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Physicochemical characterization of tetraether lipids from *Thermoplasma acidophilum*. Calorimetric studies on miscibility with diether model lipids carrying branched or unbranched alkyl chains

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The thermal properties of the main tetraether lipid of *Thermoplasma acidophilum* membranes, as monitored by differential thermoanalysis of hydrated samples, are compared with those of two synthetic diether glucolipids one of which is comprised of two phytanyl chains as apolar moieties, the other of two palmityl chains. The tetraether lipid undergoes a broad phase transition between −30°C and −5°C; the enthalpy change of the transition is low, amounting to −14 kJ · mol^{-1}. Among the two model lipids, diphytanylglucosylglycerol does not show any phase transition between −60°C and 80°C. In contrast, dipalmitylglucosylglycerol passes a sharp transition at 62°C, which is correlated with a large enthalpy change of $\Delta H = -98$ kJ · mol^{-1}. Thus, it is concluded that the presence of repetitive methyl branches along the hydrocarbon chains hinders the formation of condensed structures. At very low temperatures the two branched-chain lipids undergo a thermal transition which is not observed with the straight-chain lipid. This transition is connected with a considerable change in heat capacity. The upper and lower temperature limits of the transitions are −50°C and −90°C for the tetraether lipid and −61°C and −84°C for diphytanylglucosylglycerol. The changes in heat capacity amount to 1.26 kJ · mol^{-1} · K^{-1} (tetraether lipid) and 74 kJ · mol^{-1} · K^{-1} (diether lipid), respectively. Phenomenologically, these transitions appear to be glass transitions. Studies on lipid mixtures reveal that the bipolar tetraether lipid is able to form mixed phases with the monopolar diphytanylglucosylglycerol. In contrast, the miscibility of tetraether lipid with dipalmitylglucosylglycerol is limited depending on the experimental conditions. The miscibility of fluid tetraether lipid with dipalmitylglucosylglycerol in the gel state is very low. It is significantly increased at temperatures above 62°C which is the transition temperature of the diether lipid. However, if mixtures prepared above 62°C are cooled to lower temperatures, a metastable state is formed which slowly transforms into a stable state.

Introduction

It is well established that eucaryotic and some procaryotic cell species regulate membrane fluidity by incorporation of varying amounts of unsaturated fatty acids into their polar membrane lipids. In many procaryotes, however, particularly in spore-forming bacilli and actinomycetes, the

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fraction of unsaturated fatty acids is rather low or even zero; instead, methyl-branched hydrocarbon chains are present which are derived from anteiso- and iso-fatty acids. Introduction of methyl branches leads to increased disorder in the apolar region, hindering close packing of the hydrocarbon chains and therefore decreasing the temperature of order/disorder transition. The effect on the transition temperature depends on the position of the methyl group. As shown by Silvius and McElhaney [1], introduction of iso-branched fatty acids (position of the methyl group at C-atom $n-1$) reduces the melting temperature by about 12 K and introduction of anteiso-branched fatty acids (methyl-group at C-atom $n-2$) by about 35 K, as compared with phosphatidylcholines containing unbranched fatty acids of same chain length.

The concept of increasing chain fluidty by incorporation of methyl branches is perfected in archaebacteria which have been shown to incorporate only phytanyl residues into their polar lipids, i.e. saturated hydrocarbon chains carrying four repetitive methyl branches. Unbranched polar lipids are not present in these cells. The thermoacidophilic archaebacterium *Thermoplasma acidophilum*, isolated by Darland et al. [2] from a steaming self-heated coal refuse pile, was the first organism found to contain bipolar macrocyclic tetraether lipids, which structurally correspond to two diphytanyl ether lipids covalently linked via their apolar ends. Therefore the lipids contain two parallel C_{32} hydrocarbon chains each comprising eight methyl branches. Due to their dimension and molecular shape, tetraether lipids are assumed to span the whole membrane, thus forming monomolecular layers instead of bilayer structures. This assumption is in fact strongly supported by recent findings obtained by means of differential experimental approaches, e.g. studies on black lipid membranes [3] and film balance experiments [4].

