

# Physicochemical Characterization of Tetraetherlipids from *Thermoplasma acidophilum*

## IV. Calorimetric Studies on the Miscibility of Tetraetherlipids with Dipalmitoyl Phosphatidylcholine and Dipalmitoyl Phosphatidylglycerol

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By means of differential thermoanalysis, the miscibility of the main polar tetraether lipid of *Thermoplasma acidophilum* with two ester lipids, dipalmitoyl phosphatidylcholine and dipalmitoyl phosphatidylglycerol, resp., in the presence of excess water was studied. It is shown that with increasing fraction of tetraether lipid in the mixture, the transition range of dipalmitoyl phosphatidylcholine is broadened and the temperature of the maximum heat flow ( $T_m$ ) is shifted to lower temperatures; furthermore, the enthalpy change ( $\Delta H$ ) of the transition declines. Similar results were obtained with mixtures of tetraether lipid with dipalmitoyl phosphatidylglycerol. It is therefore concluded that the main polar tetraether lipid of *Thermoplasma acidophilum*, which essentially forms monomolecular layers, is able to form stable common phases with bilayer-forming ester lipids. Miscibility of the tetraether lipid with dipalmitoyl phosphatidylglycerol, which are both monovalent anions at neutral pH, is also observed in the presence of high proton or calcium ion concentrations.

### Introduction

The basic structure of the polar lipids of *Thermoplasma acidophilum* membranes is a tetraether lipid which structurally corresponds to two diphytanyl diether lipids covalently linked to each other via their apolar ends (for reviews see [1, 2]). Due to their molecular dimension, shape and bipolar character, tetraether lipids span the cell membranes thus forming monolayers. In some archaeobacteria additionally to tetraether lipids also diphytanyldietherlipids are present in substantial amounts; consequently the membranes of such cells are made up of monomolecular and bimolecular structures. Bipolar tetraether lipids and monopolar diether lipids therefore must be able to form stable common phases.

In a previous paper [3], we reported on DTA experiments carried out with the main membrane lipid of *Thermoplasma acidophilum*, which is a glycosyl phosphorylglycerol derivative of the tetraether [1], and two model diether glucolipids, one containing

phytanyl chains as apolar moieties, the other one hexadecyl chains. The branched lipid represents a group of polar lipids present in almost all archaeobacteria [1, 2]; the second component is an analogue not found in archaeobacterial membranes. The calorimetric data showed that, in the presence of excess water, the tetraether lipid is able to form mixed phases with the diphytanyl diether lipid. These mixtures are stable over a wide temperature range. In contrast, the ability of the tetraether lipid to mix with the unbranched ether lipid is very limited depending on the temperature. Mixed phases are only stable at temperatures above the transition temperature of the diether lipid. During storage at lower temperature phase segregation occurs.

From these observations the question arose whether the failure of the tetraether lipid to form stable common phases with the unbranched lipid is due to the presence of the unbranched hydrocarbon chains, which gives rise to the formation of highly condensed phases, or whether other peculiarities of the compound, *i.e.* ether linkages and nature of the polar head group, contribute to this phenomenon. We therefore investigated the thermal properties of mixtures of the main tetraether lipid of *Thermoplasma acidophilum* and two ester lipids containing di-

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hexadecyl chains as apolar moieties: dipalmitoyl phosphatidylcholine and dipalmitoyl phosphatidylglycerol. In the case of the latter, also the influence of free anionic charges on the miscibility with tetraether lipid was studied.

## Methods and Materials

### Organism and culture conditions

*Thermoplasma acidophilum* cultures were grown in Freundt-medium at 59 °C and at pH = 2 under moderate aeration. Cells were harvested in the late exponential phase by centrifugation and washed three times with distilled water. Details were described previously [4].

### Extraction and purification of lipids

The procedure for lipid isolation from freeze-dried cells and purification of the main glycerophospholipid followed the protocol recently described [4]. The glycerophospholipid fraction used for the experiments described in this paper was thin layer chromatographically pure as checked by using various solvent systems [5].

### Differential thermoanalysis

DTA was performed by means of a Mettler TA 3000/DSC 30 instrument equipped with a liquid nitrogen cooling device. Scans were run at rates of  $dT/dt = 0.02$  to  $0.08 \text{ K s}^{-1}$ . The buffer used for hydration of lipid samples contained 400 mM Na-cacodylate/HCl and 12.5 M ethyleneglycol. If not stated otherwise, pH was adjusted to 7.0. Further details are given in reference [3].

