

REVIEW

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The essence of being extremophilic: the role of the unique archaeal membrane lipids

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Abstract In extreme environments, mainly Archaea are encountered. The archaeal cytoplasmic membrane contains unique ether lipids that cannot easily be degraded, are temperature- and mechanically resistant, and highly salt tolerant. Moreover, thermophilic and extreme acidophilic Archaea possess membrane-spanning tetraether lipids that form a rigid monolayer membrane which is nearly impermeable to ions and protons. These properties make the archaeal lipid membranes more suitable for life and survival in extreme environments than the ester-type bilayer lipids of Bacteria or Eukarya.

Key words Cytoplasmic membrane · Permeability · Solute transport · Bioenergetics

Introduction

Since the 1960s, the number and variety of species identified that grow in extreme environments has expanded tremendously (Kristjansson and Hreggvidsson 1995). Also, more environments have been found in which microorganisms can grow. The majority of the organisms that grow in these extreme environments belong to a group with distinct characteristics. Woese et al. (1990) named this group Archaea, and postulated the Archaea as the third domain of life on earth, different from Bacteria and Eukarya (Eucarya).

Bacteria are prokaryotic organisms. Their membrane lipids are predominantly glycerol fatty acyl diesters. These organisms have a specific bacterial type of ribosomal rRNA. Archaea are also prokaryotic organisms. Their membrane lipids are predominantly isoprenoid, and their ribosomal

rRNA differs from that of bacteria. Eukarya cells are eukaryotic organisms that possess predominantly glycerol fatty acyl diester lipids and a eukaryotic type of ribosomal rRNA. This division is now generally accepted, in particular after the recent completion of the genome sequences of the Archaea *Methanococcus jannaschii*, *Methanobacterium thermoautotrophicum*, and *Archaeoglobus fulgidus* (Bult et al. 1996; Smith et al. 1997) (see <http://www.tigr.org/>). Two-thirds of the genes found in these Archaea do not have homologs in Bacteria and Eukarya, and this clearly makes the Archaea genetically different.

Despite the enormous difference in extreme and moderate environments, all organisms known so far share the same biochemical basis for metabolism and proliferation. The organization is cellular, surrounded by a lipid membrane; DNA contains the inheritable information, coding for RNA that can be translated into proteins. The autonomously replicating living organisms known so far likely originated from one common ancestor (Woese et al. 1990) (Fig. 1), and the adaptations to various environments must have taken place from that point. Here we present the adaptations of the membrane and bioenergetics of organisms, in particular Archaea, that grow in extreme environments.

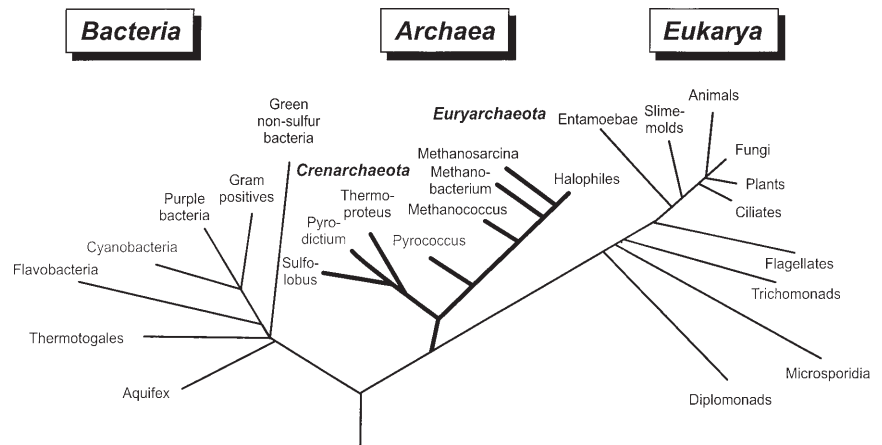
The cytoplasmic membrane

Living cells have a cytoplasmic membrane that functions as a barrier between the cytoplasm and the environment. From the onset of cellular life, hydrocarbon chains of at least 10 carbon atoms must have been present to form a membrane (Deamer 1997). Biological membranes consist of a bi- or monolayer of lipid molecules with their polar headgroups oriented toward the aqueous phase and the hydrophobic hydrocarbon chains repelled by water to the interior of the membrane. At the growth temperature of a given organism, the lipids are in a liquid crystalline state (Melchior 1982), which form a suitable matrix for proteins such as transport proteins that generate and maintain specific solute concentration gradients across the membrane.

