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ADAPTATIONS OF THE ARCHAEAL CELL MEMBRANE TO HEAT STRESS

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1. ABSTRACT

In extreme environments varying from hot to cold, acidic to alkaline, and highly saline, mainly Archaea are found. Thermophilic and extremely acidophilic Archaea have a membrane that contains membrane spanning tetraether lipids. These tetra-ether membranes have a limited permeability for protons even at the high temperatures of growth and this property makes it possible for thermophilic archaea to maintain a viable proton motive force under the extreme conditions. Ether lipids cannot be degraded easily and are highly stable which is also a requirement for life under extreme conditions. Psychrophilic and mesophilic Bacteria, and all Archaea adjust the lipid composition of their membranes so that the proton permeability of their membranes remains within a narrow range. This phenomenon is termed 'homeoproton permeability adaptation'. Thermophilic Bacteria are the only prokaryotes that are unable to control the proton permeability of their membranes. These organisms have to rely on the less permeable sodium ions in energy transducing processes in their membrane.

2. INTRODUCTION

A number of species have been found and characterized, which are able to inhabit extreme environments (1). Many of the organisms that grow in such environments belong to a group of microorganisms with distinct characteristics. Woese *et al.,* (2) named this group '*Archaea*', and postulated the Archaea as a domain of life on Earth, separate from the previously known groups Bacteria and Eucarya (eukaryotes), which were given the category of domains, equal to that of Archaea.

Cell membranes contain lipids, which in bacteria and eucarya are mainly *di-esters* from glycerol and two fatty acyl chains. In contrast, archaeal membranes contain predominantly ether lipids in which two isoprenoid chains are *ether-linked* to glycerol or another alcohol. Also the ribosomal rRNA's of Bacteria, Eucarya and Archaea differ. The proposal to classify life in domains is strongly supported by the analysis of the genome sequences of many different Archaea, starting with *Methanococcus jannaschii*, *Methanobacterium thermo-autotrophicum* and *Archaeoglobus fulgidus* (3;4) (*e.g.,* see http://www.tigr.org/). Two third of the genes found in these Archaea do not have homologues in Bacteria and Eucarya, which emphasizes the genetically different position of the Archaea.

The kingdom of the Archaea is subdivided into the subdomains euryarchaeota and crenarchaeota. The subdomain euryarchaeota consists of methanogens, extreme halophiles, thermophiles, and extremely acidophilic thermophiles (5,6). Methanogens grow over the whole temperature spectrum where life is found: from cold (psychrophiles) (7) via moderate (mesophiles) (8) to extremely hot environments (extreme thermophiles) (9). Crenarchaeota comprise the most thermophilic organism known to date, *Pyrolobus fumarii* (10) and the intensively studied extreme thermoacidophile *Sulfolobus acidocaldarius* (11). The only psychrophilic crenarchaeote discovered until now is *Cenarchaeum symbiosum* which symbiotically inhabits tissue of a temperate water sponge (12). This organism grows well at 10°C, which is more than 60°C lower than the growth temperature of all other crenarchaeota found so far.

Despite the enormous difference in extreme and moderate environments, all organisms known share the same biochemical basis for metabolism and proliferation. The organization is cellular and surrounded by a lipid

membrane. DNA contains the inheritable information, coding for RNA that can be translated into proteins. Here we present membrane features of microorganisms, in particular Archaea, that reflect adaptation to heat stress.

3. THE CYTOPLASMIC MEMBRANE AND BIOENERGETICS

The cytoplasmic membrane is crucial for the generation of metabolic energy by energy transduction. In this process, the energy of an electrochemical ion gradient across the membrane is transformed into other forms of energy or vica versa. Metabolic energy can also be obtained in the form of ATP and ADP by substrate level phosphorylation processes. Both metabolic energy generating processes are closely linked and together they determine the energy status of the cell. The energy transducing systems are located in the cytoplasmic membrane. Specific pumps translocate protons or sodium ions across the membrane into the external medium and this activity results in the generation of electrochemical gradients of protons or sodium ions (13,14). When protons are extruded, the resulting electrochemical gradient exerts a proton motive force (PMF). The PMF consists of two components: the delta pH, *i.e.,* the concentration gradient of protons, and the delta psi, the membrane potential, caused by the transport of electrical charge:

