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Lateral diffusion of the total polar lipids from *Thermoplasma acidophilum* in multilamellar liposomes

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Abstract

^{31}P NMR lineshapes of multilamellar liposomes composed mostly of a bilayer-spanning tetraether lipid are consistent with rapid axially symmetric motion about the bilayer normal. The residual chemical shift anisotropy of 36 ppm is comparable to that seen for diacylphosphatidylglycerol systems and suggests comparable headgroup motion. The lateral diffusion rates for *Thermoplasma acidophilum* total polar lipids in mutilamellar liposomes was measured by two dimensional exchange ^{31}P NMR as a function of temperature. At 55°C, near the growth temperature, the rate of lateral diffusion, D_L, is comparable to that of diester phospholipids in the L_α liquid crystalline phase, having a value of 2×10^{-8} cm^2/s. D_L decreases with temperature reaching a value of $8 - 6 \times 10^{-9}$ cm^2/s at 30°C. The activation energy E_a for lateral diffusion is estimated to be 10 kcal/mol ($\sim$ 42 kJ/mol). The lateral diffusion rates indicate that the tetraether liposomes have a membrane viscosity at 30°C which is considerably higher than that of diester phospholipids in the liquid crystalline phase. © 1998 Elsevier Science B.V.

Keywords: Total polar lipid; Tetraether lipid; Lateral diffusion; Membrane viscosity; ^{31}P NMR; (*Thermoplasma acidophilum*)

1. Introduction

Thermoacidophilic archaebacteria grow under conditions of extreme temperature and pH as a result, in part, of adaptation of their membrane composition to survive. The major roles of membrane lipids is to provide a barrier between the cytoplasm and the external environment and to provide membrane fluidity commensurate with proper membrane protein function at physiological temperatures. The lipid components of archaebacterial membranes must be actively controlled to ensure proper membrane function under the extreme environmental conditions in which they are found [1]. This suggests that the physico-chemical properties of the membrane are actively maintained to ensure proper function. Some archaea produce membrane-spanning lipids [2] which have been studied extensively as to their chemical characterization and their lipid bilayer arrangement [3–5]. In contrast to diacyglycerophospholipids and diacylglyceroglycolipids, relatively few investigations have been conducted on archaebacterial lipids to examine the static and dynamic properties of membranes composed of these lipids. One such property is lateral mobility of membrane components. Because this is one of the most important properties in a

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functional membrane, numerous studies of lipid lateral diffusion have been conducted in model [6] and biological [7] membrane systems composed of diacylglycerophospholipids. However, little is known about the lateral mobility of archaeal lipids. Vaz et al. [8] have used fluorescence recovery after photobleaching (FRAP) to measure the translational diffusion of a hydrolysed bipolar lipid from *Sulfolobus solfataricus* in 1-palmitoyl-2-oleoylphosphatidylcholine (POPC) multibilayers in the liquid crystalline state. Under these conditions the membrane-spanning tetraether lipid probe had a translation diffusion coefficient, D_t, which was $\sim 2/3$ that for a 1-palmitoyl-2-oleoylphosphatidylethanolamine (POPE) derivative which spans only one monolayer of the bilayer. A more recent study examined the pressure dependence of the lateral mobility of a pyrene-labelled PC probe in liposomes containing the major polar lipid fraction in *S. acidocaldarius* [9]. The authors concluded that lateral mobility only becomes appreciable at temperatures at or above the minimal growth temperature of about 58°C. In addition the results suggested that the membranes of archaebacterial liposomes are laterally immobile, as compared to other lipid membranes.