The present communication is part of a series in which we describe the physicochemical properties of the main tetraether lipid of *Thermoplasma acidophilum*. As depicted in Fig. 1, this lipid is a glucosylglycerylphosphoryl derivative of the basic tetraether. According to Langworthy [5], it makes up approx. 80% of the glycophospholipid fraction and 50% of the total lipid of the *Thermoplasma* membrane. As previously described, the lipid is very fluid [6,7]. At the air/water interface it forms stable monomolecular films of very low viscosity [4]. In dilute buffer the lipid easily forms large, stable liposomes the permeability of which is significantly lower than that of bilayer-forming ester lipids [6,8]. Recent calorimetric studies on several lipid fractions of *Thermoplasma acidophilum* suggest that the thermal properties of these lipids are mainly determined by the properties of the hydrocarbon chains [7]. In the present paper we compare the thermal behaviour of the main tetraether lipid of *Thermoplasma*, as monitored by differential thermoanalysis, with those of two synthetic diether glucolipids, diphytanylglucosylglycerol and dipalmitylglucosylglycerol (see Fig. 1). With regard to findings that some archaebacteria contain tetraether lipids together with diether lipids comprising phytanyl chains, but never with polar lipids equipped with unbranched hydrocarbon chains [9,10], we also studied whether the main tetraetherlipid of *Thermoplasma* is able to form mixed phases with the two model lipids.

Methods and Materials

Growth of organism and isolation of lipid

Thermoplasma acidophilum was obtained from Dr. Zillig, Max-Planck-Institut für Biochemie, Munich, F.R.G. The detailed methods of cultivation and lipid isolation have been described [10]. Briefly, cultures were semicontinuously grown in modified Freundt-medium at $T = 59°C$ and pH = 2 under controlled conditions using a 'Biostat S' fermenter (Braun AG, Melsungen, F.R.G.). Cells were harvested in the late exponential growth phase.

Lipids were extracted from freeze-dried cells following the procedure of Langworthy and coworkers [10]. The main component was isolated from the glycophospholipid fraction by means of chromatography over silicic acid columns using chloroform-methanol gradients [7].

Differential thermoanalysis

DTA was performed by means of a Mettler TA 3000/DSC 30 instrument equipped with a nitrogen cooling device (Mettler Instruments, Greiffensee, Switzerland). Samples were prepared by

adding lipids dissolved in chloroform/methanol in small portions into standard aluminium pans ($V = 40$ µl). After evaporation of the solvents under a stream of nitrogen and, subsequently, under vacuum for at least 36 h, lipid mass was gravimetrically determined using an electronic microbalance (Mettler M3). Finally, 20 µl buffer (400 mM sodium cacodylate-HCl (pH 7.0)/12.5 M ethylene glycol) were added and the pans sealed. Reference pans contained 20 µl of the buffer. Samples were kept for a total of 24 h at 70°C to ensure complete hydration of the lipids. The molar ratio between water and lipid in our preparations was approx. 100 : 1. The amount of 12.5 M ethylene glycol was sufficient to prevent freezing of the buffer down to temperatures of about −110°C [11].

Scan rates amounted to $dT/dt = \dot{T} = 0.02$ to 0.08 $K \cdot s^{-1}$; enthalpy changes were determined by means of a microcomputer which is integrated into the TA 3000/DSC 30 system. T_m values were obtained by extrapolating the observed temperatures of maximum heat flow versus $\dot{T} \rightarrow 0$ $K \cdot s^{-1}$. Due to minor fading of heat flows at low scan rates, which, however, did not exceed a few percent, data obtained under these conditions were omitted from the calculation of ΔH values when the peaks were broad. When heating and cooling scans were run repetitively, they showed no alteration of the transition parameters.