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Fig. 1. Phylogenetic tree shows Bacteria, Archaea (in bold) subdivided into Euryarchaeota and Crenarchaeota, and Eukarya. (Data from C.R. Woese)



An important feature of these membranes is that the structure is predominantly held together by noncovalent bonds such as van der Waals and coulombic interactions that make them highly impermeable to small ions. This barrier function is critical for the functioning of the cell, as the membrane has to control the concentration of molecules and ions inside the cell. Because of the low permeability, the energy requirement to transfer an ion from the aqueous phase into the apolar hydrocarbon-like interior of the membrane is high. Because the basal membrane permeability is caused by diffusional processes, the ion permeability of the membrane increases with the temperature.

Bioenergetics

The cytoplasmic membrane is crucial for obtaining metabolic energy by energy-transducing processes. Energy can also be obtained in the form of ATP and ADP by substrate-level phosphorylation. These energy-transducing processes are closely linked, and together they determine the energy status of the cell. Energy transduction in the cytoplasmic membrane involves primary or secondary transport systems such as electron transfer systems or ATPases that translocate protons or sodium ions across the cytoplasmic membrane to the external medium (Lolkema et al. 1994; Speelmans et al. 1993a). These systems, therefore, generate an electrochemical gradient of protons or of sodium ions. The resulting proton motive force (pmf) or sodium motive force (smf) can be used to drive energy-requiring processes such as ATP synthesis from ADP and phosphate, transport of specific solutes, flagellar rotation, maintenance of the intracellular pH, and turgor (Booth 1985). Obviously, this type of energy transduction can only operate if the transmembrane gradients can be maintained, and thus requires a low basal permeability of biological membranes for the coupling ions, protons, or sodium ions.

Archaea

Molecular phylogenetic surveys of organisms that thrive in extreme environments that are beginning to emerge

indicate that the evolutionary and physiological diversity of Archaea is far greater than previously assumed (Smith et al. 1997). The domain Archaea is subdivided into the subdomains Euryarchaeota and Crenarchaeota.

The subdomain Euryarchaeota consists of methanogens, extreme halophiles, and extreme acidophilic thermophiles (Belly and Brock 1972; Schleper et al. 1995). Methanogens grow over the whole temperature spectrum where life is found: from psychrophiles (Nichols and Franzmann 1992) via mesophiles (Kandler and Hippe 1977) to extreme thermophiles (Kurr et al. 1991). Crenarchaeota cultivated so far all live above 70°C; for example, the intensively studied extreme thermoacidophile *Sulfolobus acidocaldarius* (Brock et al. 1972) and the most extremophilic organism known to date, *Pyrolobus fumarii* (Blöchl et al. 1997). The only psychrophilic Crenarchaeote discovered up to now is *Cenarchaeum symbiosum*, which symbiotically inhabits the tissue of a temperate water sponge (Preston et al. 1996). This organism grows well at 10°C, which is more than 60°C lower than the growth temperature of most other Crenarchaeota studied thus far.

Extreme environments and bioenergetics

Temperature: psychrophiles and thermophiles

The ocean covers about 70% of the Earth's surface, and about two-thirds of its volume has a temperature of 2°–4°C. Thus, a great portion of life outside the earth's crust lives at that temperature. Organisms that can grow below 0°C are found in arctic regions and in high areas (Morita 1975; Baross and Morita 1978). Permafrost, where microbial communities can survive for at least some millions of years, is not essentially an extreme environment because organisms isolated from permafrost are not all psychrophiles and are occasionally even thermophiles (Vorobyova et al. 1997).

Hot environments are found in volcanic areas above and below sea level, where mineralized water comes to the surface, and in deep rock formations heated by the earth's interior. Because of the high concentration of salts such as

sulfates or carbonates, effluents can be acidic or basic. Eukarya have not been found living above 65°C, presumably because of the impossibility of forming stable organellar membranes at this high temperature. The highest temperature at which growth of bacterial species has been found is 95°C for *Aquifex pyrophilus* (Huber et al. 1992). Bacteria are outperformed by archaeal species in marine volcanic areas, and some can grow at temperatures up to 113°C (Blöchl et al. 1997).

