

The lamellar liquid crystalline phase, which exists between about 5 and 23 % water, shows a one-dimensional swelling. This means that no water is incorporated between the monoolein molecules, all of it being intercalated between the lipid bilayers. The thickness of the lipid layer and the surface area per monoolein molecule is almost independent of the water contents. If the water percentage exceeds 25 a cubic, viscous isotropic, phase is formed. The maximal water adsorption by monoolein corresponds to the boundary between the lamellar and cubic phases.

Cholesterol can be solubilized by the lamellar phase in a molar ratio of 2:1 between monoolein and cholesterol. Cholesterol has a condensing effect on monoolein which is accompanied by an increase in the lipid bilayer thickness while the thickness of the water layer is constant. Also the cubic phase can solubilize cholesterol and an addition of cholesterol can result in a transition from the cubic to the lamellar phase. The vapour pressure measurements show that cholesterol has only a slight increasing effect on the water adsorption by monoolein.

Monoglycerides are formed at lipase degradation of fats in the intestine. Within the food industry they are used as additives in order to modify texture and other physical properties. Knowledge of the phase behaviour and the interactions with other lipids is thus of interest both from physiological and technological points of view.

Phase diagrams of binary monoglyceride—water systems have been reported by Lutton.[1] Systematic studies of the phase behaviour and structural characteristics of monoglyceride systems have been performed by Larsson.[2]

The main interest in this study has been devoted to the structural parameters of the lamellar phase in the monoolein — water system and the effect of cholesterol on these.

MATERIALS AND METHODS

The monoolein used was purified by Florisil column chromatography and was found to be pure by conventional TLC. The cholesterol was recrystallized three times from 1,2-dichloroethane and the purity of it was checked by GLC.

Samples for X-ray diffraction experiments were obtained by weighing monoolein in glass ampoules and adding an appropriate amount of water. The ampoules were then sealed and left to equilibrate over a period of ten days at 25 °C.

The low angle X-ray measurements were performed with an apparatus described earlier.[3] The $CuK\alpha$ radiation used was Ni-filtered and the scattering angle was measured with a Rigaku-Denki small-angle goniometer.

The water vapour adsoption of the lipids was measured by keeping them in bottles over saturated salt solutions in a vacuum chamber, which was thermostatted at 25 °C. The relative water vapour pressure (p/p_0) in the chamber was increased gradually and the samples were kept at each relative humidity until the weight was constant.

RESULTS

The phase diagram of the monoolein—cholesterol—water system has been presented elsewhere.[4] The β-crystal form of monoolein melts at 35 °C. On cooling, an α-form is first formed which can take up about 5 % (w/w)
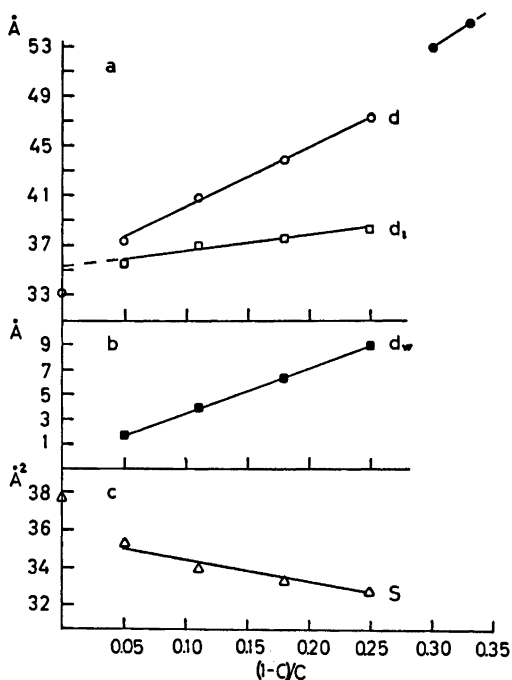
Fig. 1. Structural parameters of the lamellar monoolein − water phase calculated from X-ray data as functions of the volume fraction of monoolein (c). (a) The fundamental interplanar repeat distance, d, (O) (for comparison the dominating spacing of the cubic phase is given (●)) and the thickness of the lipid layer d_1 (□). (b) The thickness of the water layer (■). (c) The molecular surface area of monoolein (△).

In Fig. 1 the long spacings, d, are plotted against $(1-c)/c$ (c=percentage of monoolein). The first order lamellar spacing persists after the transition to the cubic phase at about 23 % water. The nature of the swelling has been analyzed by plotting log d versus log $(1/v_a)$ where v_a is the volume fraction of monoolein. The swelling was found to be principally one-dimensional (slope 1.2) for both the lamellar and the cubic phase.

