Archaeabacteria thrive in environments characterized by anaerobiosis, saturated salt, and both high and low extremes of temperature and pH. The bulk of their membrane lipids are polar, characterized by the archaeal structural features typified by ether linkage of the glycerol backbone to isoprenoid chains of constant length, often fully saturated, and with sn-2,3 stereochemistry opposite that of glycerolipids of Bacteria and Eukarya. Also unique to these bacteria are macrocyclic archaeol and membrane spanning caldarchaeol lipids that are found in some extreme thermophiles and methanogens. To define the barrier function of archaeabacterial membranes and to examine the effects of these unique structural features on permeabilities, we investigated the water, solute (urea and glycerol), proton, and ammonia permeability of liposomes formed by these lipids. Both the macrocyclic archaeol and caldarchaeol lipids reduced the water, ammonia, urea, and glycerol permeability of liposomes significantly (6–120-fold) compared with diphytanoylphosphatidylcholine liposomes. The presence of the ether bond and phytanyl chains did not significantly affect these permeabilities. However, the apparent proton permeability was reduced 3-fold by the presence of an ether bond. The presence of macrocyclic archaeol and caldarchaeol structures further reduced apparent proton permeabilities by 10–17-fold. These results indicate that the limiting mobility of the midplane hydrocarbon region of the membranes formed by macrocyclic archaeol and caldarchaeol lipids plays a significant role in reducing the permeability properties of the lipid membrane. In addition, it appears that substituting ether for ester bonds presents an additional barrier to proton flux.

Many archaeabacteria thrive in hostile environments, such as hot springs, salt lakes, and acidic or alkaline domains (1, 2). For instance, *Methanococcus jannaschii* grows optimally at 85 °C, pH 6 (3, 4), *Thermoplasma acidophilum* at 55 °C, pH 2.0 (5), and *Halobacterium salinarum* in near-saturated salt brines (2). The major role of the cell membrane is to provide a selective barrier between the external environment and the inside of the cell. Given the extreme environmental conditions in which these bacteria thrive, it is not surprising that their plasma membranes are composed of lipids that differ markedly in structure and physicochemical properties from the glycerolipids of eubacterial, animal, and plant cell membranes. In these unique bacteria, the membrane lipids are characterized by the presence of ether linkages instead of ester linkages, and they contain regularly branched phytanyl and diphytanyl chains instead of fatty acyl chains (6). The presence of ether rather than ester bonds is thought to contribute to greater chemical stability at extreme pH. Moreover the glycerol ethers contain an sn-2,3 stereochemistry that is opposite that of the naturally occurring sn-1,2 stereochemistry of glycerophospholipids of the other domains (7). The basic lipid core structures of these unique organisms are summarized in Fig. 1 (6, 7).

There are two major classes of archaeabacterial lipids, the archaeol lipids (diphytanyl glycerol diethers) and the caldarchaeol lipids (dibiphytanyl diglycerol tetraethers).1 The caldarchaeol lipids span the membrane, and liposomes made from these lipids form a monolayer as opposed to the bilayer formed with conventional glycerophospholipids (8, 9). The majority of the tetraether lipids are phosphoglycolipids containing one or more sugar residues on one pole, most commonly glucose, mannose, or galactose, and a phosphopolyol moiety, such as phosphoglycerol, or inositol on the other. The more bulky sugar residue(s) may be expected to face outward, and the phosphate residue may be expected to face toward the cytoplasmic side of the membrane (10). Depending on the growth temperature, certain thermophilic archaea are capable of controlling membrane fluidity by altering the number of cyclopentane rings from 0 to 8 in the caldarchaeol lipid chains (11). The macrocyclic archaeol lipid has so far been found only in *M. jannaschii* (6) and *Methanococcus igneus* (12).