An intriguing feature of Bacteria and Archaea is that they can grow over a wide range of temperatures. Each species has its own specific temperature range of growth. The difference between the lower and upper growth temperature is about 30°C for bacteria (Russell and Fukunaga 1990). These organisms respond to changes in ambient temperature through adaptations of the cytoplasmic membrane lipid composition (Gaughran 1947; Sinensky 1974). A change of temperature results in an alteration of the lipid composition so as to maintain the membrane in a liquid crystalline state (Russell and Fukunaga 1990). At higher temperatures, this can be done by increasing the chain length of the lipid acyl chains, the ratio of *iso* to *anteiso* branching, or by increasing the degree of saturation (Reizer et al. 1985; Prado et al. 1988; Svobodová et al. 1988). The adaptation to low temperatures in Archaea is unsaturation of lipids (Nichols and Franzmann 1992), while they respond to high temperatures by cyclization of the fatty acyl chains and a transition from diether to tetraether lipids (De Rosa et al. 1991; Sprott et al. 1991; Yamauchi and Kinoshita 1995).

High temperatures also impose a burden on organisms to the functioning of cellular metabolism, and a higher stability of enzymes and other macromolecules is required (Adams 1993). Reactions proceed faster at higher temperatures; among these is the rate of basal ion permeation across the membrane. When the coupling ions, i.e., protons or sodium ions, permeate too fast, the organism is unable to establish a pmf or smf. The proton permeability of the cytoplasmic membrane thus can be a major factor that determines the maximum growth temperature. Liposomes prepared from lipids derived from a variety of organisms growing optimally at different temperatures were compared (Van de Vossenberg et al. 1995). Analysis of the permeability characteristics of these liposomes for protons revealed that the membrane becomes highly permeable when the temperature is beyond the maximal growth temperature of the organism from which the lipids were derived. Membranes were found to be far less permeable to sodium ions. The basal sodium ion permeability characteristics depend only on the temperature and only slightly on the origin of the membranes.

The proton permeability of bacterial and archaeal membranes at the temperature of growth is maintained within a narrow window (H^+ permeability coefficient near $10^{-9} \text{ cm s}^{-1}$) no matter what the source of lipids (Van de Vossenberg et al. 1995) (Fig. 2). Homeostasis of the proton permeability, i.e., “homeo-proton permeability adaptation,” was recently demonstrated for *Bacillus subtilis* grown at the boundaries of its temperature range (Van de

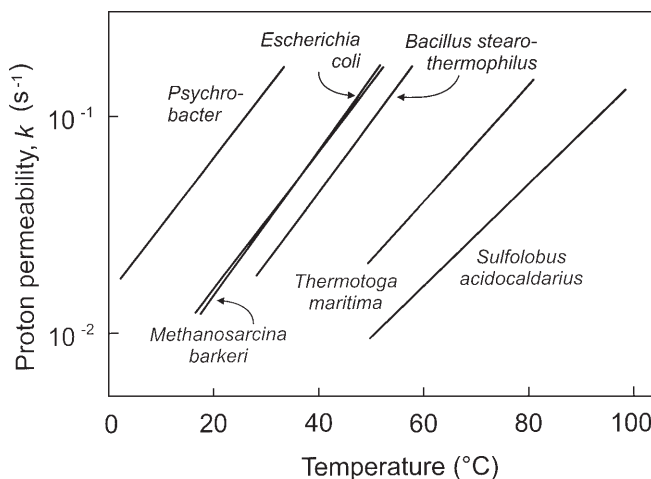


Fig. 2. Schematic representation of the temperature dependence of the proton permeability of liposomes prepared from lipids derived from Bacteria and Archaea that grow at different temperatures. At the respective growth temperatures, the proton permeability falls within a narrow window (grey bar). The proton permeability of the membranes of *Thermotoga maritima* and *Bacillus stearothermophilus* at their respective growth temperatures is higher than expected, suggesting that these organisms overcome the increased membrane proton permeability at higher temperatures by another mechanism