### Chemicals

All chemicals used were analytical grade reagents. Organic solvents purchased from Baker Inc. were of 'Resi'quality. Dipalmitoyl phosphatidylcholine and dipalmitoyl phosphatidylglycerol were obtained from Sigma Chemical Corporation, München, F.R.G.

## Results and Discussion

### Mixed phases of tetraether lipid and dipalmitoyl phosphatidylcholine

Fig. 1 shows a series of thermograms (heating curves) of the hydrated main tetraether phospholipid and dipalmitoyl phosphatidylcholine, and mixtures

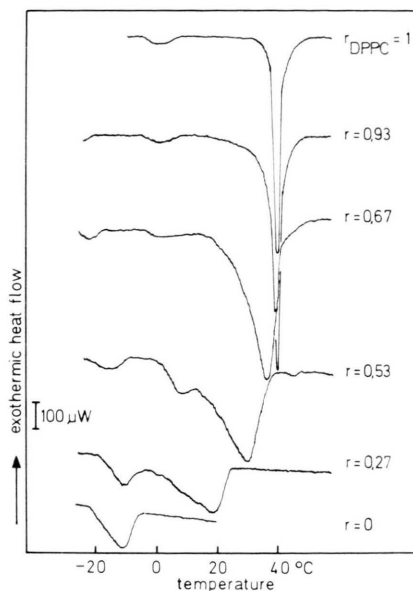


Fig. 1. DTA heating scans of tetraether lipid (MPL), dipalmitoyl phosphatidylcholine (DPPC) and mixtures of tetraether lipid and dipalmitoyl phosphatidylcholine. Scan rate  $\dot{T} = 0.08 \text{ K s}^{-1}$ ;  $r = c_{\text{DPPC}}/(c_{\text{DPPC}} + 2c_{\text{MPL}})$ .

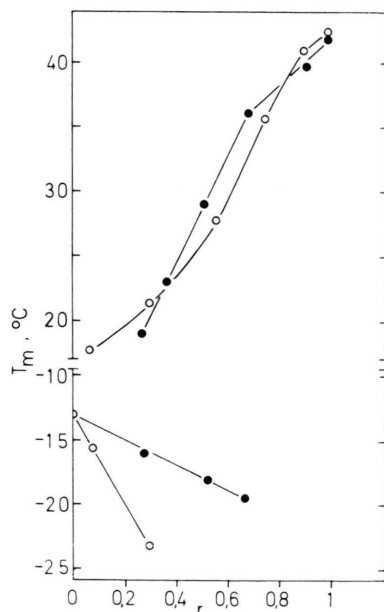


Fig. 2. Dependence of the transition temperature ( $T_m$ ) of lipid mixtures on sample composition,  $r$ . Values from figures 1 and 5.

● Tetraether lipid + dipalmitoyl phosphatidylcholine;  
○ tetraether lipid + dipalmitoyl phosphatidylglycerol.

containing both components in different molar ratios, according to the relation  $r = c_{\text{DPPC}} / (c_{\text{DPPC}} + 2c_{\text{MPL}})$ , where  $c_{\text{DPPC}}$  and  $c_{\text{MPL}}$  are the molar concentrations of dipalmitoyl phosphatidylcholine and tetraether lipid, resp. (for details see ref. [3]).

As previously described, the tetraether lipid exhibits a broad, weak phase transition at subzero temperatures [4]. Dipalmitoyl phosphatidylcholine undergoes a sharp phase transition at  $T = 42^\circ\text{C}$ ; a weak pretransition usually observed at  $35^\circ\text{C}$  is quenched under the conditions of these experiments due to the presence of ethylene glycol added to all samples as cryoprotectant.

