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THE OCCURRENCE OF DIFFERENT MOLECULAR CONFORMATIONS OF DIGLYCERIDES AT THE AIR-WATER INTERFACE

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SUMMARY

Pressure-area isotherms of 1,3-dimyristin surface films on water are reported which show that a state of transitory existence with the two acyl chains pointing in opposite directions in relation to the glycerol group is formed when compression is continued beyond the collapse point of the monolayer in which the two acyl chains are located in the same layer. A triple chain layer is formed at further compression. As the molecular geometry of many membrane lipids is related to that of diglycerides, the observed existence of an extended molecular conformation on water might be of interest for discussions on lipid phase transitions in membranes.

INTRODUCTION

Measurements showing that diglycerides can, under certain conditions, form a monomolecular surface film phase on water with the two acyl chains pointing in opposite directions in relation to the glycerol group will be reported here. The dominating membrane lipids possess the same molecular geometry, and although there are important differences in the polar head groups, it is suggested that phase transitions involving such a conformation, called extended in the following text, might be relevant for flip-flop and transport functions in membranes.

METHODS AND MATERIALS

Pressure-area (π-A) isotherms were recorded by a continuously recording surface balance of the horizontal Wilhelmy type. The diglycerides were solved in hexane-ethanol (9:1, by vol.) and spread by an Agla micrometer syringe. A compression rate of about 4 Å^2 per molecule per min were used. The purity of the diglycerides used was checked by thin-layer chromatography.

The phase behaviour of surface films of symmetric, saturated diglycerides was studied in the temperature range 5-35 °C. Short members, such as 1,3-dicaprin and 1,3-dilaurin, form only one condensed phase, which is of a liquid type. In the case of longer homologues a solid-condensed phase is also formed. π-A isotherms of 1,3-dimyristin are shown in Fig. 1. At temperatures above 28 °C, only a liquid-

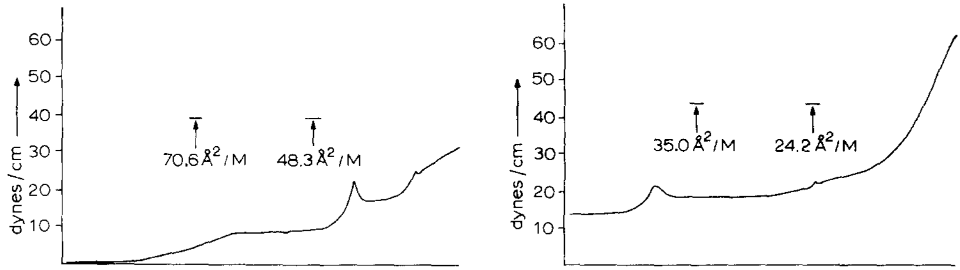

Fig. 1. Pressure–area isotherm of 1,3-dimyristin at 17.2 °C (to the left) and 21.0 °C (to the right).

condensed phase is formed, with a collapse area of about 64 Å^2 per molecule. At lower temperatures a solid-condensed phase is formed, and as can be seen in Fig. 1, the collapse area is 40.7 Å^2 at 17.2 °C. The compressibility properties and areas per hydrocarbon chain of the phases show that they are of liquid-condensed and solid-condensed types, respectively. The pressure drops after collapse of the monolayer, and approaches the value corresponding to equilibrium between the liquid-condensed and the solid-condensed phases. At about 24 Å^2/M there is a hump in the dynamic compression curve. This area is in agreement with the expected value of an extended molecular conformation, as the closest packing of hydrocarbon chains corresponds to a cross-section area of about 18 Å^2 per chain so that the smallest possible molecular area of diglyceride with the polar group in contact with water is about 36 Å^2. The hump in the dynamic compression curve is reproducible, and it is more pronounced at higher temperatures as shown in Fig. 1. At further compression at 21 °C there is a steep rise in pressure starting at about 17 Å^2/M with an inflexion point at about 12 Å^2/M. A structure with an extended molecular layer above a folded molecular layer in this compression region is suggested on the basis of these values and the general behaviour of lipid multilayers. It has thus been found that various lipids, such as fatty acids, simple esters and triglycerides, exhibit a phase with a monolayer under a double layer[1,2]. If then a triple chain layer is assumed here, the 21 °C isotherm shows a strong increase in compressibility at an area per hydrocarbon chain of about 22 Å^2 and a highest compressibility at about 18 Å^2 per chain, which is quite reasonable.

The purity of the diglyceride is a most important factor for the formation of the transition state with extended molecules. The corresponding 1,2-diglyceride does not exhibit this state according to the dynamic compression isotherms. Even when there are only minor amounts of 1,2-diglyceride present in the 1,3-diglyceride surface film, the hump in the isotherm indicating an extended conformation disappears. The reason for this is probably not that one molecule is symmetric and the other not, but that a very favourable packing of extended molecules is achieved just in the case of 1,3-diglycerides. It should be mentioned in this connection that simple long-chain esters form similar extended monolayers. In ethyl stearate[1] for example, the molecular area at collapse is about 18 Å^2, which means that the methyl groups of the extended ester groups form the contact surface with water. If the ester group is elongated, molecules with extended zig-zag conformation from one methyl end group to the other is formed up to amyl stearate.

Three crystal structures of lipids with this molecular geometry have been reported, two diglyceride crystal forms[3,4] and one ceramide[5]. All three have the extended conformation, with the polar groups in the center of unimolecular layers. X-ray low-angle data of diglycerides indicate that the same structure persists after melting[6]. There is also evidence for the existence of an extended conformation in the solid state of glycosphingolipids[7]. This indicates that a favourable molecular packing is obtained by extended molecules. It can, therefore, be expected that the unfavourable surface energy contribution from the polar groups in the case of an extended unimolecular layer in contact with water, compared to the conformation with the polar groups at the water interface, to some degree is compensated by the better packing adopted by extended molecules.

It is believed that such an extended conformation might be relevant for the structural properties of biomembranes under certain conditions. The possible significance of lipid phase transitions in membranes is frequently discussed (*cf.* ref. 8). If there is a phase transition in a lipid bilayer from the folded conformation, representing the stable state, to an extended form, followed by a transition in the reversed direction, a flip-flop rearrangement of half of the lipid molecules is achieved. Even if the extended conformation only can exist as a metastable phase of transitory existence, it is obvious that transport across the bilayer of small molecules or ions adsorbed on the polar groups might be achieved in this way. The required transition energy can for example be provided from a membrane protein by shielding of the polar groups in a surface section of the lipid bilayer, so that the lipid head groups are not anchored by polar attractive forces at the outer surfaces of the lipid layer. Under such conditions the possibilities for an extended conformation is favoured, according the solid state and surface film behaviour of simple lipids.

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