SURVIVAL STRATEGIES AND MEMBRANE PROPERTIES OF EXTREMOPHILES

Wil N. Konings, Sonja-V. Albers, Sonja M. Koning, and Arnold J.M. Driessen
University of Groningen, The Netherlands

Keywords: membranes, ion permeability, transporters, binding protein, bioenergetics, proton motive force, sodium motive force

Contents

Summary

The cytoplasmic membranes of Bacteria and Archaea determine to a large extent the composition of the cytoplasm. Since the ion and, in particular, the proton and/or the sodium ion electrochemical gradients across the membranes are crucial for the bioenergetic conditions of these microorganisms, strategies are needed to restrict the permeation of these ions across their cytoplasmic membranes. The proton and sodium permeabilities of biological membranes increase with the temperature. Psychrophilic and mesophilic Bacteria, and mesophilic, (hyper)thermophilic, and halophilic Archaea are capable of adjusting the lipid composition of their membranes in such a way that the proton permeability at the respective growth temperature remains constant (homeo-proton permeability). Thermophilic Bacteria, however, have more difficulties to restrict the proton permeation across their membranes at high temperatures and often they rely on the less permeable sodium ions for maintaining a high sodium-motive force, which then drives energy requiring membrane-bound processes.

Transport of solutes across bacterial and archaeal membranes is mainly catalyzed by primary ATP-driven transport systems or by proton or sodium motive force driven

secondary transport systems. Unlike most Bacteria, hyperthermophilic Bacteria and Archaea prefer primary uptake systems. Several high-affinity ABC transporters for sugars from hyperthermophiles have been identified and characterized. The activities of these ABC transporters allow these organisms to thrive in their nutrient-poor environments.

1. Introduction

An increasing number of microorganisms are found in extreme environments and these organisms are termed extremophiles. These extremophiles flourish in environments in which the physical parameters such as temperature, salinity, pH, or pressure are extreme with respect to the conditions in which eukaryotic organisms live preferentially. Most of these extreme environments were previously thought to be hostile for any form of life.

Most extremophiles belong to the kingdom of Archaea, but certainly also Bacteria and even some Eukarya can tolerate some of these extreme conditions.

Biological cells are surrounded by cytoplasmic membranes that function as barriers between the cytoplasm and the extracellular environment. Such membranes are usually very impermeable for most ions and solutes, a property that is essential for controlling the composition of the cytoplasm. The cytoplasmic membrane is therefore essential for maintaining the internal conditions of the cells optimal for metabolism and energy transduction. Solutes and ions have to pass the membranes for metabolism to proceed and specific transport proteins catalyze the transfer of these compounds across these membranes.

Membranes are very complex structures. They consist of a bilayer or monolayer of lipid molecules that form a matrix in which various membrane proteins float. The basic properties of these membranes are described by the fluid-mosaic model. The fluidity and permeability properties of the membranes are mainly determined by their lipid composition. Organisms are able to adapt the properties of their cytoplasmic membranes in response to changes in the environment by changing the lipid composition. The different strategies, which extremophiles use to adapt their membrane and membrane proteins to the various extreme conditions in which they grow, are described.

2. Composition of the Membrane

The lipid composition of cell membranes is very complex and differs strongly between organisms. It is tightly regulated and dependent on environmental conditions. Bacterial and eukaryal lipids are mainly composed of two acyl chains, which are bound via an ester linkage to glycerol (Fig. 1A). These lipids are organized in a bilayer such that the polar head-groups stick into the water phases while the carbon chains are directed towards the inner side of the membrane.

In contrast to bacterial and eukaryal lipids, archaeal lipids consist of two phytanyl chains, which are linked via an ether bond to glycerol or other alcohols like nonitol. Such C_{20} diether lipids also form bilayer membranes just as their bacterial and eukaryal counterparts. In extremophilic Archaea, ether lipids are found in which the phytanyl

chains of two diether lipids are fused to a C_{40} core. These lipids are called tetraether lipids. These tetraether lipids form a monolayer in which the tetraether lipids span the whole membrane (Figure 1B). Freeze-fracturing studies of biological membranes revealed that cleavage can occur between the leaflets in a lipid bilayer, but not in a tetraether lipid monolayer. In tetraether membranes, therefore, the water-facing sides of the membrane are connected and cannot be separated. Ether lipids are much more stable at high temperature and resistant to oxidation than ester lipids. Moreover, ether lipids are not susceptible to degradation at alkaline pH and enzymatic degradation by phospholipases and are more tolerant to high salt concentrations. Liposomes composed of archaeal di- or tetraether lipids are therefore more stable than those of bacterial lipids and have a lower proton permeability at a given temperature.

