Acta Chemica Scandinavica A 30 (1976) 281—284

Lecithin—Diglyceride Interaction in Surface Films on Water and in Aqueous Systems

ROSALIND FAIMAN, KÅRE LARSSON * and BALAZS SZALONTAI

Lipid Chemistry Laboratory, University of Göteborg, CTH, Rännvägen 6, S-402 20 Göteborg 5, Sweden.

The interaction between the diglyceride 1-oleyl-3-stearin and L-α-dipalmitoyllecithin in monomolecular surface films at the air/water interface was studied by the surface balance technique. This diglyceride exhibits a condensed phase with extended molecules, which means that the methyl end groups form the contact surface with water. The diglyceride-lecithin mixtures form one phase with the polar groups in contact with water over the whole composition interval. At high pressures molecular segregation occurs at a diglyceride content above the 1:1 molecular ratio and the extended molecular conformation is adopted for diglyceride molecules above the 1:1 composition. Three-dimensional aqueous phases were examined by Raman spectroscopy, with emphasis on the hydrocarbon chain structure. It was found that in liposomes a few degrees below the chain melting temperature, the transition tilted to vertical hydrocarbon chains is influenced by small amounts of diglyceride, and that these effects are closely related to the monolayer phase behavior.

The interaction between different lipid components is important for the structure and function of biological membranes. The cholesterol-lecithin interaction has therefore been extensively studied, but it is somewhat surprising that almost no other components have been examined in detail in this respect. The diglyceride-lecithin interaction is interesting from many points of view. Diglycerides are naturally present in the membranes (sometimes about 10 %). The molecular geometry is the same as in the dominating membrane lipids, and therefore variations in, for example, the lecithin-diglyceride ratio corresponds mainly to variations in the strength of the water association. Furthermore the existence of one free hydroxyl group in the diglyceride molecule, which can act as a hydrogen bonding donor, relates it to cholesterol, and effects due to the rigid steroid skeleton can therefore be obtained by comparison with the well known cholesterol-lecithin interaction. Finally, the existence of an extended molecular conformation of the saturated diglycerides as a transition state in surface films on water was reported earlier.[1] It was therefore considered of interest to see whether this form is favoured in a mixed saturated/unsaturated diglyceride and whether some lecithin can participate in the formation of this type of surface film.

The surface balance technique was used in order to study the diglyceride-lecithin interaction at the air/water interface. In order to correlate the phase transitions in monolayers with aqueous bulk phases Raman spectroscopy was used.

MATERIALS AND METHODS

Standard methods were used in the synthesis of L-α-dipalmitoyl-lecithin (DPL) and the 1-oleoyl-3-stearoyl diglyceride (OHS). The purity was checked by TLC (no 1,2-diglyceride isomer was found to be present). A continuously recording surface balance of the vertical Wilhelmy type was used, and experimental details are given in Ref. 1. A Cary 82 Raman spectrophotometer with argon ion laser excitation was used. The samples were kept in a temperature controlled cell,[2] and other experimental details have been given earlier.[3] Mixtures of OHS-DPL differing by 10 % (w/w) were prepared and the solvent used in the monolayer work was hexane-ethanol 9:1. A few corresponding bulk samples with excess water were prepared, and

---

* Present address: Div. of Food Science, Chemical Center, Box 740, S-220 07 Lund 7, Sweden.

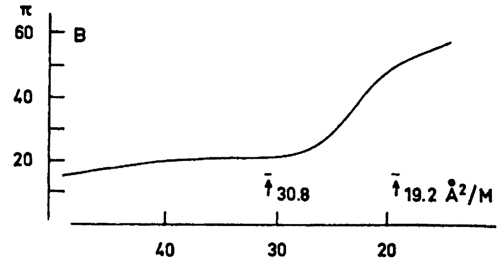

Fig. 1. Surface pressure isotherm of OHS at 20 °C. The photographically recorded isotherm is shown with surface value calibration points.

owing to the limited amount of available diglyceride the three most interesting compositions were selected. The samples contained about 75 % (w/w) of water, which means that the lipids form a dispersion of liposomes (in a gel or liquid crystalline state depending upon the temperature).

RESULTS AND DISCUSSION

The pressure-area isotherm of OHS at 20.0 °C is shown in Fig. 1. It is important to notice that the molecular area corresponding to what normally is described as a collapse point of 18.2 Å^2 per molecule corresponds to extended molecules in this monolayer phase. There is a plateau starting at a molecular area of 38.5 Å^2 per molecule. It is evident from these values that a monolayer with the glycerol group in contact with water is formed first, and on further compression an extended conformation is adopted. As mentioned earlier it is known from similar studies of saturated diglycerides that an extended conformation can be obtained as a state of transitory existence.[1] The possibility of chain segregation into a chain layer occurring with saturated chains and another with unsaturated chains means such a reduction in free energy arising from better molecular close-packing that the extended conformation is a stable state at the water/air interface. It is believed that this transition between the extended form and the ordinary bilayer structure might have relevance for certain transport functions of membranes. Most membrane lipids have the same molecular geometry (one saturated and one unsaturated chain), and a conformational change of any protein present at the water interface can in principle result in a hydrophobic environment of the polar group. Ordinary membrane lipids might under such conditions be expected to behave as the diglyceride described above.