The transport of small molecules across lipid bilayers is a fundamental biological process. Most of the biologically important transport of ions and bulky molecules with very low permeability across the lipid component of the membrane occurs through proteins. Small, uncharged molecules (e.g. water, ammonia, urea, and glycerol), however, permeate across the lipid component of the membrane at an appreciable rate. The archaeabacterial lipid membranes have been shown to exhibit low permeability to protons and 5,6-carboxyfluorescein (8). Indeed, the caldarchaeol lipids of *T. acidophilum* form liposomes that retain carboxyfluorescein even during brief autoclaving at 121 °C (13). Elferink et al. (8) showed that in liposomes made from caldarchaeol lipids of *Sulfobolus acidocaldarius*, which has an optimal growth temperature of 85 °C at pH 2.0, there is remarkable thermal and mechanical stability. Also, at temperatures below 40 °C, proton permeability was barely detectable.

1 Archaeobacterial lipids were designated using the nomenclature proposed for core lipid moieties (49); here, An refers to 2,3-di-0-phytanyl-sn-glycerol, and caldarchaeol refers to 2,2',3,3'-tetra-0-dibiphytanyl-sn-diglycerol. Variations in cores herein are shown as AnM and Cp (cyclopentane rings) designated from 0 to 8.
phatic chains (Escherichia coli lipids versus Dph-PC); 2) the ester versus the other linkages between the aliphatic chains and the glycerol backbone of the lipid (Dph-PC versus archaeol); and 3) the effects of limiting the mobility of the distal ends of the aliphatic chains (archaeol versus AM and Cp lipids).

These studies permit us to define how archaeobacteria can survive in hostile environments and the role of different components of lipid structure in impeding the flux of small molecules. These results provide novel insights into the mechanisms of permeation of biological membranes.

**EXPERIMENTAL PROCEDURES**

**Archeal Strains and Growth**—*H. salinarum* (ATCC 33170) was grown aerobically as described in Ref. 15, *Methanobrevibacter smithii* ALI (DSM2375) was grown anaerobically at 35 °C (16), *M. jannaschii* (DSM 2681) was grown anaerobically at 65 °C (17), and *T. acidophilum* 122–1B3 (ATCC 27658) was grown aerobically at 55 °C, pH 2.0 (18).

**Lipid Composition, Extraction of Lipids, and Preparation of Liposomes**—The bacteria were used as follows: *H. salinarum*, 100% archaeols (A); *M. smithii*, 60% archaeols and 40% caldarchaeols (A + C); *T. acidophilum*, 90% caldarchaeols and 10% archaeols (Cp + A); and *M. jannaschii*, 43% macrocyclic archaeols, 42% caldarchaeols, and 15% archaeols (A + C + AM). Following growth at optimal conditions (17), lipids were extracted from frozen-thawed cell paste by the Bligh and Dyer method, and total polar lipids were obtained by acetone precipitation as described earlier (18). All archaeal polar lipid extracts were analyzed by negative-ion fast atom bombardment mass spectrometry, and the analysis of ions obtained was consistent with that reported earlier (17). A comparison of n/z values of all polar lipids detected from batch to batch indicated that similar proportions of core lipids were present in each lipid mixture extracted from fresh biomass (6). 3–4 mg of lipids were dissolved in chloroform-methanol (2:1) and dried at 40 °C under a stream of nitrogen. Residual traces of solvent were removed by placing the lipids in an evacuated chamber for at least 3 h followed by bath sonication in a buffer containing 15 mM CF, 155 mM KCl, and 10 mM MOPS, pH 7.2. Caldarchaeol lipids from *T. acidophilum* and macrocyclic archaeol-rich lipids from *M. jannaschii* were bath-sonicated for 16–20 min, whereas the lipids from other archaea and commercial lipids were sonicated for 4–6 min. The liposomal suspension was left overnight and extruded through a 0.2-μm polycarbonate filter (20 passes) using the Avanti-mini extruder assembly (Avanti Polar Lipids Inc., Alabaster, AL). The un-entrapped CF was removed by passing the liposome suspension over a Sephadex PD-10 column. Liposomes were sized by quasi-elastic light scattering using a DynaPro LSR particle size analyzer. All lipid preparations behaved as homogeneous population and showed a mean diameter of 161 ± 22, 164 ± 26, 156 ± 16, 168 ± 14, 174 ± 27, and 189 ± 30 nm (n = 3) for *E. coli*, Dph-PC, A, A + C, AM, Cp, and Cp + A liposomes, respectively.