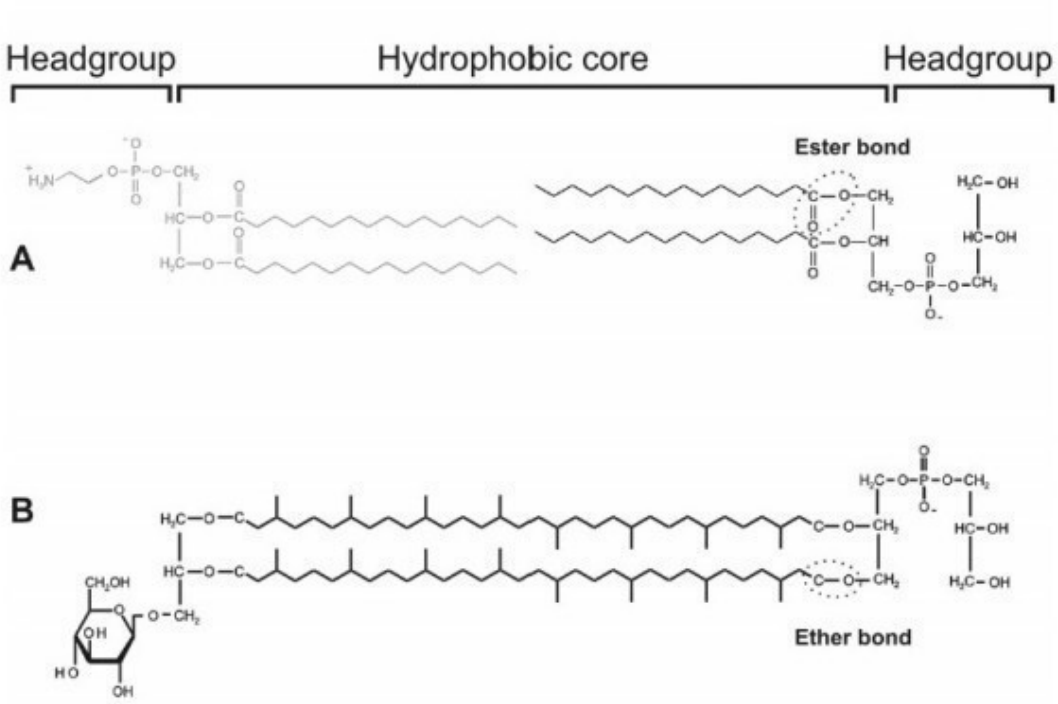

Figure 1. Lipids from Archaea and Bacteria

A) Bilayer-forming lipids in Bacteria: Phosphatidylethanolamine (PE). The acyl chain is straight (not in all cases—some bacterial lipids have a methyl branch, or a cyclohexyl group, at the end of the acyl chain, other lipids have one or more unsaturated bonds). The connection of the acyl chain to the glycerol is via an ester linkage.

B) Monolayer forming lipids in thermoacidophilic Archaea: main glycophospholipid (MPL) of *Thermoplasma acidophilum*. The phytanyl chain contains isoprenoid-like branches. The connection of the phytanyl chain with the headgroup is via an ether linkage. Archaeal membranes also contain bilayer-forming diether lipids. Some acidophilic tetraethers contain cyclopentane rings.

3. Bioenergetics

The cytoplasmic membrane plays an essential role in the generation of metabolic energy. Metabolic energy can be generated during catabolism by substrate level phosphorylation processes or by energy transduction processes in the cytoplasmic

membrane. These latter processes are catalyzed by proteins located in the cytoplasmic membrane. Specific pumps are located in the cytoplasmic membranes of Bacteria and Archaea which translocate protons or sodium ions from the cytoplasm to the external medium across the membrane thus generating electrochemical gradients of protons or sodium ions. When an electrochemical gradient of protons is generated, a force on the protons is exerted: the proton motive force (PMF). This PMF consists of two components: the chemical gradient of protons or pH gradient, the ΔpH, and the membrane potential, generated by the transport of electrical charge, the Δψ (Figure 3A). The PMF is the sum of these forces.

$$PMF = \Delta\psi - 2.3\,(R \cdot T/F)\Delta pH$$

expressed in mV, in which R is the gas constant, T the absolute temperature (K), and F the Faraday constant. The effect of 1 unit pH difference is 2.3 ($R \cdot T/F$), which equals 59 mV at 25 °C, and 70 mV at 80 °C. Under physiological conditions, the PMF is negative and the driving force on the protons is directed into the cell. In neutrophiles (around pH 7) both components of the PMF are negative. A sodium motive force (SMF) can be generated in a similar way by sodium ion pumps ($SMF = \Delta\psi + 2.3\ R \cdot T/F \log [Na^+_{in}]/[Na^+_{out}]$) (Figure 2B).