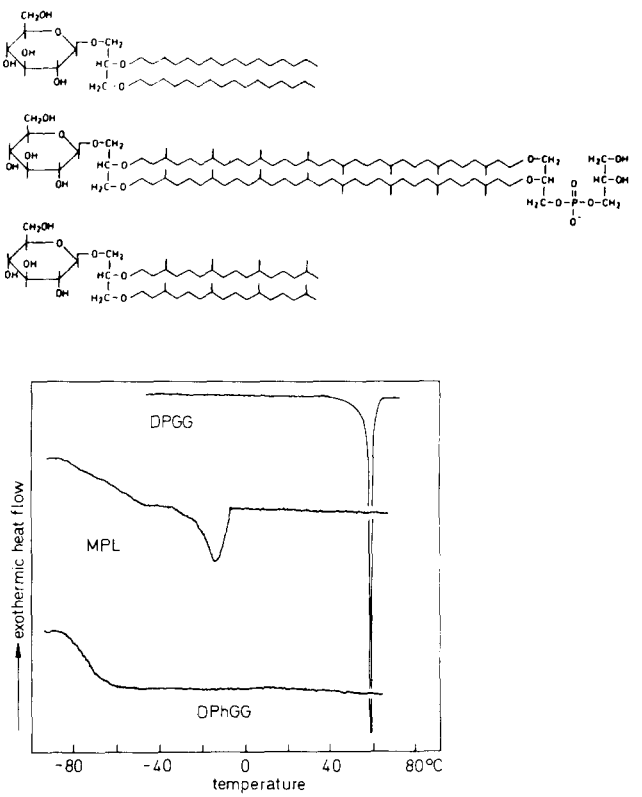

Fig. 1. DTA heating scans of hydrated dipalmitylglucosylglycerol (DPGG), diphytanylglucosylglycerol (DPhGG) and main phospholipid of *Thermoplasma acidophilum* (MPL). Scan rate $\dot{T} = 0.06$ $K \cdot s^{-1}$.

Chemicals

The model diether lipids, 1,2-diphytanyl-3-*O*-β-D-glucosyl-*sn*-glycerol (diphytanylglucosylglycerol) and 1,2-dipalmityl-3-*O*-β-D-glucosyl-*sn*-glycerol (dipalmitylglucosylglycerol) were synthesized according to Six et al. [12]. Organic solvents ('Resi' quality) were purchased from Baker Inc.; all other chemicals were of analytical grade.

Results

Role of methyl branches on thermal properties of ether lipids

Fig. 1. shows DTA scans (heating curves) of hydrated samples of dipalmitylglucosylglycerol, diphytanylglucosylglycerol and the main glycophospholipid of *Thermoplasma acidophilum*. The structures are depicted in Fig. 1. All three substances are ether lipids containing a glucosidic polar head group. The tetraether lipid is a bipolar macrocycle; the two model lipids are monopolar diethers. Diphytanylglucosylglycerol is related to the *Thermoplasma* lipid insofar as it is equivalent to the glucosidic half of the tetraether lipid. Dipalmitylglucosylglycerol is an analogue of diphytanylglucosylglycerol, the phytanyl residues of which are replaced by unbranched hydrocarbon chains of the same length.

As the thermograms show, the tetraetherlipid undergoes a broad phase transition between −30°C and −5°C. The enthalpy change of this transition is low, amounting to about $\Delta H = -14$ $kJ \cdot mol^{-1}$. Among the two model lipids, diphytanylglucosylglycerol does not show a phase transition between −60°C and 80°C. In contrast, dipalmitylglucosylglycerol passes a sharp transition at 62°C, which is correlated with a large

enthalpy change of $\Delta H = -98$ kJ · mol^{-1}, as first described by Hinz et al. [13]. All thermal transitions depicted in Fig. 1 are fully reversible (cooling curves not shown), apart from minor supercooling effects.

The occurrence of a sharp thermal transition of dipalmitylglucosylglycerol and the lack of a transition of diphytanylglucosylglycerol strongly suggest that the presence of repetitive methyl branches along the hydrocarbon chains leads to fluidization of the lipid below 62°C. This suggestion is in agremeent with data presented by Lindsey et al. [14], who could not detect a thermal phase transition of diphytanoyl phosphatidylcholine between −120°C and 120°C. Our data further suggest that the low-temperature range and the small heat content of the phase transition of the tetraether lipid can be assigned to the presence of multiple methyl branches in the apolar moiety of the molecule. Although the presence of a thermal phase transition is unquestionable, we have, however, no information about the molecular order of the tetraether lipid at temperatures below and above this transition.

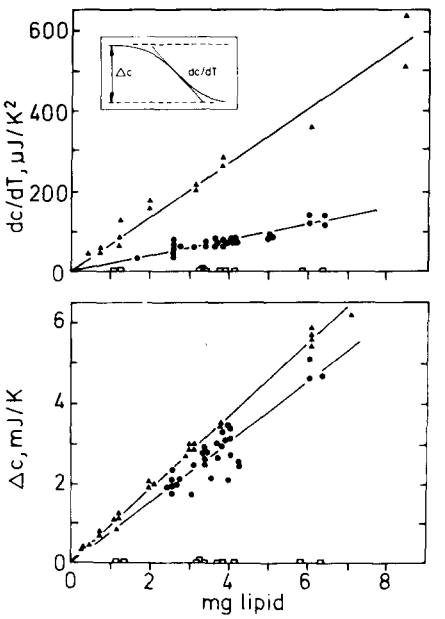

Fig. 2. 'Glass transition' of diphytanylglucosylglycerol and main tetraether lipid: Change in heat capacity, Δc (lower panel) and maximum slope at a transition of 50%, dc/dT (upper panel) plotted against lipid mass in the sample. For definitions see inset. ●, Main tetraether lipid; ▲, diphytanylglucosylglycerol; □, dipalmitylglucosylglycerol.