T. acidophilum is a thermoacidophilic archaebacterium with optimal growth conditions of pH 2 and 55–59°C. The total polar lipids (TPL) are composed of about 90% caldarchaeol (tetraether) lipids wherein the complete structure of the main polar lipid has recently been reported [10] (Fig. 1), and archaeol (diether) lipids represent the remainder [2]. Calorimetric studies of the tetraether lipid from *T. acidophilum* show a broad, weak phase transition at subzero temperatures [11]. Thus at the growth temperature the membrane is in a liquid crystalline phase well above the transition temperature of its dominant lipid. *T. acidophilum* TPL have been shown to form closed vesicle structures when dispersed in aqueous buffer [12]. Further, freeze-fracture electron microscopy of the lipid vesicles indicates a membrane spanning orientation of the tetraether lipids similar to that of the cytoplasmic membrane [12]. Since tetraether lipids dominate the TPL, these vesicles model the lipid monolayer that obtains in the membranes of *T. acidophilum*. Physical studies on such systems should provide insight into how the archaeal lipids contribute to the maintenance of membrane integrity and function under the extreme conditions of growth. In addition, the chemical stability of these lipids has attracted interest in their potential with respect to biotechnological applications such as drug delivery systems [13,14]. Such applications require more extensive characterization of the physico-chemical properties of these lipidic systems. In the present

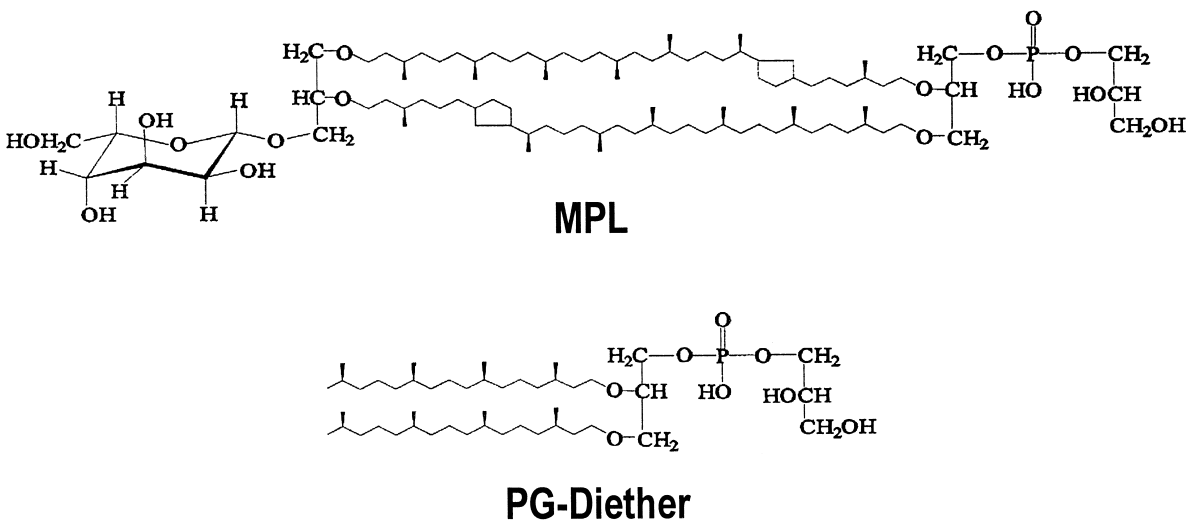

Fig. 1. Structure of the main phospholipid (MPL) showing the phosphoglycerol and β-L-gulose headgroups and one cyclopentane ring per chain [10]. Also shown is the most abundant phosphodiether lipid, present in relatively minor amount in the total polar lipid extract of *T. acidophilum*.

study we explore the lateral mobility of lipids in multilamellar liposomes composed of *T. acidophilum* TPL mixture by two dimension ^{31}P NMR exchange spectroscopy. Lateral diffusion rates between 30° and 55°C were measured.

2. Materials and methods

T. acidophilum 122-1B3 (ATCC 27658) was grown aerobically at pH 2 and 60°C and total polar lipids isolated as described previously [10].

Multilamellar dispersions of the *T. acidophilum* TPL mixture were prepared by hydrating 20 mg lipid with 0.18 ml of 10 mM Tris–HCl/160 mM NaCl pH 7.1. A second sample consisted of 46 mg of lipid hydrated with 0.35 ml 10 mM Tris–HCl/160 mM NaCl pH 7.1.

