Available online at www.sciencedirect.com

SCIENCE DIRECT

Biochimica et Biophysica Acta 1712 (2005) 1 – 8

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

http://www.elsevier.com/locate/bba

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

Tjaša Danevčič[a], Leif Rilfors[b], Janez Štrancar[c], Göran Lindblom[b], David Stopar[a,*]

[a]*University of Ljubljana, Biotechnical Faculty, Biological Centre, Department of Food Technology, Laboratory of Microbiology, Vecna pot 111, 1000 Ljubljana, Slovenia*
[b]*Umeå University, Department of Chemistry, Biophysical Chemistry, SE-90187 Umeå, Sweden*
[c]*Institute Jozef Stefan, Solid State Department, Laboratory of Biophysics, EPR Centre, Jamova 39, 1000 Ljubljana, Slovenia*

Received 9 July 2004; received in revised form 29 March 2005; accepted 31 March 2005
Available online 15 April 2005

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

* Corresponding author. Tel.: +386 1423 3388; fax: +386 1257 3390.
E-mail address: david.stopar@uni-lj.si (D. Stopar).

0005-2736/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamem.2005.03.013

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