Occurrence of a second transition at deep temperatures

The two branched-chain lipids undergo a further transition at temperatures between approximately −90°C and −60°C, which could not be observed with the straight-chain lipid. This transition is characterized by a considerable change in heat capacity. For technical reasons the lower temperature limits of these transitions can only be approximated from the scans. Measurements were limited to temperatures above −100°C in order to avoid freezing of the buffer. Since the transitions begin a few degrees above this temperature, only a small temperature range is available for estimation of the baseline of the scan. Thus, the determination of the onset temperature of these transitions involves an error in the range of ±5 K.

As depicted in Fig. 1, the deep temperature transition of the tetraether lipid begins at about −90°C and is terminated at about −50°C. The corresponding values for diphytanylglucosylglycerol are −84°C and −61°C. For further characterization of these transitions, the change in heat capacity, Δc, and the maximum slopes of the scans at a transition of 50%, dc/dT, were plotted against the masses of the lipid samples (Fig. 2). The results show significant differences between the two branched-chain lipids. The change in heat capacity of the tetraether lipid amounts to 1.26 kJ · mol^{-1} · K^{-1}, and 0.74 kJ · mol^{-1} · $^{-1}$ for the diether lipid. The maximum slope of the transition of the tetraether lipid is 31.5 ± 6.6 J · mol^{-1} · K^2, which is about 57% of that of the diether lipid (54.6 ± 8.1 J · mol^{-1} · K^2).

The differential behaviour of the three lipids, particularly of the two diether lipids, as well as the proportionality of Δc and dc/dT with lipid mass, suggest that the process is lipid-specific and excludes the effects as artifacts due to temperature-induced reorganization of buffer components. On the basis of the differential behaviour of the three lipids we propose that these transitions are glass transitions. To emphasize that this proposal is yet a working hypothesis, we will use the term glass transition in quotation marks throughout this paper.

Miscibility of tetraether lipid with diphytanylglycosylglycerol

Fig. 3 shows a series of heating curves of mixtures of tetraether lipid and diphytanylglucosylglycerol. The data suggest complete miscibility of the two lipids, as elucidated by the analysis summarized in Fig. 4. In Fig. 4a, the upper and lower temperature limits of the phase transition of the tetraether lipid, T_u and T_l, respectively, as well as the temperature of the maximum heat flow, T_m, are plotted against the fractions of diphytanylglucosylglycerol in the samples, according to the relation $r = c_{DL}/(c_{DL} + 2c_{TL})$, where c_{DL} and c_{TL} are the molar concentrations of diether lipid and tetraether lipid, respectively. In formulating this relation, it was taken into account that one tetraether lipid functionally corresponds to two diether-lipid molecules as one membrane spanning unit.

It is evident from Fig. 4a that T_l and T_u and, consequently, also the broadness of the phase transition of the tetraether lipid, are independent of the amount of the diether lipid present in the mixture. The transition maximum, T_m, however, is linearly shifted to lower temperatures as the relative proportion of diether lipid is increased. As can be derived from Fig. 3, diphytanylglucosylglyerol

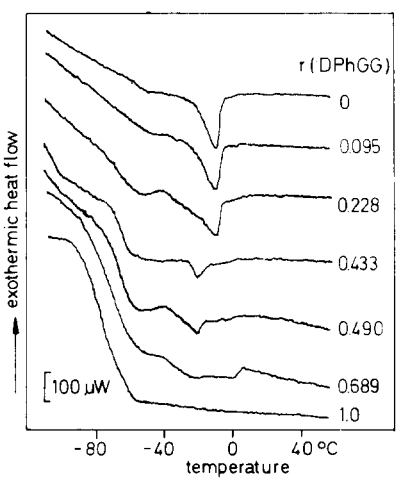

Fig. 3. DTA heating scans of mixtures of main tetraether lipid and diphytanylglucosylglycerol. Scan rate 0.06 $K \cdot s^{-1}$. Numbers indicate the fractions of the diether lipid (DPhGG) in the mixtures, according to $r = c_{DPhGG}/(c_{DPhGG} + 2c_{MPL})$.

