Voltage-dependent Porin-like Ion Channels in the Archaeon *Haloferax volcanii*  

Madeleine Besnard, Boris Martinac‡, and Alexandre Ghazi§  

From the Laboratoire des Biomembranes, URA CNRS 1116, Bât. 430, Université Paris-Sud, 91405 Orsay, France and the ‡Department of Pharmacology, University of Western Australia, Nedlands WA 6007, Australia  

Membrane vesicles isolated from the cell envelope of the archaeabacterium *Haloferax volcanii* were either reconstituted in giant liposomes and examined by the patch-clamp technique or were fused into planar lipid bilayers. In addition, cell envelope proteins were solubilized by detergent and reconstituted in azolectin liposomes, which were then fused into planar lipid bilayers. Independently of the technique used the predominant channel activity encountered exhibited the following characteristics. Channels were open at all voltages in the range approximately −120 to +120 mV and exhibited frequent fast transitions to closed levels of different amplitudes. At voltages greater than 120 mV the channels tended to close in a manner characterized by large, slow transitions of variable amplitudes. The tendency to close at high membrane potentials was much stronger at one polarity. The channel gating pattern was complex exhibiting a range of subconductances of 10–300 picosiemens in symmetric 100 mM KCl. These electrophysiological characteristics are comparable with those of bacterial and mitochondrial porins, suggesting that the archaean channels may belong to the general class of porin channels. Some channels showed preference for K⁺, whereas the others preferred Cl⁻, suggesting the existence of at least two types of porin-like channels in *H. volcanii*.

The phylogenetic tree is composed of three domains: Eucarya (eukaryotes), Bacteria (eubacteria), and Archaea (archaeabacteria) (1). Archaeabacteria comprises several different families of cells adapted to extreme environmental conditions (e.g. temperature, pH, salt concentration). Although eubacteria and archaeabacteria are both prokaryotes, it is now generally accepted that archaeabacteria are not closer, phylogenetically, either to eubacteria or to eukaryotes (2). Therefore, archaeabacteria appear to constitute an intermediary domain between eubacteria and eukaryotes.

Ion channels have been mostly studied in eukaryotic cells (3) and have also been documented in eubacteria. In particular bacterial porins and mechanosensitive ion channels are well described. Porins, large water-filled pores across the outer membrane of Gram-negative bacteria (reviewed in Ref. 4), have also been found in the cell wall of certain Gram-positive bacteria such as mycobacteria (5–7). More recently, different mechanosensitive ion channels have been described in Gram-negative and Gram-positive bacteria (8–17). These channels, most likely localized in the plasma membrane, are implicated in osmoregulation (13).

In contrast, the presence of ion channels in archaeabacteria has not been documented. A search for ion channels in Archaea thus seems to be of interest not only for the understanding of the phylogeny of ion channels, but also for a better knowledge of the physiology of these unique organisms. For our studies we chose *Haloferax volcanii* (formerly *Halobacterium volcanii*), a moderate halophilic archaean amenable to genetic studies.

We report here the existence of ion channels in the cell envelope of *H. volcanii*, that with regard to electrophysiological characteristics resemble bacterial porins. This finding indicates the importance of the porin family in the phylogeny of ion channels. Furthermore, it questions our current knowledge of the organization of the cell envelope of archaeabacteria.

**EXPERIMENTAL PROCEDURES**

**Cell Growth—** *H. volcanii* (strain NCMB 2012) cells were grown in a medium containing (in mM) NaCl (3550), MgCl₂ (170), MgSO₄ (200), CaCl₂ (6), KCl (26), NaHCO₃ (6.6), NaBr (5.4) plus 5 g of yeast extract/liter. In some cases the medium was supplemented with MnCl₂ (2.9), ZnSO₄ (1.6), CuSO₄ (0.2), FeSO₄ (1.5) plus 5 g of tryptone/liter.

**Isolation of the Cell Envelope—** One liter of the suspension was centrifuged at 10,000 × g to eliminate cell debris. The supernatant was centrifuged for 1 h at 45,000 rpm, using a Ti45 Beckman rotor. The pellet was then resuspended in 10 mM Hepes-KOH, pH 7.4, plus 5% ethylene glycol. The resulting suspension was centrifuged at 10,000 × g to eliminate cell debris. The supernatant was centrifuged for 1 h at 45,000 rpm, using a Ti45 Beckman rotor. The pellet was then resuspended in 10 mM Hepes-NaOH, pH 7.4, plus 5% ethylene glycol. Protein content was measured by the method of Lowry et al. (18). 50-μl aliquots (at 2–5 mg of protein/ml) were stored at −80 °C for further use.