PMF = delta psi – 2.303 (RT/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 59 mV at 25°C, and 70 mV at 80°C. Both components of the electrochemical proton gradient exert a force on the protons, pulling the protons across the cytoplasmic membrane. If the resulting PMF is negative, the driving force on the protons is directed into the cell. In organisms that live around pH 7 (neutrophiles) both the electrical and concentration components are negative. In analogy with the PMF, sodium ion pumps can generate a sodium motive force (SMF).

The PMF or SMF can be used to transduce their potential energy to metabolic energy requiring processes such as ATP synthesis from ADP and phosphate, transport of specific solutes across the membrane, flagellar rotation, and maintenance of the intracellular pH and turgor (15). Obviously, this type of energy transduction can only operate if the transmembrane gradient of H+ *c.q.* Na+ can be maintained. A prerequisite for this maintenance is that the biological membranes are limited in permeability for these ions.

The cytoplasmic membrane functions as a barrier between the cytoplasm and the environment. This membrane consists of a layer of lipids in which proteins are embedded. The membrane controls the movement of solutes (ions and nutrients) into or out of the cell. Biological membranes consist of a bi- or monolayer of lipid molecules and of proteins. In nature, an enormous diversity of lipids is found. The lipids have polar headgroups that stick into the water phase and hydrophobic hydrocarbon chains that are oriented to the interior of the membrane. At the growth temperature of a given organism, the membranes are in a liquid crystalline state (16). The structure of biological membranes is mainly held together by noncovalent bonds such as Van der Waals bonds and electric interactions. The barrier function of the cell membrane is critical for the functioning of the cell, as the membrane has to control the concentration of molecules and ions inside the cell. Most solutes can cross the membrane via specific transport proteins. The permeability of membranes for small solutes and ions is restricted due to the high energy that is required for the transfer of a hydrophobic solute or ion from the aqueous phase into the apolar interior of the membrane.

The lipid layer forms a suitable matrix for proteins such as transport proteins that generate and maintain specific solute concentration gradients across the membrane. The low permeability of the membrane limits the energy needed for maintaining such gradients. Organisms control the fluidity and permeability of their cytoplasmic membrane. The membrane is in a liquid crystalline state that allows optimal functioning of the membrane proteins. The rate at which protons leak inward is determined by the proton permeability and the PMF across the membrane. A proper balance between proton permeability and the rate of outward proton pumping is needed to sustain an appropriate PMF.

4. LIPIDS IN BACTERIAL AND ARCHAEAL MEMBRANES

Bacteria and eucarya contain lipids in which two acyl chains are linked to glycerol *via* ester bonds. Usually, the acyl chains are straight carbon chains (figure 1A). These lipids are organized in a bilayer in which the carbon chains are directed towards the inner side of the membrane.

The archaeal membrane lipids have some features distinct from bacterial and eucaryal membranes. The hydrophobic part of the membrane is composed of phytanyl chains and these chains are linked *via* ether bonds to glycerol or other alcohols like nonitol.

The structure of archaeal membrane lipids and the adaptation to different environments have been extensively reviewed (17-19). The Archaeal lipid chain is composed of isoprene subunits (figure 1B). These phytanyl chains contain methyl groups at every fourth carbon atom in the backbone. The reason for the higher stability of the phytanyl chain could be the reduced segmentary motion of tertiary carbon atoms (*i.e.,* rotation of carbon atoms that are bound to three other C-atoms, resulting in kinks in the acyl chain). The segmental motion in the phytanyl chains is hindered due to the methyl side groups, which is particularly pronounced in the lamellar phase and prevents kink formation in the phytanyl chains. The restriction in hydrocarbon chain mobility may also reduce the permeability of the archaeal membrane.

