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Effects of lipid composition on the membrane activity and lipid phase behaviour of *Vibrio* sp. DSM14379 cells grown at various NaCl concentrations

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Abstract

The membrane lipid composition of living cells generally adjusts to the prevailing environmental and physiological conditions. In this study, membrane activity and lipid composition of the Gram-negative bacterium *Vibrio* sp. DSM14379, grown aerobically in a peptone-yeast extract medium supplemented with 0.5, 1.76, 3, 5 or 10% (w/v) NaCl, was determined. The ability of the membrane to reduce a spin label was studied by EPR spectroscopy under different salt concentrations in cell suspensions labeled with TEMPON. For lipid composition studies, cells were harvested in a late exponential phase and lipids were extracted with chloroform–methanol–water, 1:2:0.8 (v/v). The lipid polar head group and acyl chain compositions were determined by thin-layer and gas–liquid chromatographies. ^{31}P-NMR spectroscopy was used to study the phase behaviour of the cell lipid extracts with 20 wt.% water contents in a temperature range from −10 to 50 °C. The results indicate that the ability of the membrane to reduce the spin label was highest at optimal salt concentrations. The composition of both polar head groups and acyl chains changed markedly with increasing salinity. The fractions of 16:0, 16:1 and 18:0 acyl chains increased while the fraction of 18:1 acyl chains decreased with increasing salinity. The phosphatidylethanolamine fraction correlated inversely with the lysophosphatidylethanolamine fraction, with phosphatidylethanolamine exhibiting a minimum, and lysophosphatidylethanolamine a maximum, at the optimum growth rate. The fraction of lysophosphatidylethanolamine was surprisingly high in the lipid extracts. This lipid can form normal micellar and hexagonal phases and it was found that all lipid extracts form a mixture of lamellar and normal isotropic liquid crystalline phases. This is an anomalous behaviour since the nonlamellar phases formed by total lipid extracts are generally of the reversed type.

Keywords: Lipid composition; Phase behaviour; Salt effect; Lysolipid; *Vibrio* sp

1. Introduction

The cell membrane plays an important role in many physiological processes such as solute transport, ATP synthesis and signaling. Many physical features of a lipid bilayer have been suggested to be important in determining the activity of a membrane protein embedded in the lipid bilayer, including order and dynamics of the bilayer, bilayer thickness, the free volume available within the bilayer, the charge on the lipid bilayer surface and bilayer frustration arising from the presence of lipids that prefer nonlamellar

Abbreviations: PG, phosphatidylglycerol; PE, phosphatidylethanolamine; lyso-PE, lysophosphatidylethanolamine; DPG, diphosphatidylglycerol; TLC, thin-layer chromatography; GLC, gas–liquid chromatography; 16:0, hexadecanoic acid; 18:0, octadecanoic acid; 14:1, *cis*-9-tetradecenoic acid; 16:1, *cis*-9-hexadecenoic acid; 18:1, *cis*-9-octadecenoic acid

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structures [1–4]. In addition to these nonspecific effects, specific phospholipids may bind strongly to a small number of sites on a membrane protein, acting as cofactors. The classic example is provided by cardiolipin, that is essential for the activity of many proteins important in bioenergetics [5].

It is well documented that all kinds of organisms adapt their membrane lipid composition to the prevailing environmental and physiological conditions ([4], and references therein). In general, the cells use mainly two different stress response strategies: adjustment of the acyl chain composition, and/or adjustment of the polar head group composition [6]. Studies on the Gram-negative moderately halophilic bacteria *Vibrio alginolyticus* [7], *Pseudomonas halosaccharolytica* [8] and *Vibrio costicola* [7], and on a Gram-positive halotolerant *Planococcus* sp. [9], have shown an increase in the fraction of phosphatidylglycerol (PG) or diphosphatidylglycerol (DPG), and a decrease in the phosphatidylethanolamine (PE) fraction, as a result of increasing salinity in the growth medium.