The mean diameter of the liposomes was measured using a NICOMP model 370 particle sizer (NICOMP, Santa Barbara, CA, USA) in the vesicle mode using the NICOMP distribution analysis package.

^{31}P NMR spectra were acquired at 161.1 MHz on a Varian Unity 400 spectrometer. One dimensional spectra were recorded using a Hahn echo pulse sequence [15] with broadband decoupling (gated on during acquisition). The ^{31}P $\pi/2$ pulse length was 3.2 or 7 μs, for 5 and 10 mm solenoid coil, respectively. The Hahn echo pulse spacing was 60 μs and the recycle time was 2.5 s. Two-dimensional spectra were recorded as described by Fenske and Jarrell [16]. That data sets were 1028 in the F2 dimension and 100–128 points zero-filled to 1028 in the F1 dimension. Between 64 and 128 transients were acquired for each t1 increment. The spectral width in both dimensions was 50 kHz. Spectra were processed on SPARCstation 1 and SPARCstation 5 computers using Varian VNMR software.

Correlation times t_d were extracted by simulation of 2D-exchange spectra as described by Fenske and Jarrell [16].

3. Results and discussion

^{31}P spectra of a multilamellar dispersion of TPL in Tris buffered saline are shown in Fig. 2. Although *T.*

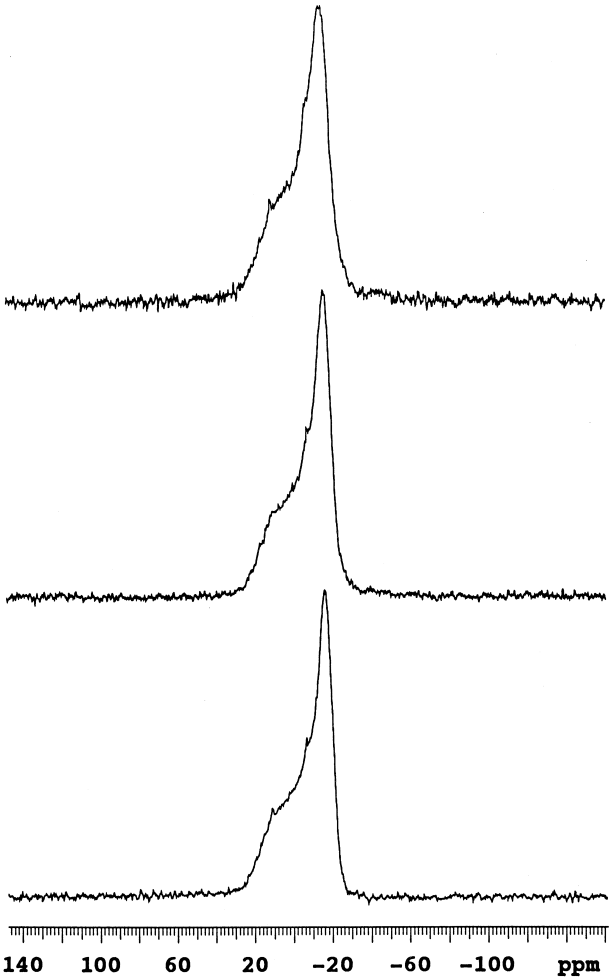

Fig. 2. ^{31}P NMR spectrum at 161.1 MHz of multilamellar dispersion of the total polar lipids from *T. acidophilum* in aqueous buffer at 30°C (bottom), 40°C (middle) and 55°C (top). The residual chemical shift anisotropy at each temperature is approximately, 36 ppm.

acidophilum grows optimally at pH 2, the internal pH of acidophiles is generally around pH 6.9 [17,18]. There is evidence that bipolar lipids are distributed asymmetrically in native membranes with most of the negatively-charged phosphate headgroup on the cytoplasmic side of the membrane [19,20]. Therefore, ^{31}P NMR measurements near pH 7 should reflect closely the biological situation. The spectra are very similar and are typical of lipid in lamellar structures undergoing axially symmetric motional averaging about the bilayer normal [21]. The residual chemical shift anisotropy is ~36 ppm between 30° and 55°C. This value is similar to that of phosphatidylglycerol above

the gel to liquid crystalline phase transition temperature [22]. This suggests that the average orientation and motional averaging of the phosphate group is most likely similar to that seen with phospholipids. Thus although tetraether lipids span the bilayer, the lipid molecules ''wobble'' about the bilayer normal with an amplitude comparable to that of phospholipids which span only half the bilayer.