Starting with the d values and knowing the volume fractions of the samples some structural parameters of the lamellar phase can be calculated. The values found for the surface area (S) per monoolein molecule in $Å^2$ per molecule, the thickness of the lipid layer (d_1) and the water layer (d_w) in Å are presented in Fig. 1. The surface area of the monoolein molecule decreases while the thickness of the lipid layer increases with increasing water contents. If the d_1 line is extrapolated to zero water contents a value of 35.5 Å is obtained while the d value for dry monoolein was found to be 33.2 Å.
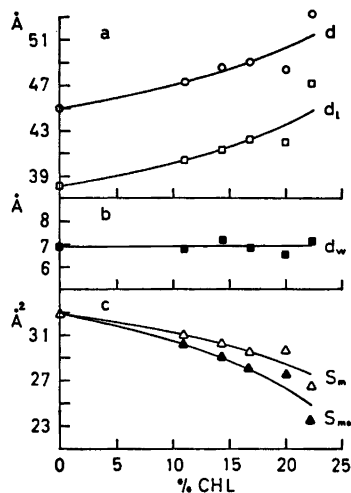
of water and form a gel-phase. A lamellar liquid crystalline phase is formed when up to about 20 % water is added to monoolein, and at higher water concentrations a cubic phase is obtained. Cholesterol can be solubilized in the lamellar phase up to a molecular ratio of 1:2 between cholesterol and monoolein. Also the cubic phase can solubilize considerable amounts of cholesterol.

The X-ray diffraction data for the monoolein − water system are given in Fig. 1. The long spacings, d, of the lamellar phase refer to the repeated distance of the bimolecular lipid and water layers.[5] For the cubic phase there are not enough diffraction lines observed for a determination of the symmetry and therefore no structural parameters can be calculated and only the dominating spacing is shown.

Fig. 2. Effects of the dry weight percentage of cholesterol on the structural parameters of the lamellar monoolein − water phase at a fixed monoolein to water ratio of 5:1. (a) The long spacings, d, (O) and the thickness of the lipid layer, d_1, (□). (b) The thickness of the water layer, (d_w). (c) The mean molecular surface area (S_m) (△) and the partial molecular surface area of monoolein (S_{mo}). (▲).
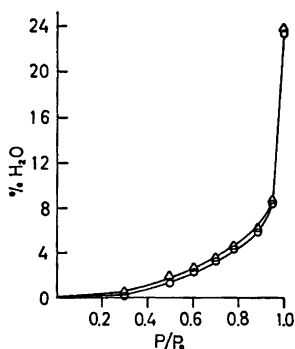
Fig. 3. Water vapour adsorption isotherm at 25 °C for monoolein (O) and monoolein—cholesterol (molar ratio 2:1) (△). The weight percentage of adsorbed water based on water and monoolein weight only is plotted against the relative vapour pressure (p/p_0).

The effect of cholesterol on the structural parameters was studied at a constant ratio of 5:1 between monoolein and water. The variation in long spacings with cholesterol contents is shown in Fig. 2. The d values increase somewhat with increasing cholesterol percentage. Assuming a constant area of 37.5 Å^2 per molecule for cholesterol[7] the mean molecular surface area (S_m) and the surface area per monoolein molecule (S_{mo}) can be calculated. Also the thickness of the lipid (d_1) and water (d_w) layers can be estimated (Fig. 2). Cholesterol has a marked condensing effect on monoolein. This effect is accompanied by an increase in the thickness of the lipid layer. The thickness of the water layer is almost unaltered by addition of cholesterol.

The results from the vapour pressure measurements are presented in Fig. 3. The water percentages are calculated on water and monoolein weights only. The maximal water adsorption by monoolein corresponds to the phase boundary between the lamellar and cubic phases. Cholesterol has only a small increasing effect on the water adsorption of monoolein.

DISCUSSION

Dry monoolein at 25 °C (gel phase) gives a d value of 33.2 Å, but if the line of d vs. $(1-c)/c$ is extrapolated to zero water contents a value

of 35.5 Å is obtained. This fact indicates that the fatty acid chains in the gel phase are tilted and in the lamellar phase perpendicular to the bilayer. The tilted to vertical chain transition will result in a decreased surface area per monoolein molecule at the bilayer surface.

The swelling of the lamellar monoolein-water phase is one-dimensional. In accordance with this the thickness of the lipid bilayer and the interfacial area per polar group are almost independent of the water contents. This means that no additional water seems to be incorporated between the monoolein molecules, all of it being intercalated between the layers. Such behaviour is typical for many nonionic swelling amphiphiles.[8] The dominating spacing of the cubic phase varies with water contents in a way similar to that of the lamellar phase. This fact seems to support the idea that the cubic phase is built up of small lamellar bilayer units.[9]

It is interesting to note that an addition of both water and cholesterol causes a transition from lamellar to cubic phase. Also the structural changes introduced by these compounds are similar; an increase in lipid bilayer thickness and a decrease in surface area of monoolein. The condensing effect of cholesterol is, however, less than that found by surface balance measurements.[4] A corresponding behaviour was also found for lecithin[7,10] and this fact stresses the differences of molecular packing between mono- and bilayers.

The maximal cholesterol solubilizing capacity of the lamellar monoolein—water phase of 2:1 between monoolein and cholesterol can be compared with the corresponding value of 1:1 for the lecithin—water lamellar phase.[10] From these values one can conclude that the molar ratio between hydrocarbon chains and cholesterol is the critical factor for the molecular interaction. The same conclusion can be drawn from monolayer studies which have shown maximal condensing effects at the same molar ratio.[12]

The water adsorption isotherm of monoolein is very different from that found for lecithin.[11] The marked water adsorption at low vapour pressure noted for lecithin, which indicates strongly bound water, is absent with monoolein. The fact that the maximal water adsorption coincides with the lamellar-cubic phase

boundary shows that additional water added to the cubic phase is free water. The effect of cholesterol on the water adsorption of monoolein is almost insignificant which indicates that there is no pronounced interaction between the polar groups of these lipids.

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