Volume 240, number 1,2, 105-109 FEB 06517 November 1988

Ion-conducting channels in a Gram-positive bacterium

Mario Zoratti and Valeria Petronilli

CNR Unit for the Physiology of Mitochondria, Institute of General Pathology, Via Loredan 16, Padova, Italy

Received 26 September 1988

The patch-clamp technique was used to obtain information on the existence and properties of ion channels in giant protoplasts obtained from the Gram-positive bacterium *Streptococcus faecalis*. The membrane proved to contain a pore with numerous conductance states, ranging from 10 pS to several nanosiemens. Application of a slight pressure differential across the membrane resulted in the activation of the channel. The pressure sensitivity points to a relationship between this channel and one recently discovered in *E.coli* spheroplasts [(1987) Proc. Natl. Acad. Sci. USA 84, 2297–2301] suggesting that pores of this type might be widespread among prokaryotes.

Ion channel; Patch clamp; Pressure activation; Conductance substate; (Gram-positive bacteria, *Streptococcus faecalis*)

1. INTRODUCTION

Ion-conducting channels play a role of some importance in bacterial physiology: channels appear to be involved in phage infection [1]; the outer membrane of Gram-negative bacteria contains porins [2], which presumably act as general-purpose filters; some prokaryotes produce and excrete pore-forming toxins [3]. A considerable body of information on the latter two classes of proteins has been obtained by studies generally involving their isolation and reconstitution. Much less is known about any ion-conducting channels that might exist in the cytoplasmic membrane of prokaryotes, where they might be involved in the entry of molecules such as glycerol [4] and antibiotics [5] and in conferring resistance to osmotic shocks. The only patch-clamp study reported so far [6,7] led to the discovery of high-conductance channels in the membranes of giant *E. coli* spheroplasts. In part to avoid ambiguity as to the location of channels, we patch-clamped the cell membrane of a Gram-positive bacterium.

Correspondence address: M. Zoratti, CNR Unit for the Physiology of Mitochondria, Institute of General Pathology, Via Loredan 16, Padova, Italy

2. EXPERIMENTAL

Streptococcus faecalis cells, strain ATCC9790, grown in a defined-composition medium [8] to approximately mid-logarithmic phase were harvested and subjected to digestion of the cell wall with lysozyme in hypertonic buffer. The resulting protoplasts were allowed to grow in the original growth medium plus 0.25 M sucrose to a diameter of 2–5 μm. They showed a tendency to revert to bacteria upon incubation without nutrients and to disintegrate during the procedure to establish the gigaseal or soon after its formation. In the latter case a membrane patch continued to adhere to the patch pipette (Hilgenberg 11411 glass) with resistances of several gigaohms. Most of our recordings were obtained from this configuration. The behavior in the cell-attached configuration was qualitatively the same. Steady transmembrane potentials were applied and currents were amplified by a list electronic EPC-5 patch-clamp unit. Signals were filtered at 1 or 3 kHz, recorded using a Racal 4 recorder and subsequently digitized at 10–100 kHz and analyzed using a Nicolet 4094A digital oscilloscope. Unless otherwise mentioned, the current records presented in this paper were obtained from membranes symmetrically bathed in 250 mM KCl, 90 mM $MgCl_2$, 10 mM $CaCl_2$, 5 mM Tris/Hepes, pH 7.2, using a filter setting of 1 kHz and a digitizing frequency of 20 kHz. Experiments were conducted at room temperature (20–25°C).

3. RESULTS

When steady voltage and/or ion concentration gradients were applied across excised patches of *S. faecalis* protoplast membrane, single-channel

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/88/$3.50

current steps of a surprising variety of sizes were observed. As shown by fig.1, the corresponding conductances ranged from about 10 pS to several nanosiemens. Many of the peaks in the histogram appear to fall at values which can be obtained by an arithmetic combination of lower ones. Higher conductance changes tended to cluster around multiples of 1 nS. Commonly the electrical activity consisted of bursts of current through a channel flickering between two or more conductance states, not necessarily including the closed state(s). We occasionally observed interruptions of the flickering behavior by short periods of more steady current flow at intermediate levels, as exemplified by fig.2. Since for statistical reasons the steady current is likely to be conducted by the same channel engaged in flickering, we interpret these observations as evidence for the existence of multiple conductance states of the channel.

