# A GLYCOLIPID CONTAINING HOPANE ISOLATED FROM THE ACIDOPHILIC, THERMOPHILIC *BACILLUS ACIDOCALDARIUS*, HAS A CHOLESTEROL-LIKE FUNCTION IN MEMBRANES

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Received 7 March 1980

## 1. Introduction

*Bacillus acidocaldarius* is unique among Bacilli in that it grows optimally at pH 3 and 65°C. The limits of conditions for growth are pH 2–6 and 45–70°C [1]. The intracellular pH is about 6 in this organism [2]. Considering these unusual prerequisites for growth, it was of interest to investigate any special features in the architecture of the cytoplasmic membrane which might enable the organism to grow under, normally, antagonistic conditions.

De Rosa et al. [3] have found that this bacterial strain possesses about 70% ω-cyclohexane fatty acids besides the usual branched-chain fatty acids characteristic of Bacilli. Besides the unusual fatty acid pattern, the complex lipids are also very exotic. In the glycolipid fraction, some pentacyclic triterpene-containing lipids are found, namely 1-(*O*-β-*N*-acyl-glucosaminyl)-2,3,4-tetrahydroxypentane-29-hopane (further called glycolipid; fig.1) and 1,2,3,4-tetra-hydroxypentane-29-hopane [4]. About 15% of the lipids contain hopane which suggests that the character of these lipids may contribute significantly to the properties of the cytoplasmic membrane.

Cholesterol was found to have a pronounced effect on the packaging of the membrane lipids [5]. Because of the structural similarity between the hopane ring system and cholesterol, we investigated a possible similarity in the function within the membrane, by inserting the glycolipid containing hopane. We first investigated this question using the monolayer technique which is basically a physical technique. The results provided an insight into how this glycolipid contributed to the thermophilic and acidophilic properties found in *B. acidocaldarius*.

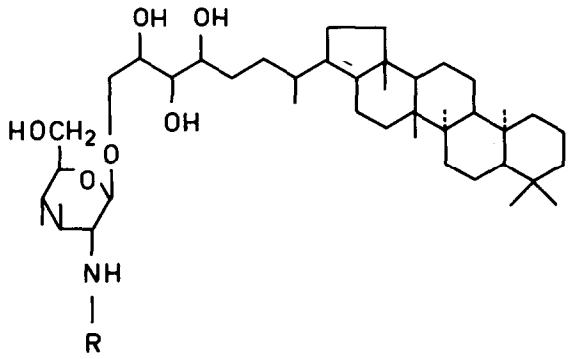


Fig.1. Structure of the glycolipid 1-(*O*-β-*N*-acylglucosaminyl)-2,3,4-tetrahydroxypentane-29-hopane according to [4,7]. R, fatty acid residue.

Lipids containing hopane are not only interesting from the viewpoint of adaptation to extreme environments. A very interesting theory has been proposed that hopanes are phylogenetic precursors and structural equivalents of sterols [6,7].

## 2. Materials and methods

*B. acidocaldarius* strain 104-IA from T. D. Brock was cultured in 200-l volumes in a fermentor with "intensor"-system type b200 (AG für Biologische Verfahrenstechnik Giovanola, Monthey, Switzerland) with 1800 l/h aeration at 50°C and pH 3 in a synthetic medium containing an additional 1 g yeast extract/l [1].

Lipids were extracted from freeze-dried cells [8]. Hydrophilic compounds were removed on Sephadex G-25 [9]. The glycolipids and acidic lipids were

separated on a silica gel column by conventional techniques. After saponification of the glycolipid fraction the nonsaponifiable residue was dissolved in a mixture of chloroform:methanol:water (1:2:0.8) and the hopane-containing lipids precipitated by slow evaporation of the solute at 37°C. The precipitate contained essentially only 1-(*O*-*β*-*N*-acylglucosaminyl)-2,3,4-tetrahydroxypentane-29-hopane and 1,2,3,4-tetrahydroxypentane-29-hopane. These compounds were fractionated by preparative thin-layer chromatography. The glycolipid was identified as a rapid Schiff-positive compound, containing different fatty acids and possessing an MW corresponding to this heterogeneity, as determined by field desorption mass spectrometry. 1,2-Dipalmitoyl-sn-glycero-3-phosphorylcholine (DPPC) was a chromatographically pure product from Fluka, Neu-Ulm, FRG.