It is evident that the thermal behaviour of the tetraether lipid is strongly influenced by the presence of dipalmitoyl phosphatidylcholine. In response to increased fractions of the latter, the transition range is gradually shifted to lower temperatures (Fig. 2) and

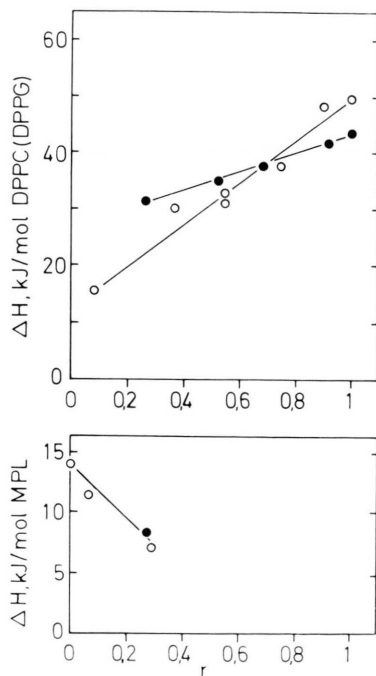


Fig. 3. Dependence of the enthalpy change ( $\Delta H$ ) of lipid mixtures on sample composition,  $r$ . Values from figures 1 and 5.

Panel a:  $\Delta H$  values of the transition above  $0^\circ\text{C}$ , referred to molar fractions of dipalmitoyl phosphatidylcholine and dipalmitoyl phosphatidylglycerol, resp.

Panel b:  $\Delta H$  values of low temperature transitions, referred to molar fractions of tetraether lipid.

● Tetraether lipid + dipalmitoyl phosphatidylcholine;  
○ tetraether lipid + dipalmitoyl phosphatidylglycerol.

the transition of the tetraether lipid is quenched (Fig. 3). This indicates that dipalmitoyl phosphatidylcholine is inserted into the tetraether lipid phase.

In turn, the transition range of dipalmitoyl phosphatidylcholine is broadened (Fig. 4) and shifted to lower temperatures, as the fraction of tetraether lipid is increased (Fig. 2); simultaneously, the melting enthalpy decreases (Fig. 3). Even administration of a very small amount of tetraether lipid ( $r = 0.93$ ) is sufficient to significantly diminish the degree of cooperativity of the transition of dipalmitoyl phosphatidylcholine and to decrease the transition temperature. These effects are strengthened as the fraction of tetraether lipid is increased. At  $r = 0.67$ , the thermogram reveals a broad endotherm with a maximum of  $T = 36^\circ\text{C}$ ; additionally a weak transition between  $-2^\circ\text{C}$  and approximately  $15^\circ\text{C}$  is observed. Whereas the lower part of this transition is an artifact attributable to melting of ice (see thermograms at  $r = 1$  and  $r = 0.93$ ), the tailing of the peak to higher temperatures indicates the occurrence of a mixed phase containing dipalmitoyl phosphatidylcholine and tetraether lipid in substantial amounts. The rela-

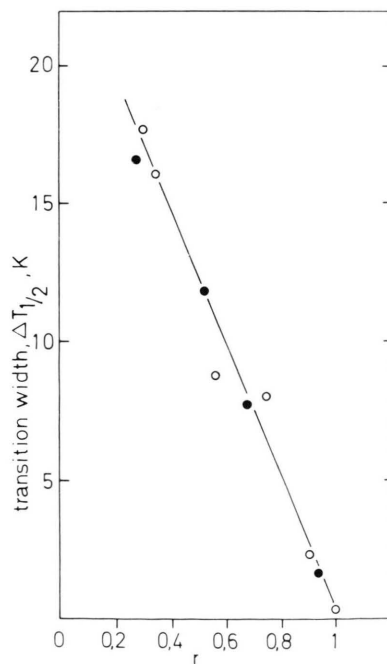


Fig. 4. Width of the transition at  $T > 0^\circ\text{C}$  ( $\Delta T_{1/2}$ ) of lipid mixtures as a function of sample composition. Scan rate  $\dot{T} = 0.02 \text{ K s}^{-1}$ .

● Tetraether lipid + dipalmitoyl phosphatidylcholine;  
○ tetraether lipid + dipalmitoyl phosphatidylglycerol.

tive proportion of this phase increases as the fraction of tetraether lipid is further enhanced. At  $r = 0.53$ , *i.e.* at approximately equal portions of monolayer and bilayer forming units, the thermogram shows two major peaks between 0 °C and 40 °C. Upon further increasing the fraction of tetraether lipid ( $r = 0.27$ ), above 0 °C only one broad endotherm with a peak maximum of  $T = 18$  °C can be detected. At 42 °C, the transition temperature of dipalmitoyl phosphatidylcholine, no indication for the occurrence of a phase transition is remained any longer. Thus, in this sample all dipalmitoyl phosphatidylcholine is integrated into mixed phases with tetraether lipid. The major part is present in the phase melting above 0 °C; a minor fraction is incorporated into the tetraether lipid phase, melting at  $T = -13$  °C.