**Water Permeability Measurements**—Osmotic water permeability (Pw) was measured at 25 °C as described (19–21). All other permeability measurements were measured at 25 °C also. Liposomes containing 15 mM CF were abruptly exposed to a doubling of external osmolarity in a stopped-flow fluorometer (SF.17 MV, Applied Photophysics, Leatherhead, United Kingdom) with a measurement dead time of less than 1 ms. The rate of water efflux from liposomes following a sudden increase in osmolarity is given by Eq. (1).

\[ \frac{dV(t)}{dt} = \frac{P_w}{MW}(C_{in} - C_{out}) \]  

where \( V(t) \) is the relative volume of the liposomes at time \( t \), \( SV \) is the surface area to volume ratio, \( MW \) is the molar volume of water (18 cm³/mol), and \( C_{in} \) and \( C_{out} \) are the initial concentrations of solute inside and outside the liposomes, respectively. Using parameters, which included the rate constant, vesicle diameter, and applied osmotic gradient, \( P_w \) was calculated using MathCad software (MathSoft Inc., Cambridge, MA) as described earlier (22).

**Solute Permeability Measurements**—Permeability measurements were performed using an osmotic pressuremeter (MathSoft Inc., Cambridge, MA) as described earlier (22).

**Permeability Measurements**—Permeability measurements were performed using a stopped-flow fluorometer (21–24). Briefly, liposomes were equilibrated in buffer (500 mosmol/kg) containing 200 mM solute (glycerol or urea) for 2 h at room temperature. In the stopped-flow device, liposomes were rapidly mixed with an equal volume of a solution with identical osmolality containing 100 mM solute. The concentration gradient results in solute efflux from liposomes followed by water efflux. Vesicle shrinkage can be monitored due to CF

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2The abbreviations used are: CF, 5,6-carboxyfluorescein; Dph-PC, diphytanoylphosphatidylcholine; \( P_w \), coefficient of osmotic water permeability; \( A_o \), standard archaeol; \( AM \), macrocyclic archaeol; \( CP \), caldarchaeol with cyclopentane ring; MOPS, (3-N-morpholino)propanesulfonic acid.
self-quenching. By use of parameters from the single exponential curve fit to the data, \( P_{\text{solvent}} \) was solved using MathCad software as described earlier (22, 23). Osmalalities of all solutions were confirmed and adjusted, if necessary, by measuring freezing point depression on a Precision Instruments Osmette A osmometer.

**Proton Permeability**—Apparent proton permeabilities were measured using pH-dependent quenching of CF fluorescence as described (23–26). Stopped-flow experiments were performed in which the liposomes were pretreated with 1 mM valinomycin and then rapidly mixed with an identical buffer acidified to pH 6.50. Valinomycin, which was used to collapse any potential difference arising as a result of proton influx, did not appear to be necessary, as permeability measurements performed in its absence did not alter the results. Buffer capacity was determined on an SLM-Aminco 500C spectrofluorometer by adding 10 mM acetate (final concentration) to liposomes as described (21). Fluorescence data from the stopped-flow device were fit to a single exponential curve, and fitting parameters were used to solve the following equation for \( P_{\text{H}^+} \),

\[
J_{\text{H}^+} = (P_{\text{H}^+})(S_\Delta)(\Delta C) = (\Delta pH)/t(BCV)
\]

where \( J_{\text{H}^+} \) is the flux of protons, \( \Delta C \) is the initial difference in concentration of protons between the inside and the outside of the vesicle, \( \Delta pH \) is the change in pH when time equals \( t \) (the time constant of the single exponential curve describing the initial change in fluorescence as a function of time), and BCV is the buffer capacity of an individual vesicle.