Single opening steps followed by more than one closing event, or the opposite sequence, were often observed in our study, under circumstances suggesting that only one channel was involved. Such occurrences were particularly common when the membrane of an excised patch was bathed by a KCl-based medium on one side and by a Tris/Hepes solution on the other. Fig.3 presents an anthology of relevant current records. Events of this type have invariably been construed as evidence for the presence of channel substrates [9]. Most of the conductance states identified from measurements on isolated events, with the exception of the lowest one (~ 10 pS), were also identified as lower levels associated in this manner with one or more higher conductances. The behavior summarized in figs 1–3 is consistent with the presence of a high-conductance channel, possessing a variety of substates. A possible model might envision a number of cooperative subunits, each with several substates and a maximal conductance of about 1 nS. The highest single-channel currents we recorded correspond to a conductance of 8.5 nS. However, many high-conductance events could not be measured because of amplifier saturation. The possibility therefore exists that the maximal conductance of the channel (or channel cluster) is still higher. The channel seems to possess both fast and slow desensitized states; often no activity was evident for periods of minutes.

Application of a slight suction to the pipette resulted in the immediate onset of electrical activity, as exemplified in fig.4. This activity at times appeared as a train of brief openings (fig.4a), at others as a cluster of superimposed steps (fig.4b).

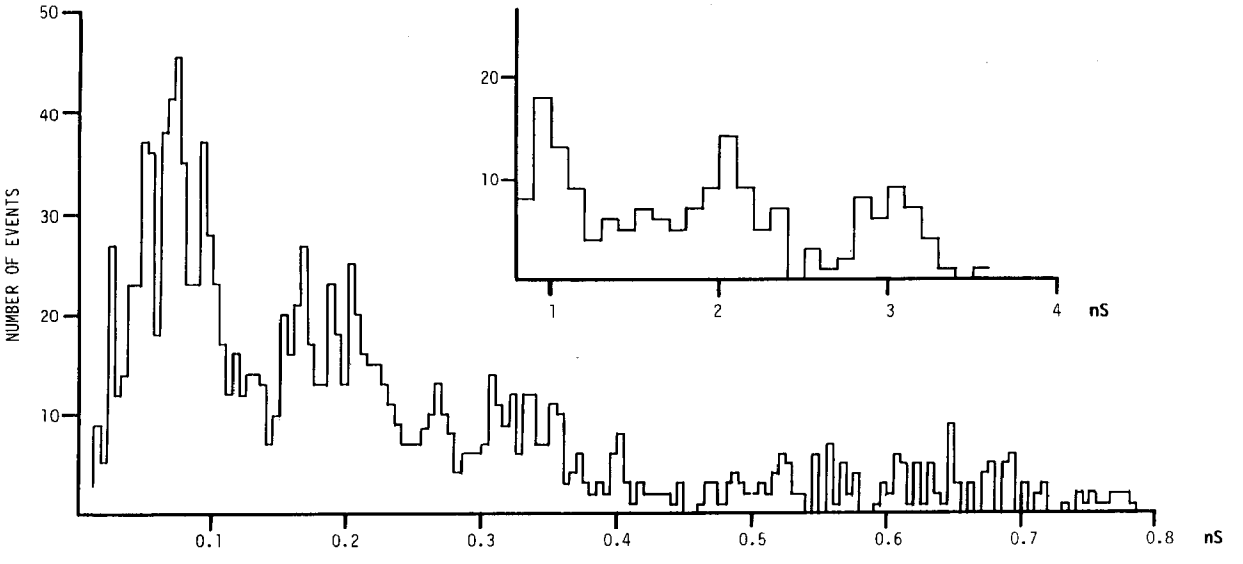

Fig.1. Histogram of conductance values, determined from stepwise changes in the current flowing through excised *S. faecalis* protoplast membrane patches. A total of 1514 events, recorded at various holding potentials using three different membrane patches are plotted in 5 pS or 100 pS (inset) bins. Stepwise current variations corresponding to conductance changes of 5.4 and 7.0 nS were also observed. Ohmic behavior was assumed in calculating conductances.