The data presented in Fig. 1 demonstrate that the tetraether lipid and dipalmitoyl phosphatidylcholine are able to form mixed phases. The homogeneity of the mixed phases increases in response to increased fraction of dipalmitoyl phosphatidylcholine. Thus, dipalmitoyl phosphatidylcholine appears to be a somewhat better solvent for the tetraether lipid than tetraether lipid for dipalmitoyl phosphatidylcholine.

#### Mixed phases of tetraether lipid and dipalmitoyl phosphatidylglycerol

Fig. 5 presents a set of heating curves of tetraether lipid, dipalmitoyl phosphatidylglycerol and mixtures thereof. Detailed data on transition temperatures, widths of the transitions, and enthalpy changes are summarized in Figs. 2–4. The results are similar to those obtained with mixtures of tetraether lipid and dipalmitoyl phosphatidylcholine, though the net charges of dipalmitoyl phosphatidylglycerol and dipalmitoyl phosphatidylcholine under the conditions of these experiments are essentially different. As a comparison between the thermograms in Fig. 1 and Fig. 5 shows, the mixed phases of tetraether lipid and dipalmitoyl phosphatidylglycerol are somewhat more homogeneous than those of tetraether lipid and dipalmitoyl phosphatidylcholine. This is also reflected by the data summarized in Fig. 3 (upper panel), depicting that the melting enthalpy of dipalmitoyl phosphatidylcholine is significantly more influenced by increasing the fraction of tetraether lipid in the mixture than the melting enthalpy of dipalmitoyl phosphatidylcholine.

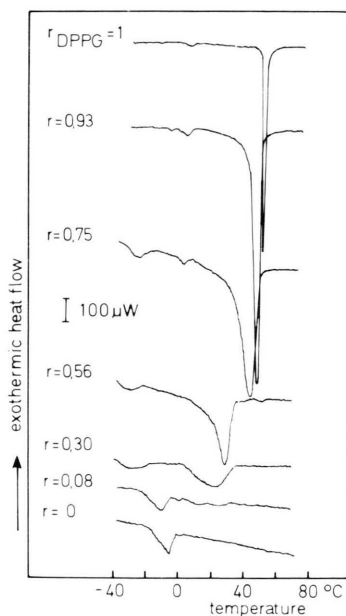


Fig. 5. DTA heating scans of tetraether lipid, dipalmitoyl phosphatidylglycerol (DPPG) and mixtures of tetraether lipid and dipalmitoyl phosphatidylglycerol (DPPG). Scan rate  $\dot{T} = 0.03 \text{ K s}^{-1}$ ,  $r = c_{\text{DPPG}}/(c_{\text{DPPG}} + 2c_{\text{MPL}})$ .

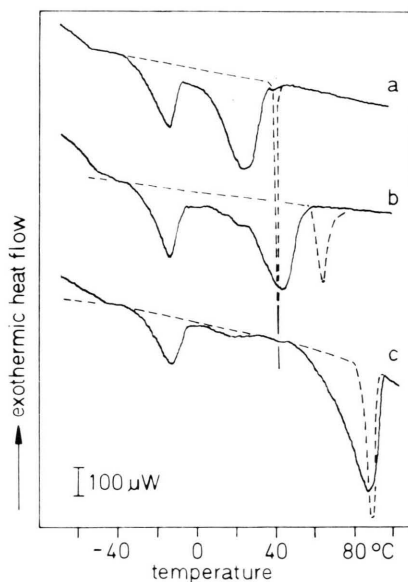


Fig. 6. DTA heating scans of pure dipalmitoyl phosphatidylglycerol (DPPG) and mixtures with tetraether lipid. — — — Dipalmitoyl phosphatidylglycerol; — — — dipalmitoyl phosphatidylglycerol + tetraether lipid;  $r = 0.36$ . (a) pH 7.0; (b) pH 2.0; (c) pH 7.0, presence of  $\text{Ca}^{2+}$  ( $c_{\text{Ca}^{2+}}/c_{\text{DPPG}} = 0.74$ ).

