Properties of ω-cyclohexane fatty acids in membranes

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The properties of di-ω-cyclohexyldodecanoylphosphatidylcholine (DCDPC) in various model membranes were studied by differential scanning calorimetry (DSC) and the monolayer technique. DCDPC shows a broad phase transition at 13°C with a total enthalpy of 4.1 kcal/mol as measured by DSC. In a DCDPC monolayer no phase transition was observed. At 25 dyn/cm the area increases linearly with temperature. The molecular area at 40°C and 25 dyn/cm equals that of dipalmitoylphosphatidylcholine. DCDPC liposome experiments show that the permeability of glycerol is strongly reduced above the phase transition in comparison with linear saturated fatty acid-containing phosphatidylcholines of comparable chain lengths. These results suggest that lipids with ω-cyclohexane fatty acids may possess a high acyl chain density at the free fatty acid end in the core of the membrane, which stabilizes the membrane and influences the permeability. This seems to be an important mechanism for membrane adaptation under various conditions for thermoacidophilic bacteria.

ω-Cyclohexane fatty acid *Membrane adaptation* Bacillus acidocaldarius

1. INTRODUCTION

Fatty acids containing an ω-cyclohexane ring are found in lipids of thermo acidophilic bacilli [1–3], in the bacteriophage φNS 11 [1], and in the mesophilic *Curtobacterium pusillum* [4]. ω-Cyclohexane fatty acids constitute up to 65% of the membrane fatty acids of *Bacillus acidocaldarius* [5] and 81.7% in *Curtobacterium* cultivated at the maximum growth temperature.

In biological membranes the role of lipids containing ω-cyclohexane fatty acids is not clear since a significant relation between temperature, pH and ω-cyclohexane fatty acids could not be established up to now [6,7]. As *Bacillus subtilis* lipids enriched with ω-cyclohexane fatty acids show a phase transition at 10°C, the role of lipids with an ω-cyclohexane ring in their fatty acid structure becomes even less clear [8].

For this reason we investigated the role of ω-cyclohexane fatty acids by studying model membranes of di-ω-cyclohexyldodecanoylphosphatidylcholine (DCDPC) with differential scanning calorimetry (DSC) and the monolayer technique. Additionally, the barrier properties of DCDPC liposomes with respect to small non-electrolyte molecules were studied. Some results of our studies are presented below.

2. MATERIALS AND METHODS

DCDPC was synthesized and characterized as in [9]. Di-ω-cyclohexyldodecanoylphosphatidic acid (DCDPA) was derived from DCDPC by treatment with phospholipase D as in [10].

1,2-Dipalmitoyl-*sn*-glycero-3-phosphatidylcholine (DPPC), 1,2-dipalmitoyl-*sn*-glycero-3-phosphate (DPPA), 1,2-dimyristoyl-*sn*-glycero-3-phosphate (DMPA) were chromatographically pure products from Sigma. All other chemicals were of analytical grade.

The phospholipid dispersions for DSC measurements were prepared as in [11]. Calorimetric scans were carried out with a Privalov calorimeter [12].

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The scan rate was 1 K·min^{-1}. The phosphate concentration of the suspension was determined by phosphate analysis as in [13].

Isobars and isotherms of monolayers were measured as in [14], using a commercial Langmuir film balance (Messgerätewerke Dr Wobser, Lauda, FRG).

The phospholipid dispersion for permeability measurements was prepared by a slightly modified procedure of [15]. The lipids (15 μmol) were pipetted into 10-ml round-bottomed flasks. Solvent was removed under nitrogen followed by high vacuum for 2 h. PC solutions contained 4 mol% of the corresponding PA. To the dry lipid film a glass bead and 1 ml of 50 mM KCl were added. The lipids were dispersed by two 30-s runs on a vortex mixer above the transition temperature of the lipids. If necessary for the complete removal of the lipid film from the glass wall, the samples were sonicated in a Bronson bath ultrasonicator for 30 s in between the vortex runs. The swelling of the liposomes in isotonic solutions of glycol, glycerol and erythritol was measured in an Eppendorf (type 1101 M) photometer at 436 nm connected to a Servogor (type RE 551) recorder. The cylindrical glass cuvette was fitted into a metal block, whose temperature was controlled. Isotonic solution (2.5 ml), was mixed in the cuvette with 30-μl samples of lipid dispersions in 1 s or less by a fast running stirrer.