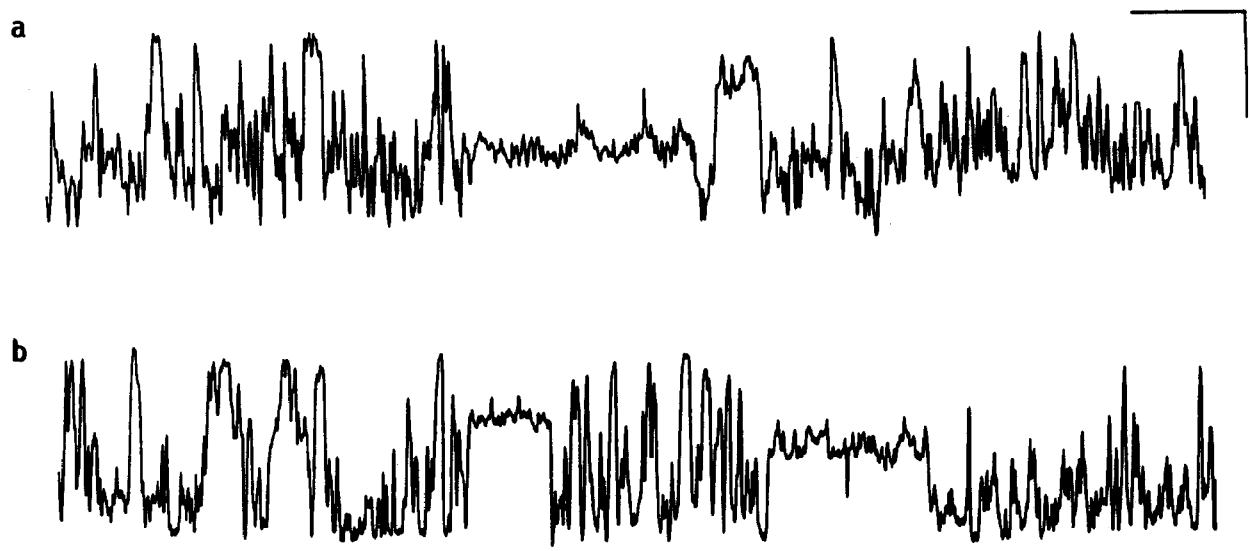

Fig.2. Examples of 'channel noise'. The sequence of negative current spikes is interrupted by less noisy periods spent in intermediate conductance states. Bars: 20 ms, 50 pA. Applied potential (V_H): a, −40 mV; b, −50 mV.