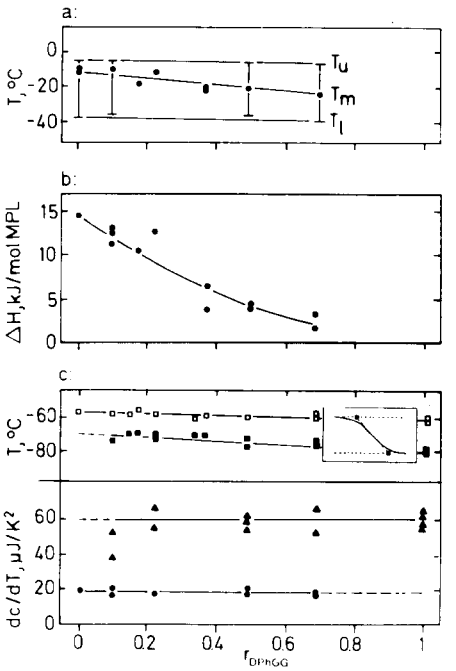

Fig. 4. Analysis of the thermograms of mixtures of main tetraether lipid and diphytanylglucosylglycerol from Fig. 3. Dependence of various parameters on the fraction of the diether lipid in the mixtures. (Panel a) Upper and lower temperature limits (T_u and T_l) and temperature of maximum heat flow (T_m) of the phase transition of the tetraether lipid. (Panel b) Enthalpy change, ΔH, of the transition, referred to the mass of tetraether lipid. (Panel c) Upper and lower temperature limits (for definitions see inset) of the 'glass transition' of diphytanylglucosylglycerol (upper part) and maximum slopes of the 'glass transitions', dc/dT, attributable to the fraction of diether lipid (▲) and tetraether lipid (●).

appears to quench the phase transition of the tetraetherlipid. The quantitative analyses presented in Fig. 4b show that the enthalpy changes, ΔH, referred to the fraction of tetraether lipid, decline to almost zero as the fraction of diether lipid is increased to one. This clearly shows that the tetraether lipid and the branched-chain diether lipid form mixed phases. There is no indication that the insertion of the diether lipid into the tetraether lipid phase is limited, or, in other words, that the two lipids are enriched in different domains.

As can be further derived from Fig. 3, even in mixed samples the 'glass transitions' of the tetraether lipid and the diether lipid can be separated

from each other on the basis of their different temperature ranges and dc/dT values. Fig. 4c shows that T_u and T_l of the 'glass transition' attributable to diphytanylglucosylglycerol are slightly shifted to higher temperatures as the amount of tetraether lipid in the mixtures is increased; the maximum slopes, dc/dT, and the changes in heat capacity, Δc, however, remain unchanged as the composition of the mixtures is altered.

Miscibility of tetraether lipid with dipalmitylglucosylglycerol

Mixtures of tetraether lipid and the straight-chain diether lipid exhibit a completely different thermal behaviour than mixtures of tetraether lipid and the branched-chain diether lipid.

The thermograms shown in Fig. 5 reveal two phase transitions which can be clearly separated from each other, one occurring in the same temperature range as that of pure tetraether lipid, the other one near the transition temperature of pure dipalmitylglucosylglycerol. This indicates that the two lipids are enriched in separate phases. However, the fact that in response to increased portions of the tetraether lipid in the mixture, the second transition range is broadened and shifted to lower temperatures suggest that a significant amount of tetraether lipid is integrated into the diether lipid domain. Yet, this amount is small because no substantial alteration of the endotherm attributable to the diether lipid could be detected. Apparently, incorporation of very little tetraether lipid is sufficient to disturb the molecular arrangement within the diether lipid phase. The scans also indicate that, conversely, a significant amount of dipalmitylglucosylglycerol is integrated into the tetraether lipid phase. This is substantiated by the quantitative data in Fig. 6b showing that ΔH of the transition of the tetraether lipid phase declines with increasing fractions of dipalmitylglucosylglycerol. Thus, dipalmitylglucosylglycerol appears to be able to form mixed phases with the tetraether lipid although to a very limited extent.

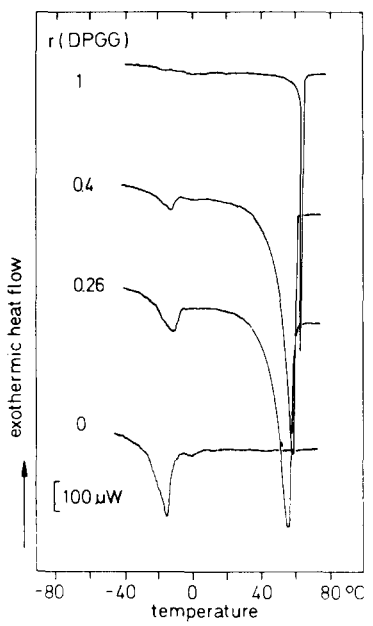

Fig. 5. DTA heating scans of mixtures of main tetraether lipid and dipalmitylglucosylglycerol. Scan rate 0.06 $K \cdot s^{-1}$. Numbers indicate the fraction of diether lipid (DPGG) in the mixtures, according to $r = c_{DPGG}/(c_{DPGG} + 2c_{MPL})$.