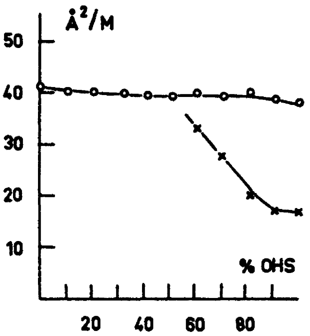

Fig. 2. Average molecular area at the collapse point *versus* composition of DPL-OHS mixtures. The collapse point corresponding to the extended molecular conformation is also shown.

The average molecular area at collapse of the monolayer *versus* composition of binary mixtures DPL-OHS is shown in Fig. 2. The solid-condensed form with the two chains of the molecules laterally arranged has a molecular area at collapse of about 40 Å^2. This is a characteristic value of vertical chains in the hexagonal form, and it corresponds to the gel phase in aqueous lecithin bulk systems (*cf.* Ref. 4). No particular condensation effect is observed at the collapse pressure as well as at lower pressure values. This is interesting with regard to cholesterol-lecithin interaction. The well-known condensation effect in this case is usually ascribed to an effect from the rigid skeletons on the hydrocarbon chain mobility. Another explanation might be hydrogen bonding. As lecithin only contains hydrogen bonding acceptors it should be expected that any hydrogen bonding donor (such as the cholesterol hydroxyl group) would stabilize the mixed bilayer structure, the strongest effect corresponding to a 1:1 ratio. A consequence of this could be condensation of mixed monolayers. The diglyceride can like cholesterol act as a hydrogen bonding donor, and as will be seen below there is evidence for the occurrence of a 1:1 complex, and the lack of condensation effects indicates therefore that cholesterol-lecithin condensation is more due to the rigid steroid skeleton.

The collapse point of the extended form discussed above for pure OHS is also shown in Fig. 2. It can be seen that there is no such phase at a diglyceride content of less than the 1:1 molecular ratio. The linear behavior of this collapse curve (see Fig. 2) shows that all the diglyceride molecules above this limit are segregated, so that above the collapse pressure of the mixed monolayer the excess of diglyceride molecules (above the 1:1 molecular ratio) is squeezed out to form separate film regions with an extended structure. The shape of the collapse curve at low lecithin content indicates that a minor proportion of the lecithin molecules are solved in the segregated diglyceride phase. It is proposed that the explanation for this behavior at high pressures is that there is a DPL-OHS hydrogen bonded molecular compound. As the lecithin molecules have many hydrogen bonding acceptors it seems probable that there is steric hindrance to hydrogen bonding associated with more than one diglyceride molecule per lecithin molecule.

It has recently been reported that DPL in excess water shows a transition from tilted to vertical chains at about 34 °C, and chain melting (gel→liquid crystal) at about 42 °C[4]). Related monolayer phases were also observed. In the binary DPL-OHS monolayer mixtures it was found that the tilted phase of DPL was stabilized at higher temperatures by the addition of OHS. An isotherm demonstrating this effect is shown in Fig. 3. The solid-condensed phase with tilted chains, which is formed at low pressures and which shows high compressibility, exists over a wide pressure range. In the case of pure DPL this tilted phase has a very narrow range of existence, whereas the high-pressure phase with vertical chains exists at a broader pressure range.[4]

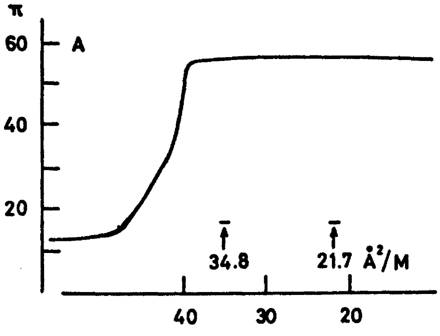

Fig. 3. Surface-pressure isotherm of a DPL-OHS 70:30 (w/w) mixture at 20 °C.

The Stokes Raman spectra DPL and DPL-OHS mixtures, in the proportions 90:10, 80:20, and 70:30 (w/w) in the presence of excess water were recorded at various temperatures in the region of vertical chain→tilted chain→disordered chain transitions, *i.e.* between 30 – 45 °C.[4] It has been shown earlier that the band intensity ratio I_{1090}/I_{1130} in the vibrational skeletal stretching region is highly sensitive to the conformation of the hydrocarbon chains particularly for the cooperative chain melting process when the all- or nearly all-*trans* configuration of the chains collapses to form the liquid crystalline state.[5,6] The ratio I_{1090}/I_{1130} for aqueous DPL is shown in Fig. 4, together with the errors allowed in the positioning of each point. There is some indication of a minor increase in this ratio, I_{1090}/I_{1130} in the temperature range of 34 – 42 °C, where the vertical →tilted chain transition is known to occur in the crystalline state [4] but the uncertainties in the experimental values, shown in Fig. 4, are too large to detect the lower temperature of this transition. The chain melting temperature at 39.5 – 41 °C is, however, clearly shown by the steep gradient of the curve, indicating that a co-operative chain melting process is occurring in this region.[5,6]