3. RESULTS

Fig.1 shows the thermotropic behaviour of DCDPC dispersions. The total thermotropic phase transition enthalpy was 4.1 kcal/mol with low cooperativity ($CU = 22$). The curve shows two maxima at 11.8 and 14.5°C. In fig.1, regions I and II are arbitrarily fitted, assuming that the transition can be decomposed into two steps. For transitions I and II enthalpies of 1.9 and 2.2 kcal/mol, respectively, can be estimated.

In fig.2a monolayer isobars from DCDPC at a surface pressure of 25 and 35 dyn/cm are shown. Surprisingly, no significant phase transition can be detected. Comparing the areas occupied by DCDPC and DPPC at a lateral surface pressure of 25 dyn/cm, both lipids have the same area requirement of 60 Å^2 at 40°C, while at 10°C, DCDPC (57 Å^2) shows an area which is 6–7 Å^2 larger than

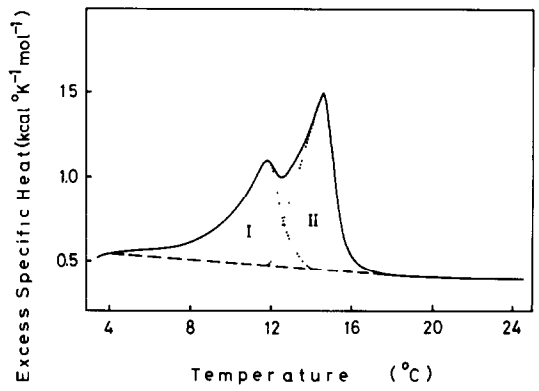

Fig.1. High-sensitivity DSC trace illustrating the thermotropic phase behaviour of multilamellar dispersions of DCDPC.

DPPC (51 Å^2). The isotherms in fig.2 show a more condensed film for DCDPC at 1°C. At higher temperatures the curves are characteristic for liquid-expanded films. The isotherms measured show no phase transition.

According to [15] liposomes behave as ideal osmometers. This implies that liposomes swell when solutes penetrate. A change of volume can be measured as a change in light scattering or turbidity [16]. Hence the initial change in absorption, i.e., the initial swelling rate of the liposomes, is a measure for the permeability of a solute [15].

Fig.3a shows the initial swelling rates of DCDPC liposomes for glycol, glycerol and erythritol as a function of temperature. The permeability depends

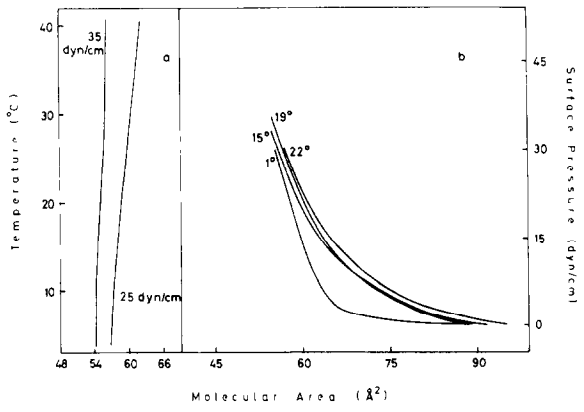

Fig.2. Isobars (a) and isotherms (b) from DCDPC monolayer films. Lateral pressure and temperature are indicated.

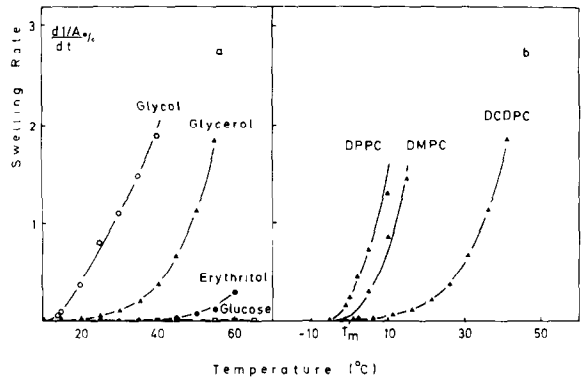

Fig.3. Initial swelling rates of DCDPC liposomes in various isotonic solutes: (a) in solutes of glycol, glycerol and erythritol; (b) in glycerol solutes with the swelling rate as a function of the reduced temperature $\Delta T = T - T_m$.

on the molecular size of the solute and increases with temperature. In fig.2b the initial swelling rates are compared with those from DPPC and DMPC. The swelling rates are compared with those from DPPC and DMPC. The swelling rates are plotted against the difference of the transition temperature, T_m, and the measured temperature. The transition temperatures of the lipids are 23.9°C for DMPC, 41°C for DPPC and 13°C for DCDPC. It is obvious that DCDPC liposomes show reduced permeabilities even 30°C above the phase transition temperature compared to DPPC and DMPC, and also a lower temperature dependence. In the region of the phase transition no significant increase of swelling rates were measured.