**Preparation of Giant Proteoliposomes—** Cell envelopes were mixed with azolectin (from soybean, type II-S, Sigma) liposomes at a lipid to protein ratio (w/w) of 50 and fused into giant proteoliposomes by dehydration-rehydration as described previously (11). Rehydration was performed in 100 mM KCl, 10 mM Hepes-KOH, pH 7.4.

**Solubilization and Reconstitution of Membrane Proteins into Proteoliposomes—** Membrane proteins from cell envelopes were solubilized using octyl β-D-glucoside; 100 μl of the cell envelope suspension was mixed with 900 μl of the solubilization buffer (10 mM Hepes-KOH, pH 7.4, 300 mM KCl, 1 mM dithiothreitol, 100 mM octyl β-D-glucoside) and incubated for 20 min. The suspension was then centrifuged at 90,000 rpm for 40 min using a TL100 Beckman ultracentrifuge. The supernatant was added to a 1-ml suspension of azolectin liposomes (1 mg of lipids/ml) in 10 mM Hepes-KOH, pH 7.4, 300 mM KCl and incubated for 20 min before the addition of Bio-Beads SM-2 (Bio-Rad) (160 mg/ml). The suspension was gently agitated for 5 h, the Bio-Beads were discarded, and the suspension was centrifuged for 30 min at 90,000 rpm, using a TL100 Beckman ultracentrifuge. The pellet was then resuspended in 200 μl of 300 mM KCl, 10 mM Hepes-KOH, pH 7.4.

**Electrophysiological Recording—** Giant proteoliposomes were examined for channel activity using the standard patch-clamp method (19). 2 μl of the giant proteoliposomes suspension was added to the patch-clamp chamber and covered with 2 ml of 100 mM KCl, 10 mM Hepes-KOH, pH 7.4. Patch electrodes were pulled from pyrex capillaries (Corning code 7740) and were not fire-polished. They were filled with 10 mV.
Porin-like Ion Channels in H. volcanii

RESULTS

Several attempts were made to record channel activities from native membranes of giant protoplasts of H. volcanii. Despite a concentrated effort to make the surface of the giant protoplasts amenable to patch-clamp recording, we were unable to form gigaohm-seal on their surface. Therefore, we decided to record ion channel activities by either reconstituting H. volcanii cell envelope in artificial liposomes or into planar bilayers.

The archaeal membrane vesicles were fused into giant liposomes by dehydration and rehydration (10, 11), and the giant liposomes were then examined by the patch-clamp technique. In parallel experiments the membrane vesicles were fused into planar lipid bilayers. In addition, cell envelope membrane proteins were solubilized using octyl β-D-glucoside, reconstituted in liposomes that were then fused into planar lipid bilayers. Independently of the technique used the predominant channel activity encountered exhibited the characteristics described below.

Channels were mostly open at all voltages in the range −120 mV to +120 mV and exhibited frequent fast transitions to closed levels of different amplitudes (data not shown). At voltages greater than 120 mV the channels tended to close in a manner characterized by large, slow (seconds) transitions (Figs. 1 and 2). The greater the magnitude of the membrane potential, the shorter the open-time duration following the voltage step. Sporadic fast closures were often superimposed on the slow kinetic closures. For some patches the channels also entered a sustained fast gating mode (millisecond) as shown in Fig. 1.

The tendency to close at high membrane potentials was greater at one polarity (positive potential for bilayer experiments and negative potential for patch-clamp experiments), indicating both a preferential insertion of the channels in the lipid bilayer and an asymmetry in the voltage dependence.

Figs. 1 and 2 show recordings from the same bilayer at positive and negative voltages. Upon application of +180 mV across the bilayer the channels reached a closed inactivated state (Fig. 1). At negative potential, full closure of the channels could not be reached, and the time course of closure was much slower (Fig. 2). In most cases the inactivated state was reversible and the channels reopened by returning to 0 mV for several seconds (Fig. 1). However, in a few instances, the channels appeared to be locked in the inactivated state, and reopening could not be obtained at any membrane potential.

In general the channel gating was complex, showing a range of conductances in between 10 and 300 picoSiemens in symmetric 100 mM KCl. While this could be attributed in part to a mixture of different channels (see below), in some cases clear cooperative events were observed, indicating that the channels can gate at different conductance states. This is illustrated in Fig. 3, which shows recordings obtained by two successive steps to −160 mV on the same patch. The most frequent transition had a conductance of 80 picoSiemens, but a clear cooperative 160-picoSiemens transition was also observed. Furthermore, channel gating at a subconductance level of approximately 10 picoSiemens was observed.