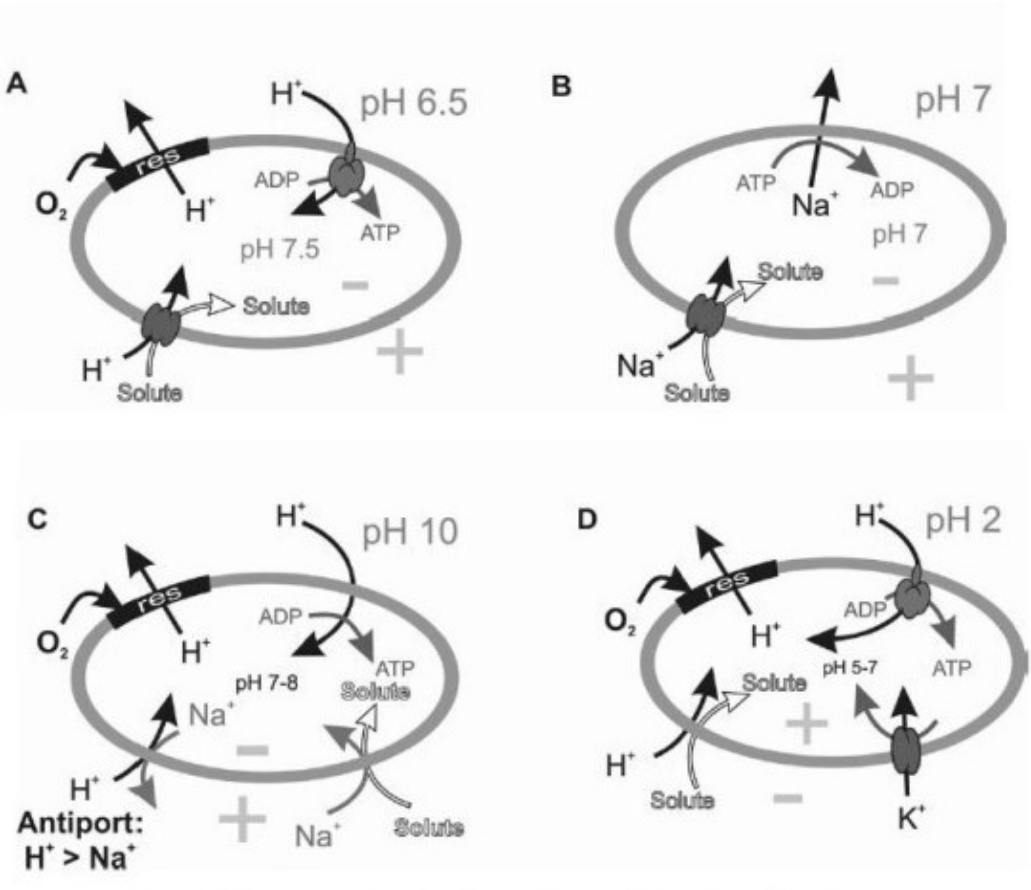

Figure 2. Energy transduction in membranes of Bacteria and Archaea
A) H+ cycling in aerobic mesophiles such as *E. coli* and *B. subtilis*. The respiratory chain excretes protons, thereby generating a PMF. This PMF drives the influx of

protons, which is coupled to ATP hydrolysis or to the uptake of solutes from the environment.

B) Na^+ cycling in anaerobic thermophilic Bacteria such as *C. fervidus*. At the growth temperature the membranes of this organism is leaky for protons. The organism relies on a sodium extruding ATPase to build up a SMF, and this SMF drives Na+-coupled uptake of solutes.

C) H^+ and Na^+ cycling in organisms such as *Bacillus alcalophilus* that live in alkaline environments: Protons are extruded by the respiratory chain. This results in a high PMF. H^+ are electroneutrally exchanged with Na^+, resulting in a reversed ΔpH (inside acid) and a high SMF. The SMF is then mainly used to drive transport of solutes into the cell.