**NH₃ Permeability**—NH₃ permeability was determined using stopped-flow fluorometry by monitoring the pH-sensitive increase in CF fluorescence when vesicles equilibrated to pH 6.8 were rapidly mixed. Fig. 2B shows that the apparent proton permeability, as seen in liposomes composed of macrocyclic archaeol and caldarchaeol lipids, showed reduced water permeability, 0.87 ± 0.040 × 10⁻³ cm/s, compared with the prior lipids. Similarly, liposomes made of caldarchaeol lipids (90% C₄) from *T. acidophilum* also showed a marked reduction in water permeability, 0.65 ± 0.06 × 10⁻³ cm/s compared with the other lipids. *E. coli* lipids showed slightly higher permeability, and lipids of *M. smithii* (60% archaeol and 40% caldarchaeol) showed an intermediate permeability.

**Statistics**—The program SigmaStat (Jandel Corp., Corte Madera, CA) was used for Bonferroni t-test, which allows for multiple comparisons. Differences in permeability values were considered significant when \( p < 0.05 \) was obtained.

**RESULTS**

**Osmotic Water Permeability (Pf)**—Fig. 2A shows representative averaged fluorescence tracings observed as liposomes shrank following their abrupt exposure to a doubling of external osmolality. For each curve, averaged data and fitted single exponential curves are shown. Fig. 2B compiles the averages from determinations taken from several different preparations of liposomes to give mean ± S.E. permeability values for each type of lipid as labeled. For the purposes of comparison, permeabilities of extracted lipids from *E. coli* were also measured. Liposomes composed of *E. coli* polar lipids (4.9 ± 0.16 × 10⁻³ cm/s), Dph-PC (4.29 ± 0.30 × 10⁻³ cm/s), and polar lipids from *H. salinarum* composed entirely of archaeol lipids (3.88 ± 0.73 × 10⁻³ cm/s) showed comparable water permeabilities (Fig. 2B). On the basis of these results, it appears that the shift from an ester to an ether linkage or the presence of methyl groups (Dph-PC versus *E. coli* lipids) does not have a major effect on the water permeability. Liposomes made of total polar lipids of *M. jannaschii*, which is mainly composed of macrocyclic archaeol and caldarchaeol lipids, showed reduced water permeability, 0.87 ± 0.040 × 10⁻³ cm/s, compared with the prior lipids. Similarly, liposomes made of caldarchaeol lipids (90% C₄) from *T. acidophilum* also showed a marked reduction in water permeability, 0.65 ± 0.06 × 10⁻³ cm/s compared with the other lipids. *E. coli* lipids showed slightly higher permeability, and lipids of *M. smithii* (60% archaeol and 40% caldarchaeol) showed an intermediate permeability.

**Temperature dependence of water permeability**—Fig. 3. A plot of natural log of rate constant (k) of water permeability versus the reciprocal of absolute temperature, T. The slope multiplied by gas constant R (1.98 cal/K-mol) gives the activation energy (see Table I). a, Dph-PC; b, *E. coli* lipids; c, archaeol lipids (*H. salinarum*); d, macrocyclic archaeol-rich lipids (*M. jannaschii*); e, caldarchaeol lipids (*T. acidophilum*).