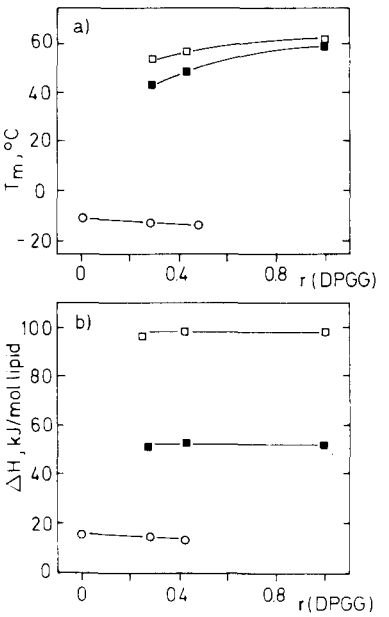

Fig. 6. Analysis of thermograms of mixtures of main tetraether lipid and dipalmitylglucosylglycerol from Fig. 5. Dependence of various parameters on the fraction of the diether lipid in the mixtures. (Upper panel) Temperatures of the maximum heat flow (T_m). (Lower panel) Enthalpy changes, ΔH, referred to the mass of diether lipid and tetraether lipid, respectively. ■, dipalmitylglucosylglycerol, pretreatment 24 h at 70°C; □, dipalmitylglucosylglycerol, pretreatment 24 h at 4°C; ○, main tetraether lipid. (Values are means of several determinations.)

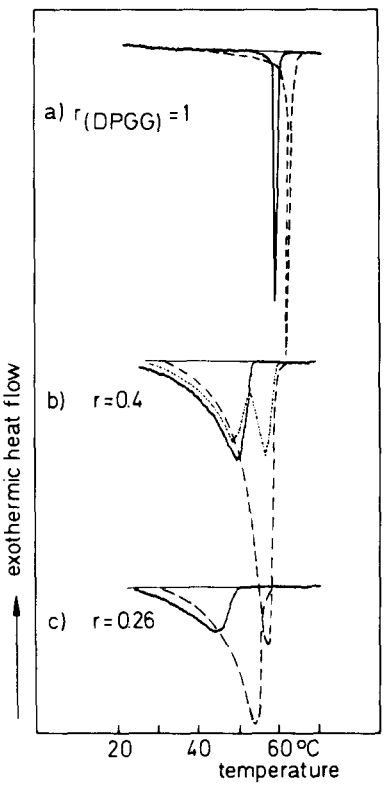

Fig. 7. DTA heating scans (scan rate 0.02 $K \cdot s^{-1}$) of pure dipalmitylglucosylglycerol (a), and of mixtures with main tetraether lipid (b: $r = 0.4$; c: $r = 0.26$) after different pretreatment: ———, 24 h at 70°C; – – – –, 24 h at 4°C; ·····, 24 h at 70°C, thereafter 3 h at 4°C.

The degree of miscibility of dipalmitylglucosylglycerol and tetraether lipid depends on the pretreatment of the sample, as can be delineated from the data presented in Fig. 7. These data are restricted to the high temperature range and therefore deal only with the endotherm of the diether-rich phase. Fig. 7a shows the heating curve of pure dipalmitylglucosylglycerol after short sotrage at 4°C. After extended storage at this temperature, i.e. at $T \ll T_m$, the transition temperature is increased from 59°C to 62°C (Fig. 6a), and ΔH is almost doubled from -52 to -98 $kJ \cdot mol^{-1}$ (Fig. 6b). This suggests that storage at $T < T_m$ gives rise to closer lipid packing. Similar effects are observed with mixtures of dipalmitylglucosylglycerol and tetraether lipids. Fig. 7b shows data obtained with a mixture of 40% diether lipid. When the sample was heated immediately after cooling from 70°C to 4°C, the scan reveals, at $T > 25$°C, a single transition with $T_m = 48.8$°C, representing melting of a mixed phase of diether lipid and tetraether lipid. The heat content amounts to -53 $kJ \cdot mol^{-1}$ diether lipid. After storage of the sample for 24 h at 4°C, the transition is shifted to higher temperatures by about 10 K. The peak width is about half that of the first one; ΔH amounts to -98 $kJ \cdot mol^{-1}$ diether lipid, which is about twice the initial value. This again suggests that during extended incubation at 4°C, a more densely packed phase is formed, presumably containing a lower portion of tetraether lipid. Similar behaviour was found with a mixture containing 26% tetraether lipid (Fig. 7c). Again, the range of the phase transition is shifted by approx. 10 K, as the samples are kept at 4°C for 24 h.