The phase structures formed by membrane lipids are affected by several factors, e.g., the chemical structure of the lipid, temperature and interaction with cations [10]. Not all membrane lipids form bilayers at the growth temperature of the cells. Lipids with small and/or poorly hydrated head groups, like PE and monoglucosyl lipids, tend to form aggregate structures with negatively curved monolayers [10], while lysophospholipids tend to form aggregate structures with positive curvature [11,12]. Such nonbilayer forming lipids introduce a packing stress when incorporated into a lipid bilayer [3,13]. It is generally accepted that most cells have a high fraction of lipids forming reversed nonlamellar phases in their membranes and it has been shown for several bacteria that they adjust their membrane lipid composition in order to maintain a proper balance between the lipids forming a lamellar phase and the lipids forming reversed nonlamellar phases [4,6,14–19].

The membrane lipids present in *Vibrio* species are PG, DPG, PE and lyso-PE [7,20]. High concentrations of NaCl have been shown to induce the formation of a reversed hexagonal phase for DPG [21,22] and PE [23,24] and a study has been performed with the aim of investigating if the salt-induced changes in the membrane lipid composition of *V. costicola* preserve the structural integrity of the lipid bilayer and thus the membrane function [24].

In this study, the marine bacterium *Vibrio* sp. DSM14379 was grown at different salt concentrations. Previous unpublished work in the laboratory showed that *Vibrio* sp. DSM14379 has a relatively high concentration of lysolipids at optimal salt concentrations. Therefore, the effects of the salinity on the growth rate, the membrane spin label reduction pro perties, the polar head group and acyl chain compositions, as well as on the lipid phase structures were studied. Two lipids (i.e., PE and lyso-PE) in model systems that can shift the phase equilibria in opposite directions were always present in the cell membrane and it is of interest to investigate how the phase properties of these lipids are balanced in total lipid extracts.

2. Materials and methods

2.1. Cell growth

The *Vibrio* sp. DSM14379 was isolated from the Adriatic sea. The cells were grown under aerobic conditions in a peptone-yeast extract medium consisting of 5 g of peptone, 1 g of yeast extract, 2 g of $MgCl_2 \times 6H_2O$ per liter of distilled water and supplemented with either 0.5, 1.76, 3, 5 or 10% (w/v) of NaCl. Cells were incubated in an Innova™ 4300 Incubator Shaker (New Brunswick Scientific, Edison, NJ) at 28 °C and 200 rpm. The fresh growth medium was inoculated with 1% (v/v) of an overnight *Vibrio* sp. DSM14379 culture grown at an appropriate salt concentration. The cells were grown until late exponential or early stationary phase, harvested by centrifugation at $10{,}000 \times g$ for 15 min at 4 °C and washed twice in a 20-mM Tris–HCl buffer (pH 7.4) with the appropriate salt concentration. The effect of salinity on the growth rate of *Vibrio* sp. DSM14379 was determined by measuring OD_{650} of the bacterial cultures during the incubation. The bacterial growth rates were obtained by fitting the experimental data with the logistic equation:

$$OD_{650}(t) = \frac{K OD_{650,t_0}}{OD_{650,t_0} + e^{-\mu t}\left(K - OD_{650,t_0}\right)} \quad (1)$$

where K is the carrying capacity, μ is the growth rate and OD_{650,t_0} is the optical density at $t=0$.

2.2. EPR spectroscopy

Cells in the late exponential or early stationary phase were centrifuged at $10{,}000 \times g$ for 10 min and resuspended in a Tris–HCl buffer with the appropriate salt concentration. The cell suspensions were mixed with 1.18×10^{-2} M 2,2,6,6-tetrametyl-4-piperidone-1-oxyl (TEMPON) solution. Typically, 100 μl of cell suspension was thoroughly mixed with 1 μl of spin label solution. The labeled sample was transferred into a quartz-glass capillary (1-mm inner diameter) and the EPR spectrum was recorded on a Bruker ESP 300 E spectrometer (Karlsruhe, Germany) with microwave frequency of 9.59 GHz and power of 20 mW, modulation frequency of 100 kHz and amplitude of 0.5 G. The scan was 20 s, the integrator time constant was 20 ms and the center of the magnetic field was 0.3415 T. The spectra were recorded at 20 °C continuously until the signal had disappeared due to spin label reduction. The spin label

reduction rate was obtained as the reciprocal of the time during which the EPR signal was reduced to the level of noise. The reduction rate was normalized to the same number of viable cells counted as colony-forming units in order to compare cell reduction potential at different salt concentrations. In the experiments where salt concentration was changed after cells were harvested, TEMPON was added 5 min after cells were resuspended in Tris–HCl buffer with the appropriate salt concentration.