Vossenberg et al., in manuscript). The growth temperature-dependent alterations in fatty acyl chain composition mainly seems to be aimed at maintaining the proton permeability of the cytoplasmic membrane at a rather constant level. In thermophilic bacteria such as *Bacillus stearothermophilus*, “homeo-proton permeability adaptation” cannot be maintained. These organisms rely on other mechanisms to maintain their pmf at a viable level, e.g., by increasing the respiration rate with increasing temperatures (De Vrij et al. 1988), or by shifting to the less permeable sodium ions as main coupling ions for energy transduction. The latter strategy is used by *Caloramator fervidus* (previously *Clostridium fervidus*) (Speelmans et al. 1993a,b). This organism can grow at a much higher temperature than *B. stearothermophilus*, i.e., at 80°C (Patel et al. 1987) versus 65°C (Esser and Souza 1974). As a result of the high proton permeability of the cytoplasmic membrane at these temperatures, *C. fervidus* is unable to maintain a constant intracellular pH, and growth is confined to a narrow niche, i.e., an environment with a pH near neutrality.

Adaptation to low temperature is governed by protein stability, membrane fluidity, and proton permeability of the cytoplasmic membrane (reviewed by Russell 1990). Also, in these psychrophiles the proton permeability at growth is maintained at the same level (Van de Vossenberg et al. 1995). Most psychrophilic organisms found so far belong to the Bacteria and Eukarya, and only few psychrophilic Archaea are known.

Salt: halophiles

In the presence of suitable osmolites, bacteria can survive osmolarities up to 0.85M NaCl (Graham and Wilkinson

1992; Boch et al. 1994; Lucht and Bremer 1994). The salt content of sea water is equivalent to about 0.5M NaCl. Higher salt concentrations are found in dry areas where evaporation rates are high. Dryness itself can be regarded as an osmotically extreme environment. Many microbes adapt to dryness by reducing the water loss or by enabling a rapid recovery upon rehydration (Reed 1986). Extreme halophiles live in environments ranging from 3M to complete NaCl saturation (Lai et al. 1991). Halophilic Archaea are so widespread in high-salt environments that they virtually define the hypersaline niche. The gram-negative bacterium *Halomonas elongata* has the widest range of salt tolerance known, and can grow between 0.05M and saturating NaCl concentration (Vreeland 1987). Despite the high external sodium concentration, halophiles keep the cytoplasm relatively free of sodium. They actively expel the sodium ions, and for osmotic equilibration they use instead the less toxic potassium ions and a wide range of compatible solutes, often zwitterionic organic molecules such as glycine betaine. The internal potassium ion level in extreme halophiles can be more than 3M, needed to maintain the osmotic pressure in a salty environment (Lai and Gunsalus 1992). In halophiles, the intracellular concentration of potassium ions can be as much as fivefold higher than that of sodium ions (Brown 1983; Perez-Fillol and Rodriguez-Valera 1986).

Halobacterium halobium can generate an electrochemical proton gradient across the membrane by respiration or by the light-driven proton pump bacteriorhodopsin (Michel and Oesterhelt 1980). This organism has a H^+/Na^+ antiporter that expels the sodium ions (Murakami and Konishi 1988). Specific transport systems are needed to accumulate potassium ions to a high intracellular concentration. The smf drives the uptake of solutes by secondary transport mechanisms (Lanyi et al. 1976).

Acidity: acidophiles and alkaliphiles

Acidic environments can be formed by geothermal processes. Volcanic sulfur is oxidized to sulfuric acid, which becomes more concentrated by the evaporation of water. In such highly acidic environments, organisms are found that can live at pH below 0, i.e., H^+ concentration greater than 1 M (Schleper et al. 1995). Only a few alkaline environments are found on Earth, mostly naturally occurring environments such as soda lakes in which sodium carbonate is the major source of alkalinity. The pH value in habitats that contain microorganisms can be higher than 10.5 (Krulwich 1995).