The rate of lateral diffusion of lipid molecules over the curved surface of TPL liposomes was measured by 2D-^{31}P exchange spectroscopy [16]. In multilamellar phospholipid systems exhibiting axially symmetric motion about the bilayer normal, the ^{31}P NMR resonance frequency relative to the isotropic chemical shift is given by [21]

$$\nu(\beta) = \Delta/3\left[3\cos^2(\beta) - 1\right] \quad (1)$$

where β is the angle between the bilayer normal and the magnetic field direction and Δ is the residual chemical shielding anisotropy. Assuming an approximately spherical liposome, diffusion of a lipid molecule over the curved surface, during a time interval t_{mix}, will lead to a change in the angle β between the local bilayer normal and the magnetic field direction, and hence a change in the resonance frequency. The 2D ^{31}P exchange experiment correlates these orientational changes as cross peaks in the 2D spectrum. The correlation time, t_d, for this orientational exchange is related to the lateral diffusion rate, D_L, by

$$D_L = r^2/6t_d \quad (2)$$

where r is the radius of the liposome. For the two TPL liposomes samples, the mean radius was determined by dynamic light scattering to be 0.77 ± 0.1 mm and 0.77 ± 0.15 mm.

Fig. 3 shows 2D exchange spectra at several mixing times. When t_{mix} is short relative to t_d, little change in β occurs and all of the spectral intensity is located on the diagonal (Fig. 3 bottom). Increasing the time allowed for lateral diffusion to occur, off-diagonal intensity increases until each frequency (each β) shows connections to all other frequencies (all bilayer orientations are connected) (Fig. 3 top). This is more readily seen by examining traces in the 2D spectrum corresponding to $\beta = 54.7°$ (Fig. 4) where as the exchange time increases the spectrum approaches that of the normal powder spectrum. The

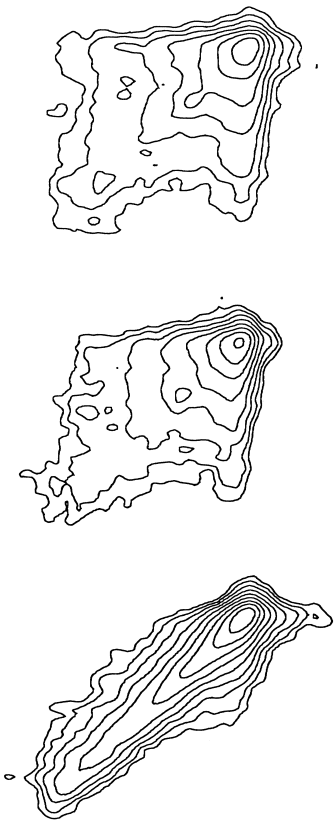

Fig. 3. 2D ^{31}P NMR exchange spectra (shown as contour plots) of TPL at 30°C as a function of the exchange time t_{mix}: t_{mix} = 0.1 ms (bottom), t_{mix} = 50 ms (middle), and t_{mix} = 100 ms (top). The horizontal and vertical directions correspond to the F2 and F1 frequency axis, respectively, with a total width of 120 ppm along each.

relatively narrow resonance at 0 ppm in the top two spectra in Fig. 4 most likely arises from either small liposomes or internal lamellae of small radius [23]. As the temperature is increased from 30°C to near the growth temperature, the rate of lateral diffusion increases as evidence by increased off-diagonal intensity for the same exchange period (Fig. 5). The correlation time is determined by simulating the exchange spectra as a function of the value of t_d/t_{mix} as described previously [16,24]. In the case of TPL exchange, it was found that simulating exchange involving bilayer orientations near $\beta = 54.7°$ were more sensitive to the value of t_d/t_{mix}. Fits of simulated and experimental spectra are given in Fig. 6 for slices from the 2D spectrum corresponding to $\beta = 90°$ and $\beta = \sim 55°$. It must be emphasized that the lipo-