2.3. Isolation and purification of lipids

The total lipids were extracted according to a modified Bligh and Dyer procedure [25]. The washed cell pellets were resuspended in chloroform–methanol–water, 1:2:0.8 (v/v) with the appropriate salt concentration. The mixture was incubated for 3 h at room temperature. During this period, it was sonicated in a water bath sonicator for 0.5 h. After incubation the mixture was centrifuged at $10{,}000 \times g$ for 10 min at 4 °C. The insoluble pellet was extracted again with the same solvent mixture, incubated for 1 h at room temperature, sonicated for 0.5 h and centrifuged. The pooled supernatants were converted to a two-phase system by adding a NaCl solution–chloroform mixture to give a final ratio NaCl solution–chloroform–methanol of 0.9:1:1 (v/v) and this mixture was centrifuged at $2000 \times g$ for 5 min at 4 °C. After centrifugation, the upper methanol–water phase was washed again with chloroform. The pooled chloroform phases were then dried in a rotary evaporator with the addition of 2-propanol to remove traces of water. The non-lipid contaminants were removed by chromatography on Sephadex G-25 Fine [26].

2.4. Determination of polar head group composition

The membrane lipids were separated by thin-layer chromatography (TLC) using pre-coated Silica gel 60 aluminium plates (Merck, Darmstadt, Germany). The chromatograms were developed with chloroform–acetone–methanol–acetic acid–water, 6:8:2:2:1 (v/v) [27]. The spots were visualized by spraying the plates with rhodamin 6 G (Merck, Darmstadt, Germany) and the phospholipids were identified with a set of reference lipids. These lipids were: 1-palmitoyl-2-oleoyl-*sn*-glycero-3-[phospho-*rac*-(1-glycerol)], 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (Avanti Polar Lipids, Birmingham, AL), bovine diphosphatidylglycerol (DPG) (Larodan Fine Chemicals AB, Malmö, Sweden), and the lysolipids 1-palmitoyl-*sn*-glycero-3-phosphoethanolamine, 1-palmitoyl-*sn*-glycero-3-(phospho-L-serine) and 1-palmitoyl-*sn*-glycero-3-[phospho-*sn*-(1-glycerol)] (Alexis, Läufelfingen, Switzerland). The phosphate content in each spot was determined as described previously [6]. The molar fractions of the lipid head groups were calculated from the total lipid phosphorus values assuming that DPG has two phosphate groups and that all the other phospholipids have one phosphate group each. The mean values and the standard deviations were calculated from two independent batches of *Vibrio* sp. DSM14379 cells with three independent phosphorus determination assays per batch.

2.5. Determination of acyl chain composition

The acyl chain compositions were determined by gas–liquid chromatography (GLC) on a Varian model 3700 apparatus equipped with a column (1.8 m long and 0.13 cm inner diameter) packed with 10% SP-2330 on Chromosorb W AW 100/120 mesh (Supelco, Bellefonte, PA). The chromatography running conditions were: initial temperature 140 °C for 5 min, a temperature gradient of 10 °C min^{-1} and final temperature 180 °C for 20 min. The flow rate of N_2 was 20 ml min^{-1}. The chromatograph was connected to a 3390 A electronic integrator (Hewlett Packard, Palo Alto, CA).