Acidophiles and alkaliphiles keep the internal pH near neutral (Booth 1985; Matin 1990) (Fig. 3). Aerobic alkaliphiles use a Na^+/H^+ antiporter in combination with H^+ -coupled respiration to regulate their intracellular pH (Krulwich 1995; Speelmans et al. 1995). An anaerobic alkaliphilic strain LBS3 that relies on sodium ions as energy-coupling ions (Prowe et al. 1996) cannot use a Na^+/H^+ antiport for pH homeostasis and thus must depend on another mechanism. Homeostasis of the intracellular pH

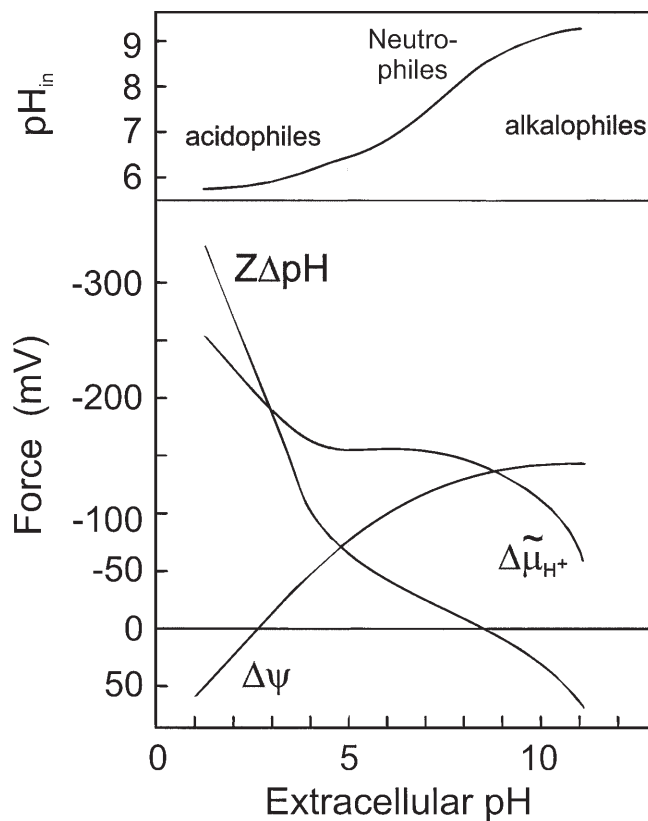


Fig. 3. Schematic representation of the pH dependence of the transmembrane electrical potential ($\Delta\Psi$), the transmembrane pH gradient (ΔpH), and the proton motive force (pmf) in Bacteria and Archaea. Also shown is the global pH dependency of the intracellular pH in these organisms

could be achieved by, for instance, a K^+/H^+ antiport (Stumpe et al. 1997).

Thermoacidophiles are only found in the Archaea. *Picrophilus oshimae*, the most acidophilic thermophile known so far, is able to grow in 1.2M sulfuric acid at 60°C (Schleper et al. 1995). This organism maintains its intracellular pH at a value of about 4.6 within an outside pH ranging from 0.5 to 4 (Van de Vossenberg et al. [in press]). This value is low compared to other extreme acidophiles that maintain an intracellular pH value above 6 (Michels and Bakker 1985; Moll and Schäfer 1988; Peebles and Kelly 1995). Acidophiles therefore have a large chemical proton gradient across the membrane. To counteract this pH gradient, the transmembrane membrane potential ($\Delta\Psi$) is inverted, i.e., positive inside. Such a steep pH gradient can only be maintained when the proton permeability of the membrane is very low. Liposomes prepared from lipids derived from thermoacidophilic Archaea are indeed extremely impermeable to protons, even at the temperatures at which the organisms are grown. However, this is not an adaptation to an acidic environment per se as lipid membranes derived from *Pyrococcus furiosus* exhibit a similar proton permeability. In this respect, in the membranes of *P. oshimae* the proton permeability even decreases at acidic pH values; however, these lipids lose the ability to assemble

into regular vesicular structures at pH values around neutrality. This loss of membrane integrity at neutral pH is an intrinsic property of the membrane lipids and likely results from the adaptation of the cells to an extreme acidic environment. As a consequence, cell viability is rapidly lost in neutral environments.