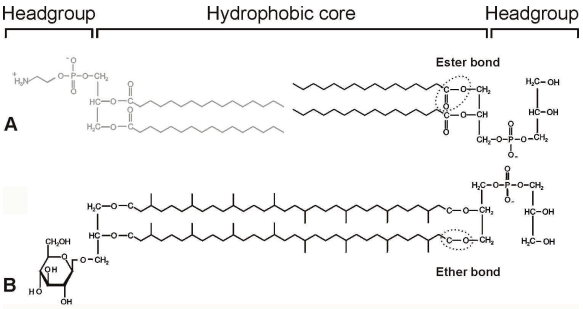

Figure 1. Lipids from archaea and bacteria. A: bilayer forming lipids in bacteria: Phosphatidylglycerol (PG) from *Escherichia coli*. The acyl chain is straight (not in all cases: some Bacterial lipids have a methyl branch, or a cyclohexyl group, at the end of the acyl chain, other lipids have one or more unsaturated bonds). The connection of the acyl chain with the headgroup is an ester. 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 an ether linkage. Archaeal membranes also contain bilayer forming diether lipids. Some acidophilic tetraethers contain cyclopentane rings.

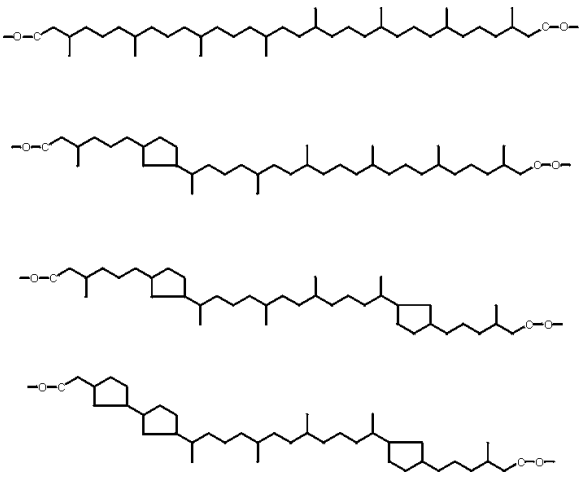

Figure 2. Cyclization of the phytanyl chains of the *S. solfataricus* tetraether lipids.
Only one of phytanyl chains is shown. The degree of cyclization increases from top to bottom.

Most of the archaeal lipid acyl chains are fully saturated isoprenoids (17,19-21). Halobacteria and most archaea growing under moderate conditions contain lipids which consist of a C_{20} diether lipid core (20, 22, 23). These lipids form bilayers in a similar way as the ester-lipids. Membrane spanning (bolaform amphiphilic) tetraether lipids are found in extreme thermophiles and acidophiles (17). These lipids have C_{40} isoprenoid acyl chains which span the entire membrane (24). Freeze-fracturing of these membranes reveals that cleavage between two leaflets of the membrane does not occur, which means that the water facing sides of the membrane are connected and cannot be separated (25-27). Tetraether lipids from *Thermoplasma acidophilum* and *Sulfolobus solfataricus* form monolayer black lipid membranes of a constant thickness of 2.5-3.0 nm (28, 29), another indication that tetraether lipids span the membrane. This monolayer type of organization gives the membrane a high degree of rigidity (27, 30).

5. PROPERTIES OF ARCHAEAL AND BACTERIAL MEMBRANES

Liposomes composed of archaeal tetraether lipids are more stable than those of bacterial bilayer lipids and have a lower proton permeability at a particular temperature (31-33). Even at extreme temperatures the proton permeability of tetraether lipids is sufficiently low to allow the generation of a high PMF (see below). A study on synthetic membrane spanning lipids revealed that in particular the bulky isoprenoid core is responsible for the lowered proton permeability (34). Ether links are far more resistant to oxidation and high temperatures than ester links. Consequently, liposomes prepared from archaeal tetraether lipids are more thermostable (35). Furthermore, in contrast to ester links, ether links are not susceptible to degradation at alkaline pH (saponification) and enzymatic degradation by phospholipases (36). The stability of liposomes of tetraether lipids is superior to cholesterol-stabilized liposomes prepared from saturated synthetic lipids that resemble bacterial lipids (37).