The lipid acyl chains were converted to their methyl esters by the following procedure. The dried lipids were treated for 2 h at 70 °C in tubes with 4 ml of 5% (w/v) H_2SO_4 in water-free methanol. After incubation, 2 ml of distilled water was added to the tubes. The methyl esters were extracted twice with 1 ml of *n*-heptane. Finally, 2 ml of a 0.85% (w/v) NaCl solution was added to the pooled *n*-heptane phases to remove traces of H_2SO_4. Thereafter, the pooled *n*-heptane phases were dried with N_2. The methyl esters were identified by a comparison of their retention times with those of reference substances. These were the methyl esters of (i) the hydroxy acids 3-hydroxydecanoate, 3-hydroxydodecanoate, 2-hydroxydodecanoate; (ii) all straight-chain saturated acids from undecanoic acid to eicosanoic acid; and (iii) the straight-chain unsaturated acids *cis*-9-tetradecenoic acid (14:1), *cis*-9-hexadecenoic acid (16:1), *cis*-9-octadecenoic acid (18:1), *cis*-9,12-octadecadienoic acid, *cis*-6,9,12-octadecatrienoic acid and *cis*-5,8,11,14-eicosatetraenoic acid. All reference fatty acid methyl esters were from Larodan Fine Chemicals AB, Malmö, Sweden.

2.6. ^{31}P-NMR spectroscopy

The lipid extracts were first dried to a thin film in a glass tube with N_2 and then dried to a constant weight in vacuum. After the addition of 20 wt.% D_2O, the samples were freeze–thawed five times to complete the equilibration. The samples were kept frozen prior to the NMR studies. ^{31}P-NMR experiments were carried out at a frequency of 162.1 MHz on a Varian CMX Infinity-400 spectrometer using a 4-mm MAS probe (Chemagnetics, Fort Collins, CO). For static ^{31}P-NMR measurements, a Hahn echo cycle pulse sequence was used with proton decoupling and a 50-μs interpulse delay. When spectra were recorded at varying temperatures (from −10 to 50 °C), the samples were equilibrated for 15 min at each temperature before recording the spectra. Between 500 and 10,000 transients were collected for each spectrum with a repetition period of 4 s.

3. Results and discussion

3.1. Growth rates

Fig. 1 shows growth rates of *Vibrio* sp. DSM14379 cells at different salt concentrations. The highest growth rate was obtained in the growth medium containing 1.76% (w/v) NaCl. The distribution of the growth rates was skewed to the right indicating that this organism can tolerate rather high salt concentrations. Based on the growth rate distribution, 0.5, 3 and 10% (w/v) NaCl were chosen as representative salt concentrations and were used for further studies.

3.2. Membrane spin label reduction process

The relative spin label reduction rates of *Vibrio* sp. DSM14379 cells labeled with TEMPON are given in Fig. 1. The relative spin label reduction rate at different salt concentrations has a similar profile as the growth rate. The maximum spin label reduction rate corresponded to the optimal growth rate, while decreased rates were observed at both salt extremes. The EPR spectral intensity is proportional to the number of spin probe radicals. In order to lose EPR spectral intensity, the spin probe must accept an electron. In the buffer solution, or in sonicated cell suspensions, the TEMPON spin label was not reduced at all. The spin label was only reduced when intact cells were present. It is generally accepted that in the cell most free electrons are found in the membrane. This is because charged molecular species that would interact with the electron and thus block electron transport are regularly excluded from the membrane. Since TEMPON is a small membrane soluble molecule, it is conceivable that it intercepts electrons in the membrane and it is therefore reduced in the membrane [28,29].

When cells were grown at 3% (w/v) NaCl, washed and resuspended in a buffer with different salt concentrations, they almost instantaneously adjusted the spin label reduction rate (Fig. 2, grey bars). Since the cells were left in the new salt solution only for 5 min before spin label was added, it is reasonable to assume that the membrane composition remained the same. The spin label reduction kinetics remained approximately the same when cells were reintroduced in the buffer with the same (Fig. 2, white and grey bars at 3% (w/v) NaCl) or lower (Fig. 2, grey bars) salt concentration. However, spin label reduction kinetics slowed down when cells were reintroduced into higher salt concentrations (Fig. 2, grey bars). The results indicate that even in the absence of a change in the membrane lipid composition the membrane spin label reduction properties can quickly respond to an alteration of ionic strength in the extracellular medium. Cells that were well adapted to either high or low salt concentrations had much lower spin label reduction potential at both salt extremes as compared to cells that had the same membrane composition (Fig. 2, white bars). We suggest that the cells do not strive to maintain a constant spin label reduction rate in their membrane when grown at different NaCl concentrations. This will be investigated in more depth in future experiments.