D) H^+ cycling in organisms such as for *Picrophilus oshimae* that live in acid environment. The respiratory chain excretes protons against a large pH gradient. The PMF is retained within physiological values by an inversion of the $\Delta\psi$ by cation influx (normally K^+). Res: respiratory chain.

The PMF or SMF can be used to drive the conversion of ADP and phosphate to ATP via the membrane-bound ATPase, transport of substrates across the membrane via specific transport proteins, the rotation of flagella, and the maintenance of the intracellular pH. Efficient generation and maintenance of a PMF or SMF is only possible if the cytoplasmic membrane has a low permeability for protons and sodium ions.

4. Bioenergetic Problems of Extremophiles

Extremophiles living in the various harsh environments face different problems in maintaining viable proton or sodium ion gradients across their membranes.

-
-
-

Bibliography

Albers S.V., Van de Vossenberg J.L.C.M., Driessen A.J.M., and Konings W.N. (2000). Adaptations of the archaeal cell membrane to heat stress. *Frontiers of Bioscience* **5**, 813–820. [This paper gives an overview about mechanism employed by archaea to resist heat stress at the level of the membrane]

Booth I.R. (1985). Regulation of cytoplasmic pH in bacteria. *Microbiological Reviews* **359**, 378. [This is a presentation of mechanisms that are used by bacteria to stabilize their internal pH.]

Elferink M.G., Albers S.V., Konings W.N., and Driessen A.J. (2001). Sugar transport in *Sulfolobus solfataricus* is mediated by two families of binding protein-dependent ABC transporters. *Molecular Microbiology* **39**, 1494–1503. [This is a description of five different binding-protein dependent sugar ABC transporter in the extreme thermoacidophilic archaeon *Sulfolobus solfataricus*.]

Elferink M.G.L., De WitJ.G., Driessen A.J.M., and Konings W.N. (1994). Stability and proton-permeability of liposomes composed of Archaeal tetraether lipids. *Biochemica et Biophysica Acta* **1193**, 247–254. [This is an extensive study of the features of tetraether lipids.]

Higgins C.F. (1992). ABC transporters: from microorganisms to man. *Annual Reviews of Cell Biology* **8**, 67–113. [This is an overview over all different classes of ABC transporters.]

Krulwich T.A. (1995). Alkaliphiles: "basic" molecular problems of pH tolerance and bioenergetics. *Molecular Microbiology* **15**, 403–410. [This report discusses the bioenergetic problems and solution of alkaliphiles.]

Lolkema J.S., Speelmans G., and Konings W.N. (1994). Na^+-coupled versus H^+-coupled energy transduction in bacteria. *Biochemica et Biophysica Acta* **1187**, 211–215. [This is a critical overview of advantages/disadvantages of sodium- or proton-coupled energy transduction.]

Mitchell P. (1961). Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism. *Nature* **191**, 144–148. [This is the first report about the coupling of solute transport to the membrane potential .]

Schäfer G., Engelhard M., and Muller V. (1999). Bioenergetics of the Archaea. *Microbiology and Molecular Biology Reviews* **63**, 570–620. [Extensive presentation of bioenergetic properties of archaea]

Singer S.J. and Nicholson G.L. (1972). The fluid mosaic model of the structure of cell membranes. *Science* **175**, 720–731. [This report presents the idea that membranes are not rigid but very dynamic.]

Speelmans G., Poolman B., and Konings W.N. (1995). Na^+ as coupling ion in energy transduction in extremophilic bacteria and archaea. *World Journal of Microbiologicy and Biotechnology* **11**, 58–70. [This is a presentation of mechanisms involved in sodium-coupled energy transduction.]

Van de Vossenberg J.L.C.M., Driessen A.J.M., and Konings W.N. (1998). The essence of being extremophilic: the role of the unique archaeal membrane lipids. *Extremophiles* **2**, 163–170. [This paper deals with the importance of the contribution of membrane lipids to the ability of organisms to survive in extreme environments.]

Van de Vossenberg J.L.C.M., Driessen A.J.M., da Costa M.S., and Konings W.N. (1999a). Homeostasis of the membrane proton permeability in *Bacillus subtilis* grown at different temperatures. *Biochemica et Biophysica Acta* **1419**, 97–104. [This report forms the base for the homeo-proton permeability adaptation.]