6. ADAPTATIONS TO HEAT STRESS

Bacteria and archaea can respond to changes in ambient temperature through adaptations of the lipid composition of their cytoplasmic membranes (38). These changes are needed to keep the membrane in a liquid crystalline state (39) and to limit the proton permeation rates. At higher temperatures, this can be done in Bacteria by increasing the chain length of the lipid acyl chains, the ratio of *iso*/*anteiso* branching and/or the degree of saturation of the acyl chain (40-42). In two members of the archaeal *Sulfolobales*, *Sulfolobus solfataricus* and *Thermoplasm*a, the degree of cyclization of the C_{40} isopranoid in the tetraether lipids is increased at higher growth temperatures (figure 2) (43). In *Thermoplasma* cells grown at 40^{o}C the ratio of acyclic/monocyclic/bicyclic chain is 62/37/1 and 25/50/24 for cells grown at 60^{o}C (44). By the increase of the cyclization of the C_{40} isopranoid chains the lipids can be packed more tightly, which results in a more restricted motion of the lipids and prevents that the membrane becomes too fluid. These two archaea already contain a high percentage of tetraether lipids in their total lipids (above 90%). In the euryarchaeote *Methanococcus janaschii* increasing temperatures induce the change from diether lipids to the more thermostable tetraether lipids (figure 3) (45). Also in this case, the cyclization of the chains tend to decrease the motion of the lipids and therefore contributes to an acceptable membrane fluidity at elevated growth temperature.

High temperatures impose a burden on the cellular metabolism, and require a higher stability of

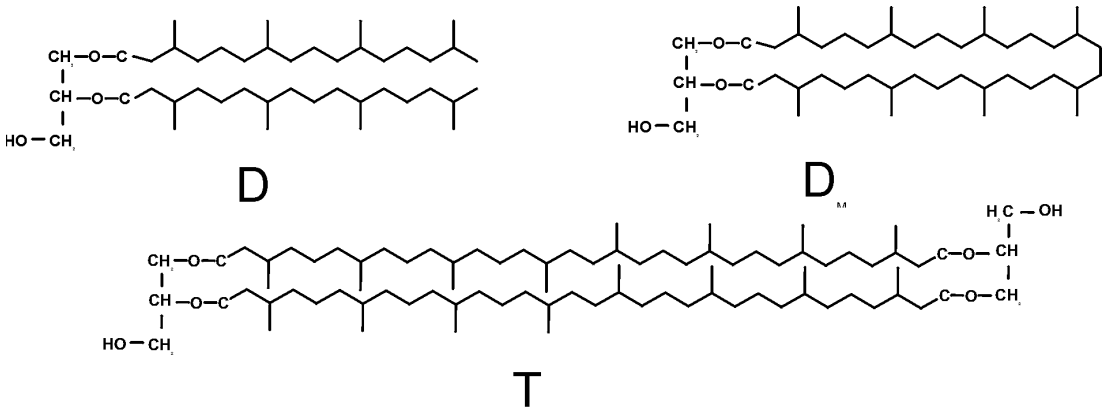

Figure 3. Structures of the ether lipid cores in *M. jannaschii*. D_M, macrocyclic diether; D, diether; T, tetraether.