4. DISCUSSION

The influence of the bulky ω-cyclohexyl groups on the transition temperature and enthalpy can be compared with the influence of methyl groups in branched (iso, anteiso) PCs of comparable length. While the enthalpy is markedly below, the transition temperature is between those of diisoheptadecanoylphosphatidylcholine (26.8°C, 9.4 kcal/mol) and dianteisoheptadecanoylphosphatidylcholine (6.6°C, 6.3 kcal/mol) [17]. The thermotropic phase behaviour of DCDPC is complex (see fig.1). Its physical basis is not yet understood. So far the DSC curves suggest that the transition is composed of two low-energy transitions of approximately the same enthalpy. Furthermore, the transition at lower temperatures seems to be less cooperative than the transition at higher temperatures.

Keeping in mind that the bilayer transition temperature is 13°C, it is surprising that no phase transition was observed in the monolayer isobars of DCDPC at lateral pressures of 25 and 35 dyn/cm. Both curves show a linear increase of the area with increasing temperature. As the isotherm at 1°C shows a more condensed DCDPC film with only little lateral compressibility, the film seems to be tightly packed and may have a relative high degree of order. Hence, the area at the low temperature, which is 5–6 Å^2 larger than that of DPPC, should be due to the bulky cyclohexane rings. This is supported by the fact that the corresponding ω-cyclohexyl fatty acid has an area of 26.5 Å^2/molecule at 20°C and 20 dyn/cm. Further investigations should clarify whether, for this system, monolayer and bilayer properties are related as observed for straight-chain lipids or whether the observation of a transition in the bilayer is a result of particular structural arrangements of the ω-cyclohexyl groups.

It is an interesting result that DCDPC shows a reduced permeability with a low temperature dependence over a wide range above the transition temperature in experiments with liposomes. There is a well established relation between membrane fluidity and the permeability of small non-electrolyte molecules [18]. Hence the reduced permeability above the transition temperature suggests that DCDPC has a lower membrane fluidity and a higher degree of order in the liquid-crystalline phase compared with DMPC and DPPC. This should be due to the bulky cyclohexane ring which leads to a high acyl chain density and a denser packing in the membrane core and thus could reduce *trans-gauche* isomerization.

These interpretations are confirmed by our monolayer findings which suggest that order decreases slowly with increasing temperature. Additionally, a densely packed membrane of DCDPC with a relatively high microviscosity above the phase transition was found in DCDPC black lipid membranes [9]. Furthermore, in di-ω-cyclohexylundecanoylphosphatidylcholine (DCUPC) and di-ω-cyclohexyltridecanoylphosphatidylcholine (DCTPC) liposomes, 1,6-diphenylhexatriene (DPH) shows a linear decrease of fluorescence depolarisation with increasing temperature and with a higher degree of order in the liquid-crystalline range compared with

PCs with straight acyl chains [19,20]. In [19,20] it could also be shown that glucose does not penetrate through bilayers of these lipids above the transition temperature. At variance with the finding that DCUPC and DCTPC are freely permeable for glucose in the region of the phase transition [19], DCDPC in our experiments does not show a significantly higher permeability at this temperature range for the fast penetrating small non-electrolytes.

Phenomenologically, the properties of the ω-cyclohexyl group of DCDPC leading to reduction of the temperature dependence of the permeability and to a less pronounced phase transition can be compared to the effect of cholesterol and hopanoids on membranes [17]. Lipids containing fatty acids with a cyclohexane ring may stabilize the membrane structure and maintain the barrier function of procaryotic membranes at high temperatures. Thus, ω-cyclohexane lipids may be an aspect of thermoacidophilic adaptation of bacterial membranes. This suggestion is confirmed by the finding that increasing temperatures, and a decreasing pH, strongly increase the amount of cyclohexane lipids (unpublished). This observation is supported by the fact that the concentration of cyclohexane fatty acids is shifted to higher amounts in response to elevated temperatures in *Curtobacterium* [4].

It is worth mentioning that the proposed membrane stabilization has been further verified by the discovery of another type of ω-alicyclic fatty acid, the ω-cycloheptane fatty acid, in a second group of thermoacidophilic bacilli [21]. Additionally, the proposed mechanism is valid also in lipids of thermoacidophilic archaebacteria. These lipids contain cyclopentane groups [22].

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