**Solute Permeability**—Liposomes were preloaded with 200 mM glycerol or urea as a permeant solute, and the efflux of the solute under isosmotic conditions was measured by a decrease in CF fluorescence. Figs. 4A and 5A show that the efflux of glycerol and urea was relatively rapid in liposomes composed of *E. coli* phospholipids, Dph-PC, and archaeols, whereas the flux was extremely slow in liposomes composed of macrocyclic archaeol and/or caldarchaeol lipids. Permeability to both glycerol and urea was comparable for liposomes composed of Dph-PC or archaeols, and liposomes made of *E. coli* lipids showed a slightly more enhanced permeability. A drastic reduction in permeability was seen in liposomes made of macrocyclic archaeol and/or caldarchaeol lipids (Figs. 4B and 5B). Urea permeability was reduced by more than 70-fold compared with Dph-PC liposomes, and glycerol permeability was reduced by ~120-fold.

**Apparent Proton Permeability**—Fig. 6A shows the internal acidification of liposomes after exposure to an acidic external buffer as monitored by entrapped CF. The permeability of archaeal liposomes (1.6 ± 0.46 × 10⁻⁴ cm/s) was approximately one-third that of Dph-PC (5.1 ± 1.3 × 10⁻⁴ cm/s), as seen in Fig. 6B. The presence of caldarchaeol lipids further decreased the apparent proton permeability, as seen in liposomes composed of 60% archaeols and 40% caldarchaeols (0.41 ± 0.12 × 10⁻⁴ cm/s) and 90% caldarchaeol lipids (0.47 ± 0.10 × 10⁻⁴ cm/s).
Table I

| Lipids                          | Activation energy (Kcal/mol) | Estimated permeability ($P_f$) cm/s |
|---------------------------------|-----------------------------|-----------------------------------|
| Dph-PC                          | 12.1                        | 0.060 at 60 °C                     |
| E. coli lipids                  | 12.2                        | 0.085 at 60 °C                     |
| $A_{12}$ (H. salinarum)         | 13.5                        | 0.061 at 60 °C                     |
| $A_{13.5}$ + $A_{15.6}$ (M. jannaschii) | 15.6          | 0.020 at 65 °C                     |
| $C_0$ (T. acidophilum)          | 15.6                        | 0.014 at 59 °C                     |

* Extrapolated permeability at their optimal growth temperature assuming that the $A_{12}$ and caldarchaeol lipids (no cyclopentane rings, $C_0$) mainly constitute the permeability barrier for water in these archaea.

Ammonia Permeability—Upon entry into the liposome, gaseous ammonia is protonated and increases the internal pH, which is measured as an increase in fluorescence of entrapped ammonia. Ammonia permeability was measured as an increase in fluorescence of entrapped ammonia due to entry into the liposome. The increase in pH due to the entry of ammonia causes a significant decrease in the permeability barrier for water in these archaea.

Glycerol permeability of liposomes. A time course of glycerol efflux under isoosmotic conditions from liposomes. Traces a–f are as described (Fig. 2A, B, coefficient of glycerol permeability, calculated as described under “Experimental Procedures.”

Ammonia permeability of liposomes. NH3 entry into liposomes causes an increase in pH, the time course of ammonia entry as described under “Experimental Procedures.”

Ammonia permeability of liposomes composed of various lipids. Fig. 7B shows that ammonia permeability of liposomes composed of E. coli lipids (20 ± 1.0 × 10−2 cm/s), Dph-PC (24 ± 3.0 × 10−2 cm/s), and archaeol (23 ± 8.4 × 10−2 cm/s) was comparable. A marked reduction in ammonia permeability was observed in liposomes composed of 60% archaeol and 40% caldarchaeol (3.1 ± 1.3 × 10−2 cm/s), macrocyclic archaeol lipids (3.8 ± 0.8 × 10−2 cm/s), and caldarchaeol lipids (2.4 ± 0.9 × 10−2 cm/s). There was an ~6–10-fold reduction in ammonia permeability of macrocyclic archaeol and caldarchaeol lipids compared with that of Dph-PC.