The formation of this second, more condensed phase is also demonstrated by the occurrence of a biphasic melting curve obtained after 3 h incubation at 4°C (Fig. 7b), representing an intermediary stage which is characterized by coexistence of the two different phases.

Discussion

In the present paper, we compare some thermal properties of the main tetraether lipid of *Thermoplasma acidophilum* membranes with those of two diether model glycolipids, one of which contains two phytanyl residues as apolar moiety, the other two palmityl chains. The two monopolar model lipids have a glucosidic polar head group. One of the two polar head groups of the bipolar tetraether is a glucosidic residue; the second head group is a glycerylphosphoryl residue, which essentially is anionic over a wide pH range and presumably also under physiological conditions.

Phase transitions of the tetraether lipid and dipalmitylglucosylglycerol

In spite of the fact that the two model lipids have identical head groups, the thermal properties, as monitored by DTA, markedly differ from each other, suggesting that these differences are due to particular properties of the alkyl chains. Within certain limits, this also holds true for the tetraether lipid, although additional influences of the anionic head group must be taken into account.

As first described by Hinz et al. [13], dipalmitylglucosylglycerol undergoes a very sharp gel-to-liquid crystalline phase transition at $T = 65°C$ *. In contrast, diphytanylglucosylglycerol remains fluid between $-90°C$ and 80°C, i.e. over the whole temperature range studied. Whereas the calorimetry data indicate a high degree of cooperativity for dipalmitylglucosylglycerol, the opposite is evident for diphytanylglucosylglycerol, implying that the presence of repetitive methyl branches along the hydrocarbon chains effectively prevents cooperative chain motion and close chain packing. This fact correlates well with observations that also diphytanoylphosphatidylcholine is unable to form gel-like structures down to a temperature of even $-120°C$ [14]. Our data are also in agreement with recent film balance studies on tetraether lipid, diphytanylglucosylglycerol and dipalmitylglucosylglycerol showing that at the air/water interface the latter forms highly condensed films, occupying a limited molecular area of 40 $Å^2$ per molecule [13], whereas diphytanylglucosylglycerol requires almost twice that value, namely about 73 $Å^2$ per molecule [4]. Still unpublished data reveal that the film viscosity of dipalmitylglucosylglycerol is very high, whereas that of diphytanylglucosylglycerol is low (Strobl, C., Regensburg, personal communication). Dipalmitylglucosylglycerol and diphytanylglucosylglycerol represent, therefore, two opposite, extreme counterparts of membrane forming lipids, which cover a wide scale of lipid 'fluidity', one forming highly condensed, rigid films, the other one giving rise to loosely packed layers which are extremely fluid.

Although the tetraether lipid comprises multiple methyl branches along the hydrocarbon chains as diphytanylglucosylglycerol, it is apparently able to undergo a thermal transition. However, the transition temperature is low; the transition range is broad, as reflected by a $T_{1/2}$ value of 10 K measured at low scan rate (for comparison of 0.2 K in the case of dipalmitylglucosylglycerol), and the enthalpy production of the transition is by a factor of 7 smaller than that of dipalmitylglucosylglycerol. Since DTA detects only phase changes, the detailed nature of the physical states of the tetraether lipid below and above the transition remains to be studied by appropriate methods, such as X-ray diffraction and NMR. Lindsey et al. [14] reported that diphytanoylphosphatidylcholine forms a partially ordered state over the entire physiological temperature range; even at 100°C it was unable to form a liquid-crystalline structure. Thus, at the moment we avoid describing the tetraether lipid transition as a gel-to-liquid-crystalline transition.

The results described in this paper clearly show that the two methyl-branched lipids are completely miscible with each other. This is indicated by the fact that the phase transition of the tetraether lipid is shifted to lower temperatures as the fraction of diphytanylglucosylglycerol in the samples is increased to one; simultaneously, the heat content, ΔH, of the phase transition of tetraether lipid declines and approaches to zero.