enzymes and other macromolecules (46). Since the basis for membrane permeation is diffusion (mainly the diffusion of water in case of proton permeation), the ion-permeability of the membrane increases with the temperature. When the coupling ions, *i.e.,* protons or sodium ions, permeate too fast, the organism will be unable to establish a sufficient PMF or SMF. The permeability of the cytoplasmic membrane thus is a major factor in determining the maximum growth temperature. Liposomes have been prepared from lipids extracted from a variety of organisms that grow optimally at different temperatures. The membranes of these liposomes become highly permeable for protons at temperatures above the growth temperature of the organism from which the lipids were derived. The sodium ion permeability is orders of magnitudes lower than the proton permeability. The basal sodium ion permeability was found to depend on the temperature and barely on the composition of the membranes (32). The most important finding of our studies is that the proton permeability of most bacterial and all archaeal membranes at the temperature of growth is maintained within a narrow window (H^+-permeability coefficient near 10^{-9} cm s^{-1}) (figure 4) (32). The proton permeability of the membranes can be restricted by adjusting the lipid composition of the membranes. The homeostasis of proton permeability, termed 'homeo- proton permeability adaptation', was confirmed in *Bacillus subtilis* grown at and within the boundaries of its growth temperature range (47). The growth temperature-dependent alterations in fatty acyl chain composition are thus mainly aimed at maintaining the proton permeability of the cytoplasmic membrane at a rather constant level. From the observations described above it is evident that the proton permeability is an important growth-limiting factor at the upper boundary of the growth temperature. In contrast, the permeability of the membranes for sodium ions at different growth temperatures was not constant, but was found to increase exponentially with temperature in a similar way for all organisms studied. However, since the sodium permeability is several orders of magnitude lower than the proton permeability, a high SMF can be generated even at high temperatures. The lipid composition of the membrane thus has only a minor effect on the membrane permeability for sodium ions, and the rate of sodium ion permeation seems mainly to be influenced by the temperature.

Unlike in psychrophilic and mesophilic bacteria and archaea, in thermophilic bacteria, the proton permeability of their membranes at the respective growth temperatures is much higher than the proton permeabilities found at the growth temperature in the other organisms (32). These thermophilic bacteria, such as *B. stearothermophilus* and *Thermotoga maritima* are unable to reduce the proton permeability of their membrane at the high temperatures at which they grow. Thermophilic bacteria thus have at their growth temperature membranes that are very leaky for protons (figure 5). Some moderately thermophilic bacteria can compensate for the high proton leak by drastically increasing the respiration rate and therefore the rate of proton pumping (48). Most thermophilic bacteria shift to the less permeable sodium ion as coupling ion for energy transduction. This strategy is used by *Caloramator fervidus* (previously *Clostridium fervidus*) (14, 49), an organism that can grow at a higher temperature than *B. stearothermophilus*, *i.e.,* 70°C *versus* 65°C (50, 51). *C. fervidus* has a Na^+ -translocating ATPase that excretes sodium ions at the expense of ATP. As a result, a SMF is generated that is the driving force for energy requiring processes such as solute transport. Due to the high proton permeability of its membrane, *C. fervidus* is unable to maintain a constant intracellular pH. Consequently, growth of *C. fervidus* is confined to a narrow niche, *i.e.,* an environment with a pH near neutrality.

7. ARCHAEAL TRANSPORT PROTEINS

Membrane proteins form a great part of prokaryotic membranes (up to 60%). Whereas a lot of information has been gathered about archaeal lipids, not much is known about archaeal membrane proteins, especially from thermophilic and hyperthermophilic archaea. Membrane proteins involved in energy transducing processes, like cytochrome oxidases and ATPases, have been described and characterized, *e.g.* from *S. acidocaldarius* (reviewed in 52), but only little information is available about solute transport proteins.

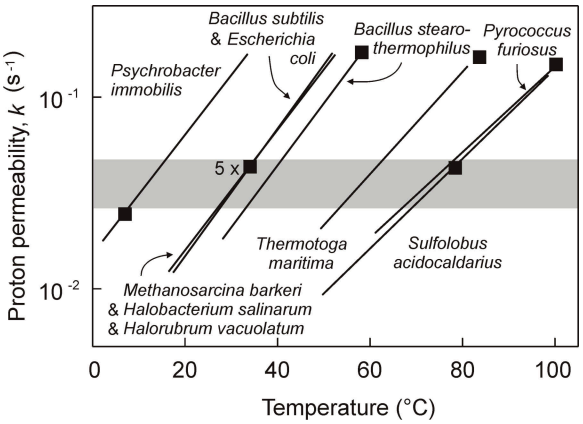

Figure 4. Schematic representation of the proton permeability in archaea and bacteria that live at different temperatures. At the respective growth temperatures, the proton permeability falls within a narrow window (grey bar). *Thermotoga maritima* and *Bacillus stearothermophilus* have a permeability that is higher than in other organisms. Both organisms overcome this problem differently.