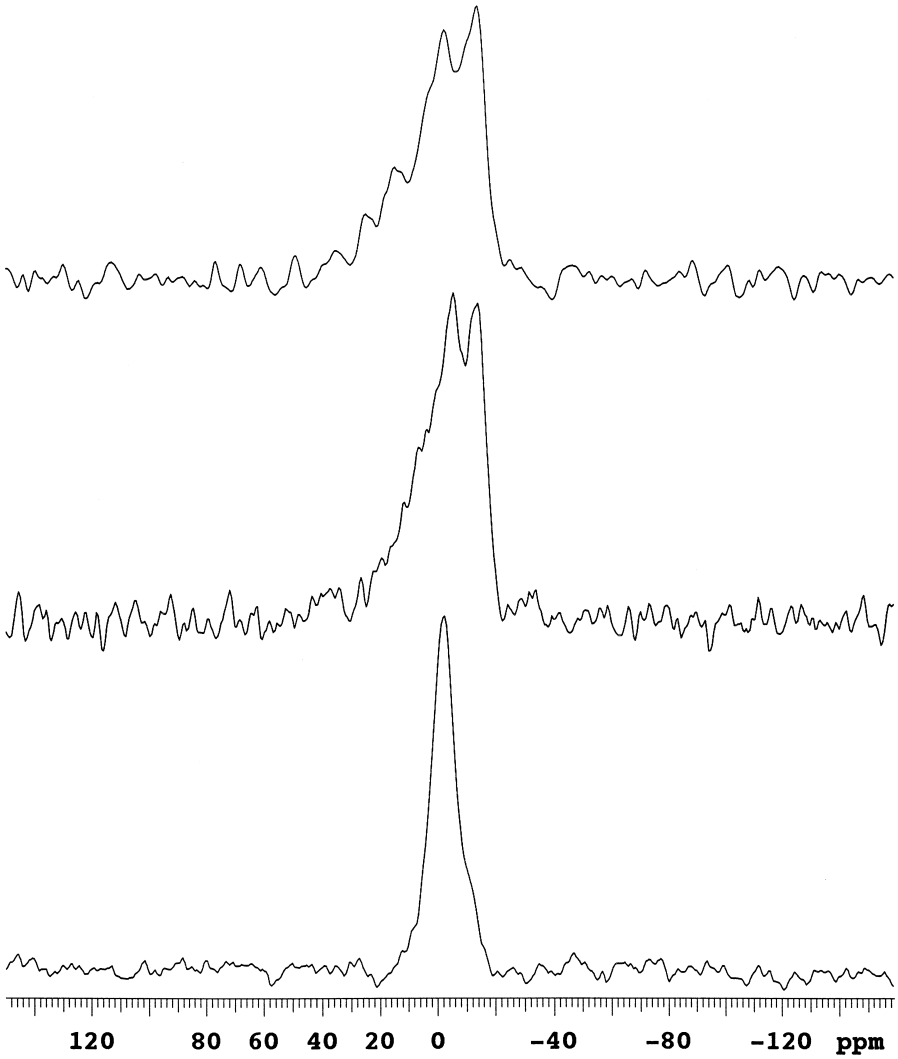

Fig. 4. Traces from the 2D ^{31}P NMR exchange spectra in Fig. 3 corresponding to bilayer normal orientations at approximately $\beta = 55°$ (see Eq. (1)). The relatively narrow line at 0 ppm may reflect the presence of liposomes of small radii or may arise from the inner lamellae of the multilamellar liposomes [23].

somes used in this study are multilamellar and have a distribution in sizes. As a result, there is actually a distribution of t_d values. The simulations shown in Fig. 6 use only a single value of t_d for each value of t_{mix} so that the best fit represents an average value for the correlation time for each exchange period. It has been shown previously that values of D_L estimated using this method are in good agreement with those estimated with FRAP methodology [16].