The selectivity of the channels was examined in bilayer experiments performed in asymmetric (500/100 mM KCl) and symmetric solution (500 mM KCl). I-V curves for unitary currents were difficult to obtain because of the different conductances encountered at each membrane potential. Instead, we
chose to plot the total initial current through a given bilayer obtained during steps to various values of the membrane potential. Between steps the membrane potential was returned to 0 mV to ensure proper reopening of the channels. The current thus corresponded to that flowing through all the fully open channels present in a bilayer. In a series of nine different bilayer experiments, the following reversal potentials (in millivolts) were obtained: 124, 120, 120, 112, 24, 28, 214, 218, 223. The two extreme cases are shown in Fig. 4. These results suggest that at least two different types of porin-like channels with opposite selectivity are present in *H. volcanii* envelopes.

**DISCUSSION**

We report here for the first time the existence of ion channels found in the cell envelope of an archaebacterium. The electrophysiological characteristics of these channels, documented by two different techniques, can be summarized as follows: 1) the channels that have large conductances are maximally open at or around 0 mV and close at positive and negative voltages, 2) voltage dependence is asymmetric, 3) the channels exhibit both fast and slow kinetics, and 4) the channels exhibit several subconducting states. The same electrophysiological characteristics can be found in eubacterial porins as documented for OmpF and OmpC (20) and PhoE from *Escherichia coli* \(^1\) and also in mitochondrial porins (reviewed in Ref. 21). This suggests that all these channels may belong to the same family of

\(^1\) Berrier, C., Besnard, M., and Ghazi, A. (1997) *J. Membr. Biol.*, in press.
Corynebacterium glutamicum would be facilitated by porins. Mycobacterial cell wall (24). Diffusion through this bilayer of another bilayer (23), explaining the low permeability of the and it is possible that mycolic acids and other lipids form part to contain lipids in their cell wall in the form of mycolic acids, fied in the cell wall of Gram-positive bacteria such as nels. However, porin-like channels have been recently identi- Gram-positive bacteria were long supposed to lack porin chan- 167–196. Berrier, C., Besnard, M., Ajouz, B., Coulombe, A., and Ghazi, A. (1996) Eur. J. Biochem. 256, 559–565. Suhharnev, S. I., Blount, P., Martinac, B., Blattner, F. R., and Kurup, C. (1993) Biophys. J. 65, 1–7. Szabo, I., Petrovili, V., and Zoratti, M. (1993) Biochim. Biophys. Acta 1112, 29–38. Berrier, C., Coulobe, C., Szabo, I., Zoratti, M., and Ghazi, A. (1992) Eur. J. Biochem. 206, 559–565. Suhharnev, S. I., Martinac, B., Arshavsky, V. Y., and Kurup, C. (1993) Biophys. J. 65, 1–7. nase of excitable membranes, 2nd Ed., Sinauer Associates, Inc., Sunderland, MA. 6. Trias, J., and Benz, R. (1993) J. Membr. Biol. 151, 175–187. Lowry, D. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275. Hamill, O. P., Marty, A., Neher, E., Sakmann, B., and Sigworth, F. J. (1981) Pfluegers Arch. 391, 85–100. Berrier, C., Coulobe, A., Houssin, C., and Ghazi, A. (1992) FEBS Lett. 306, 251–256. Benzi, R. (1994) Biochim. Biophys. Acta 1197, 167–196. Niederweis, M., Maier, E., Lichtinger, T., Benz, R., and Kramer, R. (1995) J. Bacteriol. 177, 5716–5718. Nikaido, H., Kim, S. H., and Rosenberg, E. Y. (1993) Mol. Microbiol. 8, 253–2510. Kessel, M., Widhaber, I., Cohen, S., and Baumeister, W. (1988) EMBO J. 7, 1549–1554. Blaurock, A., Stoeckenius, W., Oesterhelt, D., and Scherphof, G. (1976) Cell Biol. 71, 1–22. Sumper, M., Berg, E., Megele, R., and Strobel, I. (1990) J. Bacteriol. 172, 711–7118. Lechner, J., and Sumper, M. (1987) J. Biol. Chem. 262, 9724–9729. Brennan, P. J., and Nikaido, H. (1995) Annu. Rev. Biochem. 64, 29–63.