*Influence of protons and calcium ions on the miscibility of tetraether lipid and dipalmitoyl phosphatidylglycerol*

In order to study the influence of free anionic charges of the polar head groups on the miscibility of tetraether lipid and dipalmitoyl phosphatidylglycerol, a series of measurements was performed on samples of pure dipalmitoyl phosphatidylglycerol and mixtures of dipalmitoyl phosphatidylglycerol and tetraether lipid in the presence of different amounts of protons and calcium ions. The thermograms are shown in Fig. 6.

Lowering the pH from 7 to 2, a value at which most of the phosphate groups of tetraether lipid and dipalmitoyl phosphatidylglycerol have lost their charge, gives rise to a shift of the phase transition of pure dipalmitoyl phosphatidylglycerol by 22 K, which is in agreement with data previously published [4, 6, 7]; in contrast to this, the phase transition of pure tetraether lipid remains almost unaffected [4]. Thermograms of mixtures of tetraether lipid and dipalmitoyl phosphatidylglycerol reveal two main phase transitions at pH 7 and pH 2. Whereas the transition at subzero temperatures is not substantially influenced by the increased proton concentration, the range of the second transition, which is attributable to the dipalmitoyl phosphatidylglycerol rich phase, is shifted by 18 K; the temperature of the maximum heat flow increased from 26 °C to 44 °C. The width of the peak is slightly increased;  $\Delta H$  is diminished by approximately 10%. We can therefore conclude that removing of free negative charges from the polar headgroups slightly increases the miscibility of tetraether lipid and dipalmitoyl phosphatidylglycerol, an effect which can be attributed to the reduction of repulsive negative charges or disruption of a net work of hydrogen bonds among the polar headgroups of dipalmitoyl phosphatidylglycerol. The addition of calcium ions in molar excess at pH 7 provokes a pronounced increase in the transition temperatures of pure dipalmitoyl phosphatidylglycerol [6, 7], whereas the transition of tetraether lipid is only little affected [4]. The  $T_m$  value of the mixed phase of tetraether lipid and dipalmitoyl phosphatidylglycerol in the presence of calcium ions is increased by 42 K, being now close to the  $T_m$  of pure Ca-dipalmitoyl phosphatidylglycerol. The transition range of the Ca-dipalmitoyl phosphatidylglycerol-tetraether lipid phase, however, is considerably broadened whereas the width at half maximum

height remains constant. The transition now starts at = 45 °C, *i.e.* at a temperature 45 K below the transition maximum of the pure Ca-dipalmitoyl phosphatidylglycerol complex. Thus, although in the presence of calcium ions the transition temperature of pure dipalmitoyl phosphatidylglycerol and that of the sample containing dipalmitoyl phosphatidylglycerol and tetraether lipid are very similar, the broadness of the transition of the mixed preparation indicates that even at high calcium concentrations tetraether lipid is not segregated from the dipalmitoyl phosphatidylglycerol-rich domain. Possibly, dipalmitoyl phosphatidylglycerol and tetraether lipid are linked to each other by calcium bridges whereby the formation of a stable mixed phase is favoured.

### Conclusions

The data presented in this paper show that the membrane spanning, main glycopospholipid from *Thermoplasma* is able to form mixed phases with bilayer-forming ester lipids such as lecithin and dipalmitoyl phosphatidylglycerol. Thus, although a large number of hydrophobic interactions between the stretched methylbranched hydrocarbon chains are established maintaining the monomolecular tetraether lipid layer in a highly ordered stable state, ester lipids with unbranched hydrocarbon chains can be incorporated forming rather homogeneous mixed phases. There are no calorimetric indications that the tetraether lipid and the bilayer-forming lipids are segregated into different domains, as was observed with mixtures of tetraether lipid and dihexadecyl glucosylglycerol, a model diether lipid with unbranched alkyl chains [3]. Mixtures with dipalmitoyl phosphatidylglycerol are somewhat more homogeneous than mixtures with dipalmitoyl phosphatidylcholine. Miscibility with dipalmitoyl phosphatidylglycerol has been shown to be possible at various degrees of ionization of the polar head groups.

These results support earlier observations that the tetraether lipid is able to form stable liposomes with various ester lipids, such as dimyristoyl phosphatidylcholine, dipalmitoyl phosphatidylcholine, distearoyl phosphatidylcholine, dipalmitoyl phosphatidylglycerol, cardiolipin and phosphatidylinositol [8, 9].

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