The values of t_d which gave best fit simulations were 160, 100, and 50 ms for 30°, 40°, and 55°C, respectively. The second sample of TPL gave similar results: 200, 100 and 50 ms at the same temperatures, respectively. Using Eq. (2) and an r of 0.77 μm, values for the lateral diffusion rate D_L were estimated to be 5×10^{-9}–6×10^{-9}, 0.99×10^{-8} and 2×10^{-8} cm^2/s, at 30°, 40°, and 55°C, respectively. Assuming an Arrhenius relationship between the rate of lateral diffusion and temperature, an activation energy E_a of 10 kcal/mol ($\sim$ 42 kJ/mol) is estimated. The lateral diffusion rates are significantly smaller than those of phospholipids in the liquid crystalline phase which are typically between 10^{-7} to 10^{-8} cm^2/s [6]. The corresponding activation ener-

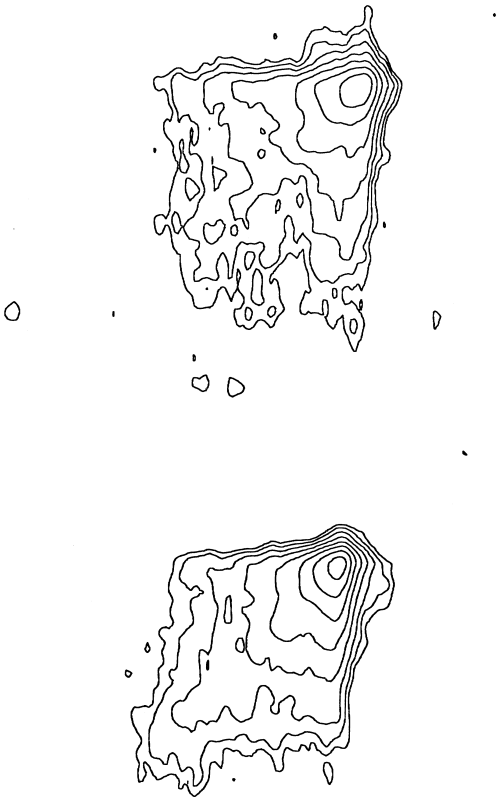

Fig. 5. 2D ^{31}P NMR exchange spectra of TPL at 30°C (bottom) and 55°C (top) after an exchange time, t_{mix}, of 50 ms.

gies range between 3 and 17 kcal/mol, with the majority having values near 7 kcal/mol [6].

Pressure studies of the lateral mobility of a probe molecule in bipolar tetraether lipids from *S. acidocaldarius* concluded that bilayers composed of these lipids do not gain ''fluidity'' until near the growth temperature [9]. The present study shows more directly and quantitatively that liposomes composed of the total polar lipids from *T. acidophilum* show a similar behaviour. The lateral diffusion rate becomes comparable to those of diacylglycerophospholipids in the liquid crystalline phase at temperatures near the growth temperature of the organism. It should be noted that at 55°C, approximately 65° above the nominal lipid phase transition temperature, the lateral diffusion rate is 2×10^{-8} cm^2/s. Diacylglycerophospholipids at comparable temperatures above their gel-liquid crystalline phase transition temperatures exhibit values of D_t at least an order of magnitude greater[6]. Vaz and coworkers have reported that a derivative of the membrane spanning lipid from *Sulfolobus solfataricus* in multibilayers of POPC exhibited D_t values that were ca. 2/3 that of a POPE analogue [8]. Interestingly, the membrane spanning probe had the same activation energy of ~28 kJ/mol as the POPE analogue, most likely because such measurements are reflecting the nature of the lipid matrix.