3.3. Polar head group composition

The present paper is the first report on the membrane lipid composition in *Vibrio* sp. DSM14379. Fig. 3 shows the polar head group composition of lipids isolated from cells grown at three different NaCl concentrations. At an optimal growth rate, four major head groups were present in the total lipid extracts: 45.4±7.7 mol% PE, 19.1±1.0 mol% lyso-PE, 27.5±4.1 mol% PG and 8.0±4.3 mol% DPG. It is noteworthy that at an optimal growth rate *Vibrio* sp. DSM14379 membranes contain an exceptionally high fraction of lyso-

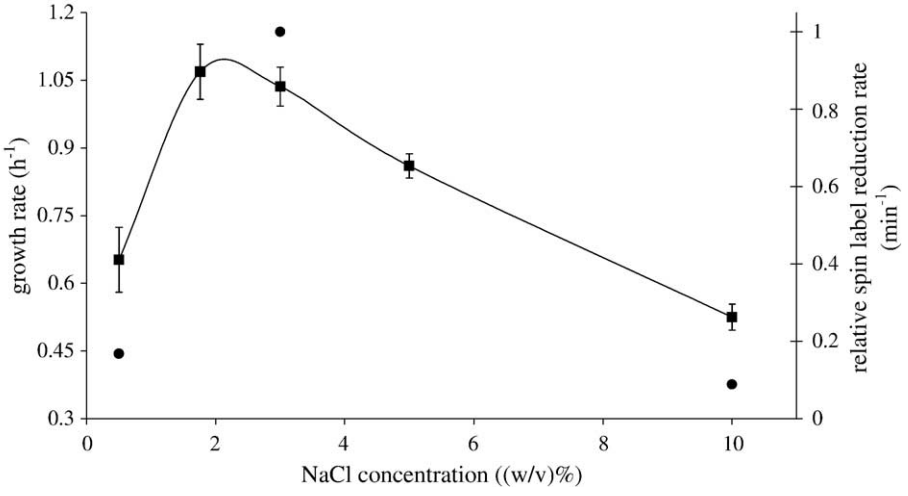

Fig. 1. The effect of NaCl concentration on growth rate (squares) and TEMPON spin label reduction rate (circles) of *Vibrio* sp. DSM14379 cells. The spin label reduction rate was normalized to the same number of viable cells at different salt concentrations. The mean values and standard deviations of growth rates were calculated from five independent batches of *Vibrio* sp. DSM14379 cells.

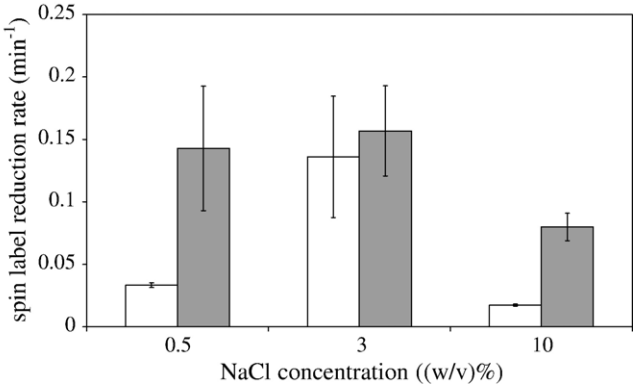

Fig. 2. The TEMPON spin label reduction rate of *Vibrio* sp. DSM14379 cells grown at 3% (w/v) NaCl, washed and resuspended in Tris–HCl buffer with 0.5, 3 and 10% (w/v) NaCl (gray bars) studied by EPR spectroscopy at 20 °C. Spin label reduction is normalized to the same number of viable cells. Spin label was added to the cell suspension 5 min after salt concentration has been changed. The membrane lipid composition of the cells was the same in this set of experiments. The other set of experiments (white bars) shows the spin label reduction rate of *Vibrio* sp. DSM14379 cells grown in a peptone-yeast extract medium with different NaCl concentrations. The spin label was added immediately after the cells were harvested. The membrane lipid composition of the cells was different in this set of experiments.