Isobars and isotherms of monolayers were measured according to Blume [10] using a commercial Langmuir film balance (Messgerätewerk Dr. Wobser, Lauda, FRG) equipped with a continuous measuring system. Isobars were always recorded with increasing temperature. The preselected surface pressure was kept constant to about ±2 dynes/cm using an automatic pressure control system of the film balance. The lipids were applied to the surface from a stock solution with dichloromethane or chloroform:methanol (4:1).

## 3. Results

First we measured the properties of the hopane glycolipid in the monolayer. The isotherm was compared to that of DPPC. This lipid shows no transition from a liquid-expanded to a liquid-condensed phase with increasing pressure, as is observed for pure DPPC (fig.2). Compared to cholesterol the molecule possesses a slightly higher compressibility (experiment not shown). This higher value of compressibility is presumably due to the fatty acid residue in the glycolipid. We determined the molecular area for the glycolipid as 53 Å$^2$ at 20°C. This value is below the theoretical one for two reasons which include the relatively large error in the determination of the concentration in the stock solution and the probable inclusion of an unknown number of water and/or solvent molecules bound to the glycolipid. However in our opinion it seems justified to calculate the molecular area as a first approximation from the cross

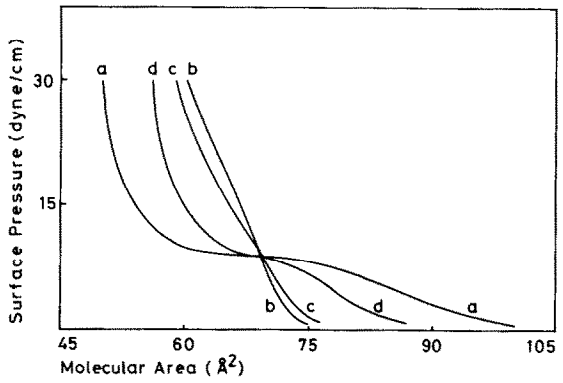


Fig.2. Isotherms at 23°C of (a) DPPC, (b) glycolipid, (c) 1:1 molecular mixture of these compounds, and (d) calculated isotherm of the 1:1 molecular mixture.

sections of the hopane ring system (40 Å$^2$) and the fatty acid chain (20 Å$^2$). The value of 60 Å$^2$ for the molecular area at a pressure of 25 dynes/cm and a temperature of 20°C is therefore preliminary. Because we are measuring relative changes in the molecular areas, our estimates may have to reassessed to some extent, when the true value of the glycolipid is known. The relative changes, however, will be very similar.

In fig.2 the calculated and measured isotherms of a 1:1 molecular mixture of DPPC and glycolipid are shown. At low surface pressure in the liquid-expanded phase of DPPC the measured area is less than the calculated one. Thus the density of the mixed lipid monolayer has increased. This phenomenon is called the condensing effect in the case of sterols [5].

When we measured isobars at 25 dynes/cm, we observed a condensing effect at temperatures above the transition temperature (32.5°C) of DPPC. In curve e of fig.3 the calculated areas of a 1:1 mixture of DPPC and glycolipid at these temperatures show higher values in comparison to the measured values (curve d of fig.3). Whereas at measured areas below the transition temperature, we found larger areas in comparison to the calculated ones. This is a consequence of a fluidizing effect of the glycolipid on the monolayer.

In fig.4 we have summarized the condensing effect at a high temperature and the fluidizing effect at a low temperature with different molecular mixtures of DPPC and glycolipid.

Increasing amounts of cholesterol in lipid bilayers and monolayers finally abolish the phase transition