Woese C.R., Kandler O., and Wheelis M.L. (1990). Towards a natural system of organisms: proposal for the domains Archaea, Bacteria and Eucarya. *Proceedings of the National Academy of Sciences (USA)* **87**, 4576–4579. [In this paper the authors proposed that archaea should not be longer a subdomain of the archaea but a distinct kingdom.]

Biographical Sketches

Wil N.Konings was born in Maastricht, the Netherlands in 1937. From 1958 he studied Biology at the University of Groningen and continued in 1965 with a PhD study in Biochemistry also at the University of Groningen. In 1969, he received a PhD on the thesis "Structure and Function of Hemocyanin." He subsequently went for a two-year postdoctoral research period to the National Institute of Health, Bethesda, Maryland, USA where he worked with Dr.Ernest Freese on the mechanisms of sugar and amino acid transport in *Bacillus subtilis.*

In 1971, he was appointed as scientific officer in the Department of Microbiology of the University of Groningen where he started a research group on solute transport in microorganisms. In 1976, he became Associate Professor, and in 1980, full Professor in Microbiology.

During his scientific career, he has fulfilled many professional duties such as Chairman of the Department of Microbiology, Chairman of the Faculty of Biology, Vice-Dean of the Faculty of Mathematics and Sciences, Chairman of the Groningen Biomolecular Sciences and Biotechnology Centre, and Vice-President of the International Union of Microbiological Societies. He was elected Member of the Royal Dutch Academy of Arts and Sciences. In 2001, her Majesty Queen Beatrix knighted him in the Order of the Dutch Lion.

His research has focused on the molecular mechanisms of solute uptake and excretion of endproducts of metabolism in pro- and eukaryotic microorganisms. Major topics of research have been: amino acid and peptide transport in the lactic acid bacterium *Lactococcus lactis;* energy-transducing exchange transporters in the Gram-positive *Bacillus subtilis*; multidrug resistance transporters in bacteria and eukaryotes, and solute transport and bioenergetics of extremophiles. He has supervised more than 50 PhD students and has authored or coauthored more than 500 scientific publications. He has been lecturer and keynote lecturer at many international scientific meetings.

S.-V. Albers studied biology at the University of Wuerzburg, Germany, and graduated on genetic elements of *Sulfolobales* in the lab of W. Zillig, Max Planck Institute for Biochemistry, Martinried, Germany. In 1996, she started her PhD study on sugar transport in the archaeon *Sulfolobus solfataricus* in the lab of Prof..dr. Konings and Prof.dr. A.J. M. Driessen, Groningen The Netherlands. She obtained her PhD in 2001, and currently holds a Postdoctoral position in the group of A.J.M. Driessen.

S.M. Koning graduated from the Free University of Amsterdam, The Netherlands. At the moment she is a PhD student in the lab of W.N. Konings and A.J.M. Driessen in the Department of Microbiology, University of Groningen, The Netherlands, where she studies sugar transport in the hyperthermophilic archaeon *Pyrococcus furiosus.*

Arnold Driessen was born in 1958 in Horst, the Netherlands. From 1997 to 1983, he studied biology at the University of Groningen, and in 1987 obtained his PhD on the thesis “Amino acid transport in lactic streptococci” under the supervision of Prof.dr. Konings. He then became scientific officer in the Department of Microbiology at the University of Groningen. In 1989-1990, he went as postdoc to the University of California at Los Angeles where he stayed with W.T. Wickner to work on the molecular mechanism of bacterial protein translocation. After returning to the University of Groningen, he became associate professor in 1992, and received the PIONIER award from the Netherlands Organization for Scientific Research (NWO). In 1997, he became full professor in microbiology, and since 2000, he holds the NWO-ALW Van der Leeuw Chair in Microbiology, and heads a group that works on the enzymatic and energetic mechanism of protein translocation in bacteria and archaea, and structural and functional studies on solute transport in microorganisms.

In 1988, he was honored with the Kluyver Award of the Dutch Society of Microbiology, and in 1993, he obtained the Federation of European Biochemical Societies (FEBS) Anniversary Prize of the Society for Biological Chemistry. He is author and coauthor of about 200 publications in refereed international journals and chapters in books, and speaker on invitation on many national and international meetings. He is a member of the editorial boards of *Molecular Microbiology*, *Archives in Microbiology*, and the *Journal of Molecular Membrane Biology*.