PE; the fraction is much higher than those previously reported for other closely related *Vibrio* species [7]. Lysolipids, depending on the conditions, can form normal micellar solutions or normal nonlamellar phases [11]. These lipids therefore destabilize a lipid bilayer structure and they have been shown to increase membrane water permeability and induce fusion between cells [12,30–32].

A change in the salt concentration influenced the lipid head group composition (Fig. 3). The fraction of PG increased between 0.5 and 3% (w/v) NaCl but did not change significantly at a higher NaCl concentration. The fraction of DPG exhibited a maximum at the optimum growth rate which implies that the total fraction of anionic lipids was 22, 36 and 32 mol% at 0.5, 3 and 10% (w/v) NaCl.

The PE fraction correlated inversely with the lyso-PE fraction, with PE exhibiting a minimum and lyso-PE a maximum at the NaCl concentration that gave the optimum growth rate. PE and PG/DPG are the end products of the two major branches of phosphoglyceride synthesis in many Gram-negative bacteria [33]. Thus, it appears that the synthesis in the PG/DPG pathway increases at the expense of the PE pathway at the NaCl concentration that gave the optimum growth rate.

Our results are partly in agreement with the results reported for *V. costicola* [7]. PE and PG together comprised 73–81 mol% of the membrane lipid in this organism and DPG, lyso-PE and lyso-PG are minor lipid components. In addition, the molar ratio PE/PG decreased from 2.4 to 0.8 in media containing 0.5 M and 3 M NaCl, respectively. In *Vibrio* sp. DSM14379 PE and PG together constituted 73–94 mol% of the membrane lipids. The molar ratio PE/PG decreased from 3.5 to 1.7 in media containing 0.5% and 3% (w/v) NaCl and then increased to 2.2 in media containing 10% (w/v) NaCl. Finally, at comparable salt concentration, the molar ratio PE/PG decreased with increasing salt concentration in *V. costicola*, while it increased in *Vibrio* sp. DSM14379.

3.4. Acyl chain composition

Table 1 summarizes the acyl chain composition of the lipid extracts isolated from cells grown at different salt concentrations. On average, we have identified approximately 90% of all acyl chains present in the lipid extracts. The results indicate that 18:1 was the dominating acyl chain in all extracts investigated. The fraction of 18:1 decreased significantly, and the fraction of 16:1 increased slightly, with

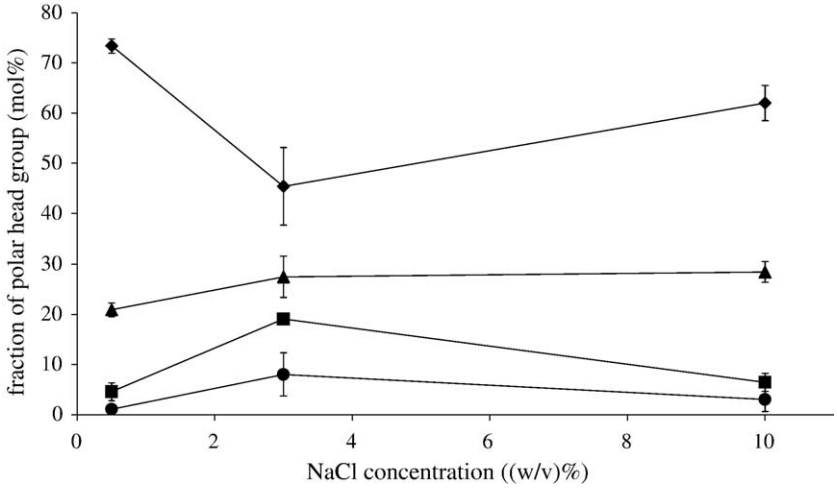

Fig. 3. The influence of NaCl concentration on polar head group composition in total lipid extracts isolated from *Vibrio* sp. DSM14379 cells; lyso-PE (squares), PE (diamonds), PG (triangles) and DPG (circles).