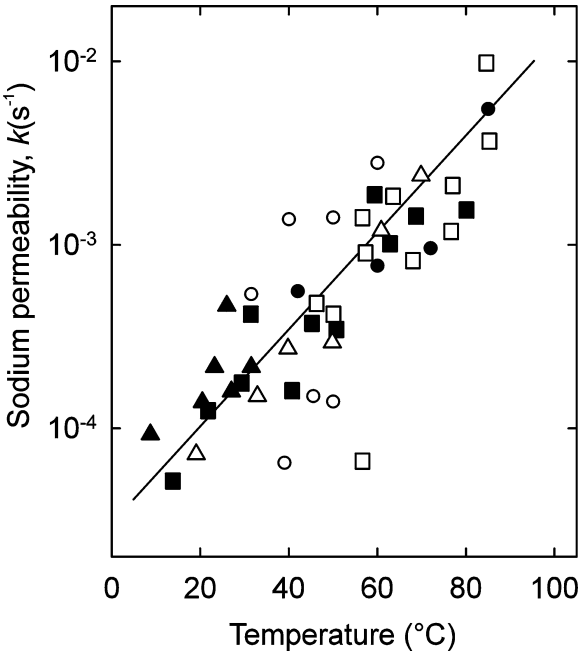

Figure 5. Temperature dependency of the sodium permeability of liposomes derived from various bacteria and archaea. *P. immobilis* sp (▲), *M. barkeri* (Δ), *E. coli* (❍), *B. stearothermophilus* (■), *T. maritima* (●), and *S. acidocaldarius* (❑).

Transport of solutes across membranes is classified in three groups dependent on the driving force: (i) primary transport, (ii) secondary transport and (iii) group translocation. The first group of transport systems uses primary energy sources such as ATP to drive the uptake of solutes against a concentration gradient. Very well studied examples are the ABC- (ATP-binding cassettes) transporters, which in all three domains of life can transport a wide variety of substrates (53). Secondary transporters use electrochemical gradients of protons or sodium ions during transport of solutes. Three modes of secondary transport can be distinguished, uniport (equilibration along an electrochemical gradient), symport (substrate is co-transported with ion) and antiport (substrate is exchanged for ion or another substrate). Group translocation systems chemically modify the substrate during the transport process. The latter system is found in bacteria, where sugars are phosphorylated during transport into the cell (phosphoenolpyruvate dependent phosphotransferase system, PTS system).

Up until now no PTS systems have been found in archaea. Interestingly, all sequenced archaeal genomes contain a large number of binding-protein-dependent ABC-type transporters and show a small number of genes with homologies to secondary transporters (3, 4). Recently, it has been reported that maltose and trehalose uptake in the hyperthermophilic *Thermococcus litoralis* occurs via a binding-protein-dependent ABC transporter (54). The thermoacidophilic *S. solfataricus* harbors an ABC transporter for glucose and for many other sugars (55, 56). Taken together thermophilic archaea seem to favor binding-protein-dependent ABC-transporters for sugar uptake. The need for such transport systems might relate to the nutrient-poor environments, such as hydrothermal vents or sulfuric hot springs, in which these organisms live. Binding proteins can scavenge solutes at very low concentrations due to the high binding affinities (K_d < 1microM). In contrast secondary transport systems exhibit binding affinities in the micro or millimolar ranges and are thus less suitable for growth in extreme environments.

8. PERSPECTIVES

It can be concluded that of all extreme conditions organisms have to face, temperature has the most pronounced effect on the membrane. Lipids and membrane proteins of hyperthermophilic archaea are well adapted to this environmental stress factor. Their features make them attractive for biotechnical applications. Because of their long term stability archaeal lipids could, *e.g.*, be used in liposomes as tool for drug delivery. Lipids and membrane proteins may form matrices for the construction of biosensors. Since expression of archaeal membrane proteins was achieved in *E. coli* (unpublished data), these proteins will be suitable for structural and functional analysis (*e.g.*, 3D crystallization).

9. ACKNOWLEDGEMENT

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