Physicochemical Characterization of Tetraether Lipids from *Thermoplasma acidophilum*

II. Film Balance Studies on the Monomolecular Organization of the Main Glycophospholipid in Monofilms

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The bipolar main tetraether lipid (MPL) of *Thermoplasma acidophilum* has been shown to form typical liquid expanded films at the air-water interface. The limiting molecular area at the collapse pressure is approximately $A_c = 73 \text{Å}^2$ per molecule. Monopolar diphytanyl diether lipids were found to occupy the same area at high surface pressure as MPL. Thus, it was concluded that in the monofilm only one of the two polar headgroups of the MPL molecules is hydrated, i.e. that the single MPL molecules are oriented upright. The packing properties of MPL in the monofilm are determined by the properties of the branched alkyl chains only; the polar head groups do not contribute to the space requirement in the film. The collapse pressure of the MPL film is approximately $39 \text{ mN m}^{-1}$ at $8^\circ \text{C}$. At a surface pressure of $\pi = 30 \text{ mN m}^{-1}$ and $20^\circ \text{C}$ the film is stable for many hours.

Introduction

The main membrane lipids of the archebacterium *Thermoplasma acidophilum* have been described to be macrocyclic, stretched tetraethers, asymmetrically substituted by a variety of polar head groups [1, 2]. As shown in Fig. 1, the basic structure consists of two repetitively methyl-branched, saturated C40 hydrocarbon chains, linked to two glycerol moieties by ether bonds. Structurally the hydrocarbon chains correspond to two phytanol residues covalently linked head-to-head. From this basic structure derivatives have been described containing up to four cyclopentane moieties instead of methyl branches [2]. The length of the stretched tetraether amounts to approximately 4 nm, thus corresponding to the width of the apolar core of the membrane. Due to this and the bipolar nature of molecules, it has been claimed that the tetraether lipids span the *Thermoplasma* membranes thereby forming a monomolecular layer instead of a bilayer [2]. First experimental support for such assumption came from observations, that *Thermoplasma* membranes cannot be freeze-fracture within the apolar plane (Verkeij, personal communication, [3]). Recently, Gliozzi and coworkers reported that neutral tetraetherlipids from the archebacterium *Sulfolobus acidocaldarius* form stable black lipid membranes which appeared to be organized as monolayers.

In a recent paper [5] we described that the main lipid component of the *Thermoplasma acidophilum* membrane, a glycophospholipid (MPL, Fig. 1), is able to form large, stable liposomes. The permeability of the liposomal membranes for hydrophilic solutes is by about one order of magnitude lower than that of membranes derived from bilayer-forming lipids, e.g. lecithins or phosphatidylinositol. On the other hand, calorimetric studies revealed that hydrated MPL, due to the large number of methyl branches, is a very fluid lipid forming much less condensed structures than lipids with unbranched hydrocarbon chains [6]. In connection with the permeability data, the calorimetric studies would be...
in line with the assumption that the MPL membranes are organized as monomolecular layers.

In the present paper we report on monolayer studies to elucidate this hypothesis. Film balance experiments deliver direct and accurate information on packing properties and limiting molecular areas of membrane lipids in planar layers. In addition to the bipolar MPL, two monopolar model diether lipids were studied as reference substances, 1,2-di-O-diphytanyl-sn-glycerol (DPhG) and 1,2-di-O-diphytanyl-3-O-β-D-glucosyl-sn-glycerol (DPhGG). The structures are presented in Fig. 1: DPhG represents one half of the basic tetraether structure, DPhGG the glycosidic half of MPL. By comparing the molecular space occupied by MPL and the two model lipids at high surface pressure, it could be shown that in the monomolecular film only one of the two polar headgroups of MPL is hydrated, which means, that at the air-water interface the MPL molecules are oriented upright.

Methods and Materials

Growth of Thermoplasma acidophilum and isolation of its main glycophospholipid

The procedures for cell growth, lipid isolation and purification of the main glycophospholipid, MPL, which represents approximately 50% of the total membrane lipid and 75% of the glycoprophospholipid fraction, was recently described [6]. MPL used for the experiments reported in this paper was thin layer chromatographically pure, as checked by means of several solvent systems [6]. According to Langworthy [2], 24% of the MPL hydrocarbon chains are bicyclic, 50% monocyclic and 26% acyclic.

Synthesis of model diether lipids

1,2-di-O-diphytanyl-sn-glycerol was prepared according to Kates et al. [7]. 1,2-di-O-diphtylanyl-3-O-β-D-glucosyl-sn-glycerol (DPhGG). The product (M = 815.31) is a colourless gel. The elementary analysis showed 72.0% C and 12.0% H; the corresponding theoretical values are 72.19% C and 12.12% H, respectively. The purity was further checked by infrared spectroscopy, thin-layer chromatography, and differential scanning calorimetry.