Table 1
Acyl chain composition (mol%) of the total lipid extracts from *Vibrio* sp. DSM14379 grown at different salt concentrations

Acyl chain	NaCl concentration of the growth medium (w/v %)		
	0.5	3	10
13:0[a]	0.4	0.4	0.4
14:1	1.2	1.1	0.5
16:0	4.9	3.6	13.3
16:1	2.5	3.7	6.1
17:0	4.3	2.4	3.7
18:0	7.9	10.5	14.6
18:1	71.1	64.6	52.7
Unidentified	7.7	13.7	8.7
Unsaturated acyl chains[b]	81.0	80.4	64.9
Saturated acyl chains[c]	19.0	19.6	35.1
Average acyl chain length[d]	17.7	17.8	17.5

NOTE: The mean values were calculated from two independent batches of *Vibrio* sp. DSM14379 cells (two to four replicate GLC analyses of each batch).

[a] The acyl chains are denoted as *n*:*k* where *n* is the number of carbon atoms and *k* is the number of *cis* double bonds.

[b–d] Unidentified acyl chains not included.

[d] Average number of carbon atoms in the acyl chains.

increasing salinity. The decrease in the total fraction of unsaturated acyl chains was compensated by an increase in the saturated acyl chains 16:0 and 18:0. Acyl chains with a hydroxyl group attached either to carbon atom two or three occur in some *Vibrio* species [34]. The hydroxy acids 3-hydroxydecanoate, 3-hydroxydodecanoate, 2-hydroxydodecanoate were used as reference substances in the GLC analysis, but none of these fatty acids could be found in *Vibrio* sp. DSM14379.

The acyl chain composition of the membrane lipids in *V. costicola* differs quantitatively from the composition in *Vibrio* sp. DSM14379. *V. costicola* synthesizes much larger fractions of 16:0 and 16:1 and much smaller fractions of 18:0 and 18:1 [7]. Moreover, in *V. costicola*, the fractions of 16:1 and 18:0 did not change significantly with increased NaCl concentration, while the fraction of 16:0 was lowest at the optimal salinity of 1 M and higher at 0.5, 2 and 3 M NaCl. 18:1 showed the opposite trend as compared to 16:0.

The data for both the acyl chain and polar head group compositions indicate that the membrane lipid composition of *Vibrio* sp. DSM14379 changed substantially as a response to the change in salt concentration. It is possible that these changes may have an effect on the growth rate as well as on the spin label reduction rate because vital physiological processes in the cell are controlled by the membrane (e.g., solute transport, signaling, electron transport). The lipids PE, PG and DPG have been reported to be crucial for several membrane-associated processes in *Escherichia coli* [35–37]. One of the most significant results is that the fractions of PE and lyso-PE are inversely related to each other and correlate very well with both the growth rate and the rate of electron transport in the membrane of *Vibrio* DSM14379 (Figs. 1 and 3). Since these lipids are able to form nonlamellar structures, we have concentrated on the phase behaviour of lipid extracts in further studies.

3.5. Phase equilibria in total membrane lipids

Among the four major membrane lipids synthesized by *Vibrio* sp. DSM14379, both PE and lyso-PE have high propensities to form nonlamellar phases. PE can form reversed cubic and reversed hexagonal phases [15,38] while lyso-PE can form normal micellar and hexagonal phases [39]. In order to study the lipid phase behaviour at different salt concentrations, we have performed solid-state ^{31}P-NMR experiments on total lipid extracts.

Fig. 4 illustrates ^{31}P-NMR spectra recorded at 25 and 50 °C from total lipid extracts containing 20 wt.% D_2O. It is generally found that a lamellar phase gives rise to a spectrum with a high field peak and a low field shoulder. A hexagonal phase generates a spectrum with a low field peak and a high field shoulder. Finally, a narrow symmetrical NMR signal is generally obtained from an isotropic liquid crystalline phase, for example, a cubic phase [40].