In contrast, the miscibility of tetraether lipid and the unbranched dipalmitylglucosylglycerol is limited and depends on the experimental procedure. As the calorimetric data show, tetraether lipid and dipalmitylglucosylglycerol in the gel state are miscible only to a small degree. Heating scans of mixtures, started at $T = -100°C$, i.e. far below the transition temperatures, reveal two phase transitions which can be attributed to domains of almost pure tetraether lipid and diether lipid, respectively. The fact that both transition temperatures slightly decrease as the fraction of the second component is increased, indicates that some incorporation of the diether lipid into the tetraether lipid phase, and vice versa, must have occurred. The relative proportion of the minor component, however, can be assumed to be very small, because we failed to detect a significant decrease in the ΔH values referred to the amounts of dipalmitylglucosylglycerol in the mixtures (Fig. 7b).

The miscibility of dipalmitylglucosylglycerol and tetraether lipid appears to be significantly increased when both lipids are in the fluid state.

* A comparison between the calorimetric data published by Hinz et al. [13] and by us shows that the T_m values measured by us are 3–6 K below the data obtained by Hinz et al., whereas the enthalpy changes are almost doubled. There is good experimental evidence (Blöcher, D., unpublished data; Six, L., unpublished data) that the different experimental conditions are responsible for these discrepancies: (a) the presence of a high concentration of antifreeze (ethylene glycol) in our samples, (b) the high salt concentration, and (c) the high lipid concentration used by us.

This is delineated from calorimetric data obtained with mixed samples, which were incubated for 24 h at 70°C and then rapidly cooled down to 4°C. If heating curves are run immediately after cooling, the scans reveal a broad transition in a temperature range which is significantly below that of mixtures formed at low temperatures. Obviously, the homogeneity of the mixture formed at $T > T_{m(diether\,lipid)}$ is maintained for a short period during storage at 4°C. Nevertheless, the incorporation of tetraether lipid into the diether lipid domain remains rather low, as no substantial differences in the $\Delta H_{(diether\,lipid)}$ values in mixed samples and pure preparations could be detected. Further modification of the treatment of the samples suggest that the mixed phase represents a metastable phase which is maintained only at high temperature. Upon prolonged storage at 4°C, it is transformed into a stable phase with a transition temperature of approx. 10 K above that of the former one. The width of the new transition is about half that of the first one; the ΔH value is doubled and is again in the same range as that of pure dipalmitylglucosylglycerol, treated in the same manner.

These data can be interpreted by assuming that segregation of a small portion of tetraether lipid from the diether lipid rich domain occurs, which gives rise to closer packing of diether lipid into a structure similar to the stable gel state of pure diether lipid.

Occurrence of a thermal transition at sub-physiological temperatures

Apart from the phase transition described as yet, the tetraether lipid as well as diphytanylglucosylglycerol pass a thermal transition between approx. −90°C and −60°C, i.e. far below the physiological temperature range. This transition might be a glass transition. Several observations support such a proposal: (1) The physical parameters of the transitions, i.e. the lower and upper temperature limits, the width of the transition, and the change in heat capacity are significantly different for the two lipids. (2) The changes in heat capacity and the slopes of the transitions are proportional to lipid mass. (3) Transitions were also observed when the composition of the medium was altered (replacement of 70% ethylene glycol by 50% dimethyl sulfoxide or 60% methanol plus 10% ethylene glycol as antifreeze;data not shown). (4) The lipid specificity of the process is further established by the fact that we were unable to detect a transition with straight-chain analogues of diphytanylglucosylglycerol, i.e. dipalmitylglucosylglycerol (Fig. 1) and distearylglucosylglycerol (data not shown). All three diether lipids have identical polar head groups. Until now, however, the characterization of these transitions must remain at the level of a phenomenological description, because nothing is known about the changes in the molecular organization of the membrane induced by cooling to low temperatures.

Our data may provide a physicochemical explanation for the occurrence of tetraether lipids with certain diether lipids in several archaebacteria; these lipids are comprised of phytanyl residues, but lack unbranched hydrocarbon chains. Due to the number and position of their methyl branches, diphytanyl ether lipids are well suited to form homogeneous mixed phases with membrane spanning tetraether lipids, thus yielding composite membranes made up of monolayer as well as bilayer structures. In contrast, ether lipids with unbranched hydrocarbon chains and tetraether lipids lack the ability to form mixed membranes which would remain stable over the whole physiological temperature range.

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