Monolayer experiments

The measurements were performed by means of a Langmuir-trough (MGW-Lauda, model FV-1/2, Lauda-Königshofen, FRG). Films were compressed by moving the barrier at a rate of 1 cm² s⁻¹, corresponding to 2–5×10⁻⁵ nm² molecule⁻¹ s⁻¹. π (surface pressure) versus A (film area) diagrams were plotted using a Hewlett-Packard xy-recorder model 7004 B. Temperature in the subphase was monitored by means of a Pt-100 sensor. Monolayers of the lipid were spread from solutions in methanol/ chloroform on a subphase of fresh, tetradistilled...
(quartz) water. Normally, 50 μl of a solution containing approximately 1 mg lipid ml⁻¹ were applied using a micro-syringe (Hormuth & Vetter, Karlsruhe, FRG). Measurements started 1 min after application of the probe.

**Results and Discussion**

**Formation and stability of monomolecular films from anionic tetraether glycophospholipids**

Figure 2 shows a π/A diagram of MPL and the two model diether lipids, run at 8 °C. As shown by differential scanning calorimetry, the liquid crystalline-to-gel transition of hydrated MPL occurs between −5 °C and −15 °C [5, 6]. Hydrated DPhG and DPhGG do not form a gel phase; they showed only a glass transition at $P < -65$ °C (Ring *et al.* unpublished data). Thus, at 8 °C, all three hydrated lipids are in the liquid-crystalline state. According to Gaines [9], MPL forms a typical liquid expanded film, as the two model lipids do. The collapse pressure $\pi_c$ of MPL amounts to approximately 39 mN m⁻¹, being in between the $\pi_c$ values of DPhG (25 mN m⁻¹) and DPhGG (48 mN m⁻¹). These values are within the same range as those of ester [10] and ether [11] lipids containing unbranched chains, if the collapse occurs directly from the liquid expanded state; condensed monolayers of such substances usually collapse at considerably higher film pressures up to 70 mN m⁻¹ [10, 11].

Long term stability of the MPL film can be derived from Fig. 3. At 20 °C and a surface pressure of $\pi = 30$ mN m⁻¹, i.e. at a film pressure being about 10 mN m⁻¹ below $\pi_c$, approximately 1.4% of the initial film area is lost within the first two hours. After that time, the film is in an equilibrium state since no further net loss of the film was detected within the next ten hours. This is in accordance with observations that liposomes derived from pure MPL remain stable for several days [5]. Considering that, under those experimental condition, MPL is essentially anionic, the high stability of the MPL film may appear surprising. Apparently, the stabilizing forces, consisting of hydrophobic interactions between the long hydrocarbon chains in the apolar core of the layer and, possibly, hydrogen bonds between glycosidic head groups in the polar region, are strong enough to overcome the destabilizing repulsive forces between the charged head groups. This is supported by differential scanning calorimetry data showing that the thermal properties of hydrated MPL are almost completely independent of pH and/or presence of CA²⁺ or Mg²⁺ [6].

**Upright orientation of the tetraether glycophospholipid at the airwater interface**

Based on Fig. 2, the molecular area of MPL at $\pi_c$ and 8 °C is approximately $A_c = 73$ Å² per molecule. The same value was found for DPhG and DPhGG. This finding is only compatible with the assumption that, under these experimental conditions, only one of the two polar headgroups of MPL is anchored in the subphase, i.e. the single molecules in the monofilm must be oriented upright. The data do not allow to conclude whether the orientation of the asymmetric molecules is uniform or not, i.e. whether the direction is parallel or antiparallel. A comparison of

![Fig. 2. Pressure-area diagrams of MPL, DPhG, and DPhGG at 8 °C.](image)

![Fig. 3. Stability of a monomolecular MPL film at $T = 23$ °C and $\pi = 30$ mN m⁻¹ during prolonged incubation.](image)
the data summarized in Fig. 2 further suggest that
the packing density of the three lipids at \( \pi = \pi_c \)
is determined only by the properties of the alkyl
chains. Neither the introduction of a glucosidic head
group (DPhG -> DPhGG) nor the introduction of a
phosphoryl-glyceryl-residue (MPL) significantly
modulate the space requirement at high surface
pressure. This is in agreement with the recent data
by Hinz et al. [12] demonstrating that diether glyco-
lipids containing unbranched alkyl chains occupy a
limiting area of \( A_c = 40 \, \text{Å}^2 \) per molecule, a value
corresponding to twice the value determined for the
corresponding fatty acids. This indicated that even
in lipids exhibiting a much smaller space require-
ment than MPL or the diphytanyl model lipids
studied by us, the glycosidic head group does not
contribute to the space requirement in the monofilm
at high surface pressure. Lastly, our data suggest
that replacement of methyl branches in the hydro-
carbon chains by cyclopentane rings does not
appear to influence the molecular space require-
ment within the limits of detection of the method.

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