Pressure: barophiles

Barophiles live in the deep sea, in deep rock formations, and in oil wells. High pressure has a relatively low influence on the microorganisms: *E. coli* can easily withstand 500 bar, the pressure found at 5000m below sea level. Mutants can even grow at much higher pressures. Serious growth inhibition only takes place at a few thousand bar (Hauben et al. 1997). Nevertheless, many microorganisms from the deep sea display high-pressure-adapted growth characteristics. Phylogenetic studies have revealed that many barophilic bacteria currently in culture collections belong to a distinct subgroup of the genus *Shewanella* (Kato and Bartlett 1997). Because of the difficult growth and experimental conditions, little is known about barophiles in general and of Archaea in particular.

Archaea in extreme environments

Archaea are found at the limits of the extreme environments described. The reason for this could be contained in the unique structure of the archaeal membrane lipids, which have some features distinct from those of bacterial and eukaryal membranes. The majority of the lipid acyl chains are fully saturated isoprenoids that possess ether links with glycerol (De Rosa et al. 1991; Koga et al. 1993; Yamauchi and Kinoshita 1995; Kates 1996). Halobacterial lipids consist of a C₂₀ diether lipid core (Kates 1993, 1996; Upasani et al. 1994).

Membrane-spanning (bolaform amphiphilic) tetraether lipids are found in extreme thermophiles and acidophiles. These lipids have a C₄₀ isoprenoid acyl chain that spans the membrane (Relini et al. 1996). Tetraether lipids assemble into vesicular structures. Freeze-fracturing does not reveal that cleavage between two leaflets of the membrane does not occur (Choquet et al. 1992; Elferink et al. 1992; Beveridge et al. 1993). Liposomes containing a high concentration of tetraether lipids (45% and more) usually fracture across or along the outer surface, while liposomes tetraether lipids containing a substantial amount of diether lipids (20%) exhibit internal fracture planes with particles. These particles are likely tetraether complexes ripped from the upper face of the fractured membrane. Tetraether lipids cross the membrane in a monolayer type of organization, giving the membrane a high degree of rigidity (Elferink et al. 1992; Thompson et al. 1992). In addition, the extensive hydrogen-bonding networks between the polar glycolipid moieties further adds to the stability of these membranes. Black lipid membranes from tetraether lipids from *Thermoplasma acidophilum* and *Sulfolobus solfataricus*

form a monolayer of a constant thickness of 2.5–3.0nm irrespective of the type of solvent used (Gliozzi et al. 1983; Stern et al. 1992). Since the stretched tetraether lipids span the membrane, the membrane thickness is rather constant.

Hydroxylated ether lipid cores have been identified only in some methanogenic Archaea (Sprott et al. 1990) and in the haloalkaliphile *Natronobacterium* (Upasani et al. 1994). Hafenbradl et al. (1993) reported the existence of an unsaturated diether lipid in the hyperthermophile *Methanopyrus kandleri*. This lipid, 2,3-di-*O*-geranylgeranyl-*sn*-glycerol, accounts for at least 25% of the lipids. It represents a more primitive type of membrane lipid than the corresponding fully saturated lipid 2,3-di-*O*-phytanyl-*sn*-glycerol and possibly is a biosynthetic precursor. In this thermophile, only a very small amount of tetraether lipid is found (Hafenbradl et al. 1996). The unsaturated lipid resembles terpenoids, suggesting that *M. kandleri* may be a rather primitive archaeon. This suggestion is also supported by the deep position of the organism in the 16S rRNA-based phylogenetic tree. The bioenergetic and biophysical consequences of a membrane that contains this kind of lipid(s) are not known.

Another type of unsaturated lipid, and probably an adaptation to the low growth temperature, was found in the psychrophilic Archaeon *Methanococoides burtonii* (Nichols and Franzmann 1992). This lipid bears a double bond that can distort the membrane order, and thereby may lower the transition temperature (Suutari and Laakso 1992).

The structure and adaptation of archaeal membrane lipids to different environments have been extensively reviewed (De Rosa et al. 1991; Yamauchi and Kinoshita 1995; Sprott et al. 1997).