Discussion

Water permeability and solute permeability across various membranes and model systems have been measured (14, 19, 23, 27), but the molecular mechanisms of its permeation are not well understood. The unique structural features of archaeobacterial lipids allowed us to test the effects of presence of ester bond compared with ether bond, phytanyl chains compared with acyl chains, and the affects of restricted mobility of the phytanyl chain in macrocyclic lipids (AM and CP). Our results show that the water permeability is not affected by the presence of an ether bond instead of ester bond or isoprenoid chain instead of acyl chain, as E. coli lipids, Dph-PC, and archaeol lipids exhibited similar permeabilities (Fig. 2B). Permeability studies of model bilayer systems indicate that the region of the acyl chain adjacent to the headgroup is the site likely to offer the most resistance for water and solute permeation (19, 28, 29). The midplane region of the bilayer formed by acyl chains farthest from the headgroup exhibit higher mobility (fluidity) and is thought to offer a reduced resistance to permeation. However, in macrocyclic archaeol lipids, in which the mobility of the distal aliphatic chain might be restricted due to linkage of terminal carbon atoms within the same lipid molecule (Fig. 1), the water permeability was reduced by ~5-fold (Fig. 2B). Similar results (~6.5-fold reduction) were also seen in monolayer liposomes of caldarchaeol lipids with restricted midplane mobility (Figs. 1 and 2B).

Water diffusion is satisfactorily described by the “mobile kink” hypothesis, which assumes that the rapidly diffusing small pockets of free volume carry the permeant across the membrane. This hypothesis requires the formation of gauche-trans-gauche kinks and their propagation by fast lateral diffusion of lipids (30). A large reduction in water permeability was shown in DPPC lipids in gel state compared with liquid crystalline state (26, 31–33) and is thought to be due to reduced number of gauche conformers in gel state. It has also been shown that rigidification of the outer leaflet of dipalmitoylphosphatidylcholine liposomes with the rare earth metal praseodymium causes a significant reduction of water and solute per-
meabilities (26). The lateral diffusion of the main polar caldarchaeol lipid of *T. acidophilum* at 30 °C, 5 × 10⁻⁹ cm²/s is 2 orders of magnitude lower than that of phospholipids in liquid crystalline phase, which are typically around 10⁻⁷ cm²/s (34). Recent fluorescence studies in giant liposomes formed from caldarchaeol lipids of thermoacidophilic archaeabacterium *S. acidocaldarius* also showed that the lipids are rigid and tightly packed (35). It is difficult to imagine the formation of g-t-g kinks in bipytanyl chains of a tetraether lipid that is tethered at both ends and that contains branched methyl groups and cyclopentane rings. These results, taken together with our permeability data, suggest that the reduced water permeability might be due to the low probability of occurrence of a rapidly diffusing kink given the tight lipid packing of caldarchaeol lipids.

Water permeability shows strong temperature dependence in model membrane systems and cells lacking water channels (31). Because the optimal growth temperatures for *T. acidophilum* and *M. jannaschii* are in the range of 55–79 °C, the water permeability was calculated for caldarchaeol and macrocyclic archaeal-rich lipids at optimal growth temperatures, by extrapolation of the measured permeability data of Fig. 3. The calculated *Pₚ* values were found to be in the range of 0.014–0.030 cm/s (see Table I). These values are remarkably close to that reported for native cell membranes expressing water channels such as erythrocytes (0.022 cm/s) and AQP2 containing endosomes (0.016 cm/s) (36, 37). A BLAST search of archaeabacterial genomic data base revealed the presence of putative water channels (0.016 cm/s) (36, 37). Because the optimal growth temperatures for *M. jannaschii* and *E. coli* are in the range of 55–79 °C, the water permeability might be due to the low probability of occurrence of a rapidly diffusing kink given the tight lipid packing of caldarchaeol lipids.