The I_{1090}/I_{1130} intensity ratio *versus* temperature was also plotted for each of the DPL-

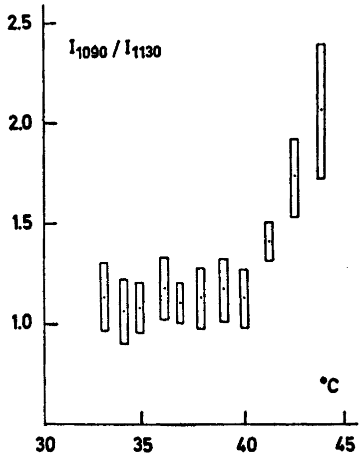

Fig. 4. Intensity ratio *versus* temperature of the 1090/1130 cm^{-1} bands in the Raman spectra of pure DPL. The experimental errors are indicated.

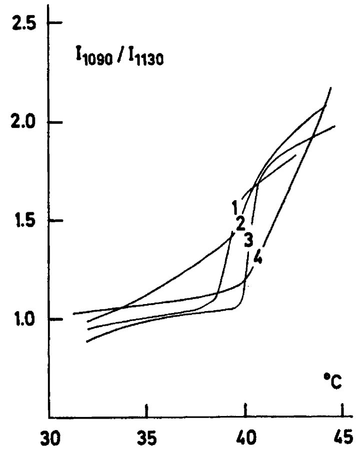

Fig. 5. The intensity ratio 1090/1130 cm^{-1} *versus* temperature of mixtures of DPL-OHS 10:90 (1), 20:80 (2), 30:70 (3) and of pure DPL (4).

OHS ratios and are shown in Fig. 5. The 90:10 ratio is designated 1, the 80:20 ratio 2, the 70:30 ratio 3, and the pure DPL curve is included for a comparison and designated 4.

Two interesting effects are observed from the addition of diglyceride to DPL, as can be seen in the Fig. 4. Firstly, there are significant shifts in the chain melting temperatures for each ratio. The magnitude of this shift may be taken as a measure of the lattice disturbances introduced by the presence of the diglyceride. This is because the gradient of the curve produced can be related to the degree of purity of the lipid and hydrocarbon chain saturation.[2,6] This result can be correlated with the features observed in the monolayer behavior described above. That is, the existence range of the vertical chain phase appears to be reduced by the presence of the diglyceride, *e.g.* in the case of the addition of 10 % (w/w) of diglyceride (curve 1) there is a shift of 1.5 – 2.0 °C to a lower temperature.

Secondly, there is a reduction in the gradient of the crystalline→liquid crystalline transition in the presence of 10 % OHS (curve 1) and 20 % OHS (curve 2) compared with the pure DPL (curve 4) but not with the addition of 30 % OHS (curve 3). The presence of *cis* C=C double bonds is known to disturb the chain packing in the crystalline state so that there is some degree of disorder already inherent in the crystalline form before transition to the liquid crystalline state occurs.[2,6] The magnitude of this disturbance in the all-*trans* conformation appears to be largest in the case of the addition of 20 % OHS (curve 2) where the gradient of I_{1090}/I_{1130} *versus* temperature is least.

In the case of the 70:30 ratio of DPL:OHS the gradient of the curve 3 is steeper than in the case of the other mixtures and, moreover, than in the case of the pure DPL (curve 4). This suggests an increase in the hydrocarbon chain order as the concentration of diglyceride is increased towards the 1:1 molecular ratio.

In conclusion it is interesting to note that this Raman spectroscopic behavior displayed by DPL-OHS mixtures at concentrations ≥70:30 is found to be contrary to the Raman spectroscopic behaviour observed in DPL-cholesterol mixtures and in fact other lipid-cholesterol mixtures.[2,7] In the case of the DPL-cholesterol mixture, the gradient of the curve I_{1090}/I_{1130} *versus* temperature decreases linearly with increasing concentrations of cholesterol even when the ratio of DPL-cholesterol is 1:1. This evidence tends to suggest that whereas the diglyceride forms a 1:1 molecular complex with the lipid in the bilayer, the presence of cholesterol in the ratio of 1:1 appears to form a looser type of association with the lipid.

Acknowledgement. The authors would like to acknowledge grants from the Natural Science Research Council, a UNESCO fellowship (for B.S.) and an EMBO fellowship (for R.F.) which made the work for this publication possible.

REFERENCES

1. Larsson, K. *Biochim. Biophys. Acta 318* (1973) 1.
2. Faiman, R. *Thesis*, University of Bradford 1974.
3. Larsson, K. and Rand, R. P. *Biochim. Biophys. Acta 326* (1973) 245.
4. Rand, R. P., Chapman, D. and Larsson, K. *Biophys. J. In press.*
5. Lippert, J. L. and Peticolas, W. L. *Proc. Nat. Acad. Sci. USA 68* (1971) 1572.
6. Faiman, R. and Long, D. A. *J. Raman Spectrosc. In press.*
7. Faiman, R., Larsson, K. and Long, D. A. *To be published.*

Received September 18, 1975.