Biophysical properties of archaeal tetraether lipid membranes

Liposomes composed of archaeal tetraether lipids are more stable than bacterial bilayer lipids and have an extremely low proton permeability (Elferink et al. 1994; Van de Vossenberg et al. 1995; Van de Vossenberg et al. 1998). A study on synthetic membrane-spanning lipids revealed that in particular the bulky isoprenoid core is responsible for the lowered proton permeability (Yamauchi et al. 1993). Ether links are far more resistant to oxidation and high temperature than ester links. Consequently, liposomes prepared from tetraether lipids are more thermostable (Chang 1994). The higher stability of tetraether liposomes is superior to cholesterol-stabilized liposomes prepared from saturated synthetic lipids (Choquet et al. 1996). Ether lipids are also resistant to enzymatic degradation by phospholipases (Choquet et al. 1994).

The rate of proton permeation across biological membranes is higher than that for other monovalent cations (Deamer and Nichols 1983; Deamer and Bramhall 1986; Van de Vossenberg et al. 1995). The proton permeability increases only tenfold over a pH range of 1 to 11 (Nichols and Deamer 1980; Nagle 1987), while the permeation of other ions increases linearly with the concentration. Three

models have been proposed to explain the mechanism of proton permeation across the membrane. One model assumes that protons permeate by solubility and diffusion, but the proton permeation rates predicted on that assumption cannot account for the rates observed experimentally (Marrink et al. 1996). High proton permeation rates have also been attributed to weakly acidic contaminants, which can act as proton carriers (Gutknecht 1987). Lipid hydrolysis and oxidation are considered possible origins for weak-acid protonophores. Experimental data show that such protonophores indeed provide a possible mechanism for the transport of protons. However, the pH dependence of protonophores differs from that of proton permeation (Deamer and Nichols 1983). The clear correlation between the proton permeability and the growth temperature of the organism (see Fig. 2) makes contaminating weak acids as a possible major source for proton permeation less likely. The third possible mechanism concerns proton permeation through a transient water wire (Nagle and Morowitz 1978). In this model, protons cross the membrane by hopping along a transmembrane hydrogen-bounded chain of water molecules. The lifetime of such a water pore must be very short, allowing the transport of only one proton. This mechanism is theoretically feasible, but permeation can occur at appreciable rates only when the proton concentration at the emerging water wire is very high and thus not rate limiting (Marrink et al. 1996). Because of the rigidity of tetraether membranes, one would expect that in these membranes formation of a water wire is a less likely event. This may explain the low proton permeability of tetraether lipid membranes (Elferink et al. 1994; Van de Vossenberg et al. 1995, Van de Vossenberg et al. 1998).

Studies on the impact of membrane thickness on ion permeability (Paula et al. 1996) indicate that the potassium and proton permeability decreases sharply with increasing acyl chain length. Water and neutral solutes likely permeate by a solubility/diffusional mechanism. In membranes composed of lipids with a short acyl chain length, protons and potassium ions may mainly permeate via water-filled pores, possibly caused by defects of the membrane bilayer organization. However, at chain lengths greater than 16–18 carbon atoms, potassium ions seem to pass only via diffusion, while for passive proton permeation, the acyl chain length must be greater than 20–22 carbon atoms.

Ionophores and uncouplers can mediate ion fluxes across membranes; however, not all these compounds are effective in tetraether lipid membranes. For instance, valinomycin (K^+ -ionophore), nonactin (Na^+ -ionophore), and gramicidin (H^+ , K^+ , Na^+ -ionophore) increase the ion conductance of a black lipid membrane prepared from tetraether lipids, while the Na^+/H^+ antiporter monensin is ineffective (Stern et al. 1992).

Hardly any information is available on the impact of ionic strength on the permeability behaviour of membranes. Artificial ether model lipids are more stable in high salt concentrations (up to 5M) than ester lipids. Liposomes composed of artificial ester lipids tend to aggregate, and therefore are unable to keep trapped solutes in their lumen. Also, the leakage of sodium ions at high osmolarity is higher

in artificial ester lipids as compared with ether lipids (Yamauchi et al. 1992).

Taken together, the higher rigidity and stability, lower permeability to protons, and the higher salt tolerance make archaeal lipid membranes better suited to extreme environments than the ester type of bilayer lipids of Bacteria or Eukarya.

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