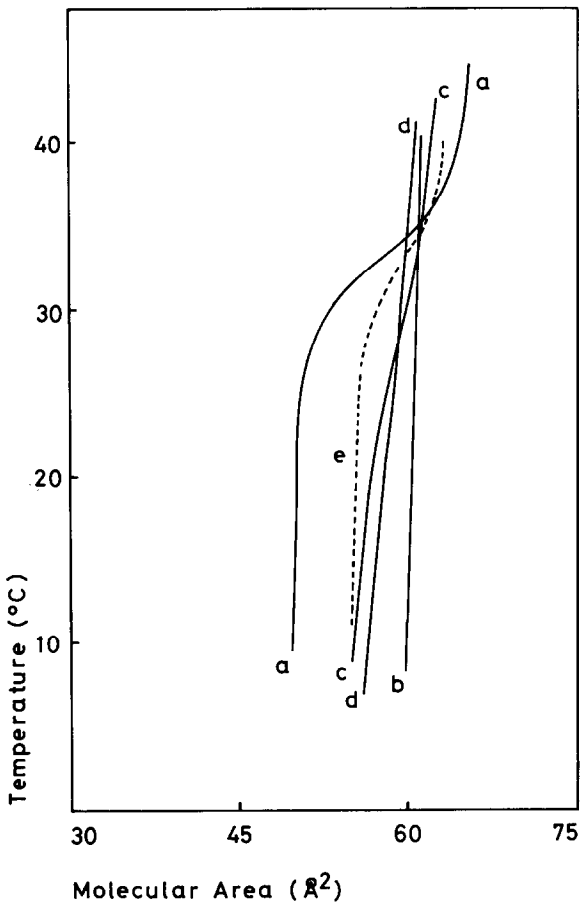


Fig.3. Isobars at a surface pressure of 25 dyne/cm of (a) DPPC, (b) glycolipid, (c) 2:1 molecular mixture, (d) 1:1 molecular mixture of these compounds, and (e) the calculated areas of the 1:1 mixture.

[5]. We also observed this effect with the glycolipid. When isobars were measured at 25 dynes/cm at different molecular ratios of DPPC and glycolipid, we observed a quenching of the phase transition (fig.3).

## 4. Discussion

We have shown that the hopane glycolipid of *B. acidocaldarius* has very similar effects on a monolayer of a synthetic lipid as has been reported for cholesterol [5]. Essentially the same effects could be shown for 1,2,3,4-tetrahydroxypentane-29-hopane, the aglycone of the glycolipid ( E. Kannenberg et al., to be published).

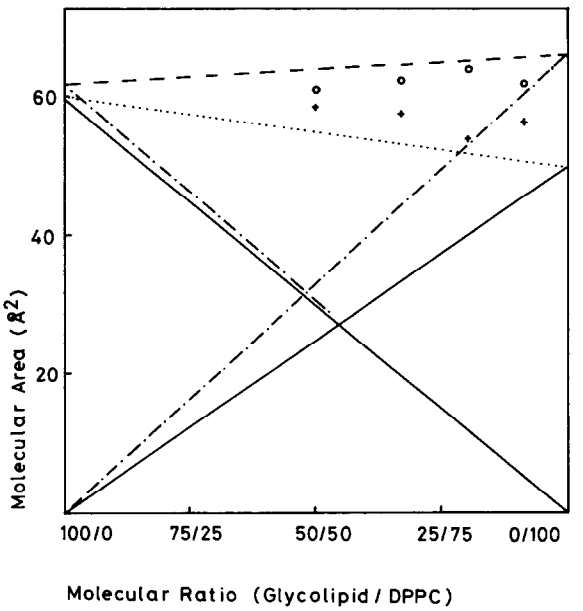


Fig.4. Effect of the glycolipid on the molecular area at a surface pressure of 25 dyne/cm at 23°C and 40°C in different mixtures with DPPC. ——, Molecular area at 23°C; –·–·–, molecular area at 40°C; . . . . ., calculated areas of the mixture at 23°C; – – –, calculated areas at 40°C; +, measured areas at 23°C; and ○, measured areas at 40°C.

Our experiments provide a basis for understanding the function of an important component of the membrane of the thermophilic, acidophilic *B. acidocaldarius*, and perhaps other bacteria which contain hopane glycolipids. Thus with the condensing action of the hopane glycolipid, the mobility of the acyl chains of the lipids in the cytoplasmic membrane will be decreased, and thereby provide a more stable membrane. The stability of membranes containing a high percentage of ω-cyclohexane fatty acids without hopanes, is not very high. This membrane possesses a phase transition temperature at 10°C as we have shown previously [11]. It is likely that a condensing substance would be of considerable advantage for survival at low pH, since passive diffusion of protons and solutes through the membrane would be diminished. Thus it would be easier for the organism to establish an approximately neutral cytoplasmic pH. In future experiments we shall attempt to demonstrate this supposed action, by investigating permeability of liposomes from synthetic lipids and lipids from *B. acidocaldarius*.

## Acknowledgements

We thank Klaus-Peter Zeller from the Institute of organic Chemistry at the University of Tübingen for the field desorption mass spectra, Hans Zähner (Microbiology I, SFB 76) for providing the facilities to produce large amounts of bacteria, and Karl Nill for engaged technical assistance. This work was supported by Deutsche Forschungsgemeinschaft.

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