Vaz et.al. [8] have suggested that membrane spanning lipids diffuse as ''stiff rods'' which can be described by the continuum fluid hydrodynamic model presented by Saffman[25,26]. For a cylindrical particle of radius, a, and height, h, D_t is given by

$$D_t = (kT/4\pi\eta h)[\ln(\eta h/a\eta') - \gamma] \quad (3)$$

where η is the membrane viscosity, $\eta'(\eta' < \eta)$ is the aqueous viscosity and γ is 0.5772. k is Boltzmann's constant and T is degrees Kelvin. Using Eq. (3) and the diffusion rates of a tetraether lipid probe, the membrane viscosity of POPC was calculated to vary from 2.5 poise at 15°C to ~1 poise at 45°C [8].

A recent monolayer study suggests that the head group area for a diether lipid is approximately 1.5 nm^2, giving a radius of about 0.7 nm [27]. Although it has been suggested that tetraether lipids can span up to 7.5 nm [28], black lipid membrane studies indicate that the *T. acidophilum* tetraether lipid form monolayers of 2.5–3.0 nm [29] thickness. Similarly, *S. acidocaldarius (Caldariella acidophila)* tetraether lipids form monolayers that are 2.7 to 2.9 nm thick [30]. For the TPL from *T. acidophilum*, assuming h to be 5 nm and a to be 0.7 nm, membrane viscosities of 10, 6, and 2.8 poise are estimated for 30°, 40° and 55°C, respectively. These values are significantly greater than the 1–2 poise values seen for typical phospholipids in the liquid crystalline phase [8]. The D_t value for the TPL at 55°C, is comparable to those seen for plasma membrane extracts [7] suggesting that at or above the growth temperature the membrane viscosity is compatible with membrane function. Reconstitution of the leucine transport system from *Lactobacillus lactis* into liposomes composed of *S. acidocaldarius* tetraether lipids shows that at 25°C transport activity was significantly lower than for the transport system in phospholipid liposomes [31]. The authors concluded that the bilayer spanning tetraether lipids decreased membrane fluidity which resulted in lower transport activity. The present results support this conclusion in that the membrane