The line shapes of all ^{31}P-NMR spectra are more or less complex. However, it seems as all lipid extracts form a mixture of lamellar and isotropic phases at both temperatures, and that the fraction of the isotropic phase, around 0 ppm, is largest in the extract from cells grown with 3% (w/v) NaCl. Because of the low water content and the polar head group composition, it is speculated that the isotropic phase is a normal cubic-like phase. A micellar solution phase also gives rise to an isotropic spectral component but it is formed at much higher water contents [3], and the

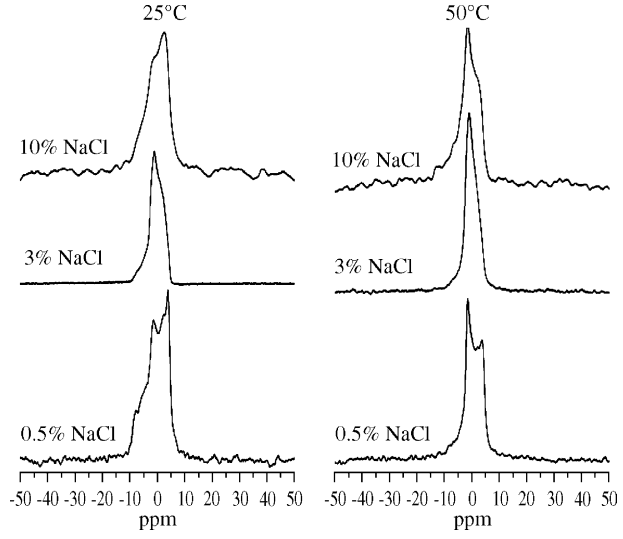

Fig. 4. ^{31}P-NMR spectra of total lipid extracts isolated from *Vibrio* sp. DSM14379 cells grown at different salt concentration and hydrated with 20 wt.% D_2O. The spectra were recorded at 25 and 50 °C and normalized to the same line height.

presence of a large fraction of lyso-PE in all lipid extracts makes a normal phase more probable than a reversed phase [3,11,12]. With this suggestion in mind, it is reasonable that the lipid extract from cells grown with 3% (w/v) NaCl is most prone to form a normal cubic-like phase: this extract contains the smallest fraction of PE, the largest fraction of lyso-PE and the largest fraction of PG plus DPG (Fig. 3). The fraction of the normal isotropic phase is larger at 50 °C as compared to 25 °C in all three lipid extracts (Fig. 4). This result would not be expected in a system consisting of one lipid and water above the phase transition temperature of the lipid. However, the total lipid extracts consists of a large number of components and the observed results therefore do not contradict our speculation that the lipid extracts form a normal isotropic liquid crystalline phase.

Our results from the phase equilibria studies of total lipids from *Vibrio* sp. DSM14379 differ from those obtained on *V. costicola* lipid extracts [41]. The total lipids from this organism grown at 1 M NaCl showed a mixture of lamellar and reversed hexagonal phases at 25 °C, while the total lipids from 3 M NaCl cultures showed only lamellar phases [41]. Moreover, a ^{31}P-NMR study on *Escherichia coli* lipid extracts showed that the lipids form only a lamellar liquid crystalline phase at the growth temperature and that reversed cubic or reversed hexagonal phases are formed only at high temperatures [6]. Finally, the phospholipids from the Gram-negative *Megasphaera elsdenii* forms a reversed hexagonal phase above the growth temperature [18]. These observations are in sharp contrast with our results that indicate that a normal nonlamellar phase is predominantly formed by the membrane lipids of *Vibrio* sp. DSM14379 at the growth temperature irrespective of the salt concentration used. This anomalous phase behaviour suggests that membrane proteins must have a decisive role in keeping the structural integrity of the lipid bilayer in these cells.

In conclusion, the *Vibrio* sp. DSM14379 has its highest spin label reduction rate around 3% (w/v) NaCl which closely matches the maximum growth rate of the bacterium. In response to changes in the salt concentration, the membrane can react both quickly by changing its spin label reduction potential as well as in the long term by adjusting its chemical composition. The observed high propensity for the formation of a normal nonlamellar phase in *Vibrio* sp. DSM14379 lipid extracts is due to a high fraction of lyso-PE.

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