Solute permeability across macrocyclic archaeal and caldarchaeol lipids was also markedly reduced, by 70–120-fold for urea and glycerol compared with Dph-PC (Figs. 4B and 5B). A significant reduction in urea and glycerol permeability was also shown in phosphatidylcholine liposomes containing sphingomyelin and cholesterol (23). It is known that permeabilities of biological membranes and model lipid bilayers depend strongly on the degree of packing of lipid chains in the membrane (19, 38) and the size of the permeating solute (39, 40). Membranes that are highly ordered show very low permeability and exhibit a steep dependence on size of the solute (23, 38, 41). The gas ammonia is known to rapidly diffuse across cell membranes.

Rigidification of the outer leaflet of the bilayer was shown to cause a significant decrease in ammonia permeability (26). Our results (Fig. 6B) suggest that the rigid packing of lipids causes a significant reduction in ammonia permeability of ~6–10-fold in liposomes made of either macrocyclic archaeal lipids or caldarchaeol lipids. The observation that the fold reduction of ammonia permeability is similar to that of water (~6–8-fold) suggests that the rate-limiting steps for permeation of both water and ammonia might be similar.

Apparent proton permeabilities have been studied in various model systems, and yet the mechanisms of permeation are not well understood. The proton permeability was only weakly influenced by fluidity of the bilayer (19, 42). By contrast, rigidification of the outer leaflet of the dipalmitoylphosphatidylcholine liposomes by the rare earth metal praseodymium led to a significant decrease in apparent proton permeability (26). These apparently anomalous results suggest that proton flux occurs by a mechanism distinct from that of water and solute. The apparent proton permeability data in Fig. 6B show that unlike water and solute permeability, a ~3-fold reduction in permeability is seen in archaeal liposomes compared with Dph-PC. It has been suggested that protons move as hydrogen-bonded clusters of water molecules (water wires) dissolved in the hydrophobic core of the membrane (43). We speculate that the presence of an ether bond, the oxygen of which lacks a lone pair of electrons, might disrupt the hydrogen-bonding network, leading to a barrier for proton diffusion in that region. Low proton permeability and sodium permeability at high salt concentrations were also shown in liposomes made of archaeal lipids from the extreme halophile *H. salinarum* and haloalkalophile *Halorubrum vacuolatum* (44). The results in Fig. 6B show a further reduction in proton permeability of 10–17-fold in macrocyclic archaeal and caldarchaeol lipids compared with Dph-PC lipids. Caldarchaeol lipids from thermoacidophilic archaeon *S. acidocaldarius* showed reduced proton permeability compared with archaeol lipids from the mesophilic *E. coli* or thermophilic *Bacillus stearothermophilus* (8). Komatsu *et al.* (14) reported proton permeability values in the range of 10⁻⁸ cm/s for caldarchaeol liposomes composed of polar lipid fraction E from *S. acidocaldarius*. Various proton permeability values have been reported in the literature from 10⁻⁴ to 10⁻⁶ cm/s for proton permeability based on the experimental conditions chosen. Small pH gradient leads to permeability values in the range of 10⁻⁴ cm/s (45), and a large pH gradient leads to permeability values in the range of 10⁻⁶ cm/s. These extremely low permeability coefficients have been hypothesized to occur as a result of formation of diffusion potentials (46). The apparent proton permeability measurement in this study employed well established methods using a small pH gradient of 0.5 pH unit (19, 26, 47). The presence of sugar headgroups on these lipids may have an effect on solute and ion permeability, but the magnitude of the effects observed cannot be explained merely by the presence of sugar groups. Negative membrane surface charge was shown not be a factor for proton permeation in egg phosphatidylglycerol liposomes (14). Our proton permeability data are consistent with a tightly packed bilayer, which could reduce the occurrence of proton wires and thereby further decrease the proton permeability. However, the mechanism of proton permeation is not clearly understood. The low proton permeability and ion impermeability of the membrane are important features in the bioenergetics of the archaeabacteria, considering that ATP synthesis is driven by proton/ion gradients (48) that need to be maintained at extremes of external pH conditions.

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