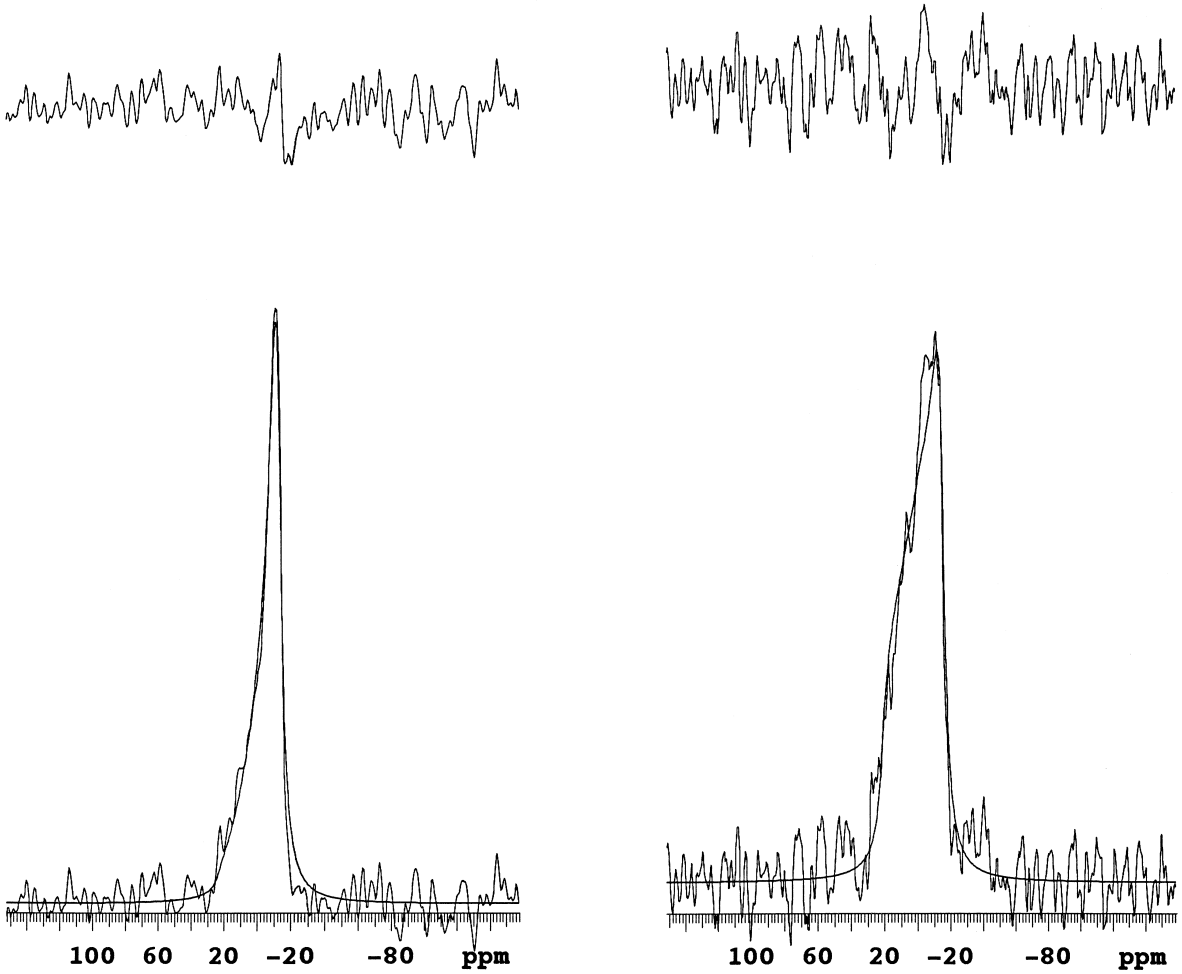

Fig. 6. Comparison of simulated and experimental 2D ^{31}P NMR exchange spectra at 55°C and $t_{mix} = 50$ ms. For simulated spectra $t_{mix}/t_d = 1$. Left spectra correspond to traces from the 2D spectrum associated with bilayer normal oriented at $\beta = 90°$ with respect to the magnetic field direction; top is the difference spectrum between simulation and experiment. Right spectra correspond to traces from the 2D spectrum associated with bilayer normal oriented at $\beta \sim 55°$ with respect to the magnetic field direction; top is the difference spectrum between simulation and experiment.

viscosity of tetraether lipid systems at 25°C may be as much as ten fold higher than that of typical phospholipids [8] and lipid extracts of plasma membranes [7].

The present results are consistent with studies which show that the thermal stability of liposomes composed of tetraether lipids is considerably greater than that of diester lipids [31–33]. The permeability of tetraether liposomes has been shown to be less temperature-sensitive than diester lipids [12,34,35]. The present results indicate that the viscosity (and thus the permeability barrier) of tetraether liposomes decreases with increasing temperature reaching that typical of liquid crystalline diester phospholipids at temperatures which are near the growth temperature of *T. acidophilum.*

4. Conclusion

In addition to chemical stability of the molecules per se, the chemical structure of archaebacterial lipids produce membranes systems which are compatible with extreme environments. The present study demonstrates that the lateral mobility of tetraether lipid molecules, and hence the membrane viscosity, is

maintained in a range consistent with membrane function. Using the estimated value of E_a for lateral diffusion, one may estimate that at 20° above the growth temperature of *T. acidophilum* the lateral diffusion rate would be increased to $2.5 \times 10^{-8}\,cm^2/s$ corresponding to a membrane viscosity of approximately 2.25 poise. Thus at temperatures substantially above the growth temperature, these lipids may be expected to allow membranes to have a ''fluidity'' comparable to those seen for the biological membranes classified within the other domains of life [7]. From a biotechnological perspective, liposomes prepared from these types of tetraether lipids are attractive as potential drug delivery systems [32,34] in which membrane permeability is a critical issue. From the present results, the ''fluidity'' at elevated temperatures is predicted to be comparable to that seen for glycerophospholipid liposomes under physiological conditions. Thus one might speculate that archaebacterial lipids will form liposomes which will entrap drugs over a broad temperature range and solvation conditions. In the case of *T. acidophilum* total polar lipids, liposomes may be expected to have low permeability at temperatures up to at least 80°C.

Acknowledgements

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