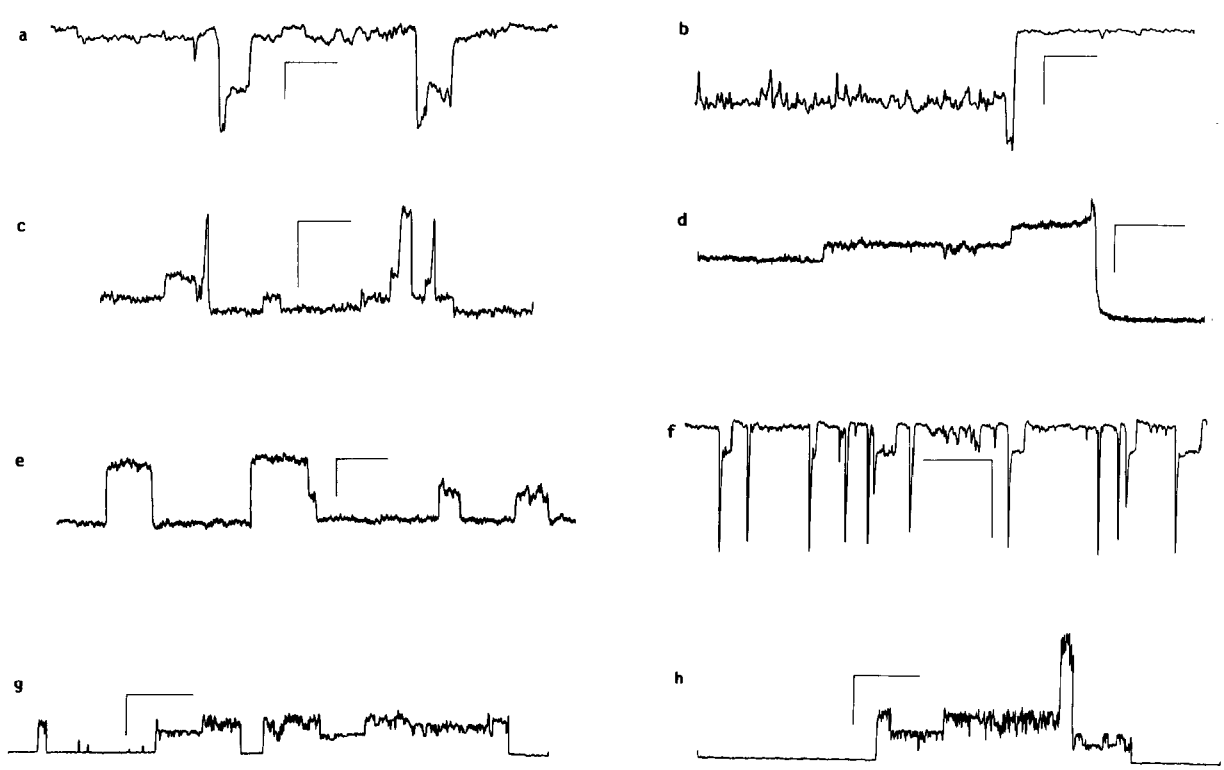

Fig.3. Multiple conductance states. (a and b) V_H, −81 mV; bars, 10 ms, 100 pA. (c) V_H, 77 mV; bars, 20 ms, 50 pA. (d) V_H, 40 mV; bars, 100 ms, 50 pA. (e and f) The pipette contained 350 mM Tris/Hepes, pH 7.2. (e) V_H, 30 mV; bars, 20 ms, 100 pA. (f) V_H, −100 mV; digitized at 100 kHz; bars, 20 ms, 20 pA. (g and h) Suction-induced currents; V_H, 67 mV; bars, 50 mS, 200 pA.

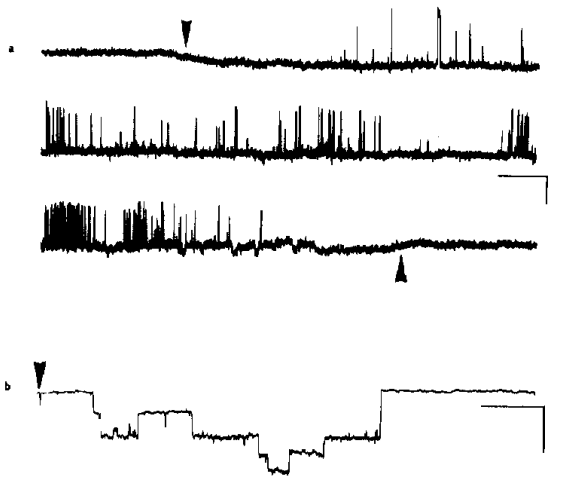

Fig.4. Suction-induced channel activity. Approximately when indicated, a slight suction by mouth was applied (downward arrowhead) to the pipette interior, or released (upward arrowhead). (a) V_H, 20 mV; bars, 200 ms, 20 pA. Suction caused a downward shift of the baseline. The first segment shown is separated by the other (continuous) two by a few seconds. (b) The pipette contained 350 mM Tris/Hepes, pH 7.2. Analogous events were observed in symmetrical solution A. V_H, −20 mV; bars, 100 ms, 100 pA.

The occasional presence of the familiar multiple opening/single closing (or viceversa) pattern in events of the fig.4b type suggests that not all the current steps represented transitions of independent channels. Furthermore, when the membrane patch exhibited this behavior, the clusters of superimposed steps were often separated by periods of total inactivity, a behavior not to be expected from a population of independent channels. In pressure-induced events membrane conductance changes in steps of 1 or 2 nS were often much in evidence, but nearly always steps of various sizes contributed to the waveform (fig.4b). As many as 13 superimposed current steps, with a total conductance of 17 nS, were observed. Also, the pressure-induced currents often showed a flickering behavior, and a variety of substates could be clearly identified (fig.3g,h, and fig.4b). The conductance levels generally corresponded to those of frequently observed spontaneous events. It seems therefore probable that the same protein is responsible for both spontaneous and suction-induced current conduction. Upon release of suction, the stimulated electrical activity most often ceased, but in a few cases (of the fig.4a type) it persisted for many seconds.

4. DISCUSSION

A striking characteristic of this channel is its high maximal conductance, which suggests a relationship to porins [2]. The latter are known to occur as triplets merging into a single channel [10], a structure shared by the VDAC channel of the outer mitochondrial membrane [11]. VDAC is furthermore known to exhibit multi-molecular interactions [11], resulting in the formation of arrays in the negative membrane. If functional and topological interactions among units possessing a number of conductance states existed in the case of the *S. faecalis* channel, they might well explain the cooperative behavior observed and the high conductances. Other cases of organization of channels in clusters with numerous components are known [12,13].

The behavior exemplified in fig.4b is reminescent of the observations by Martinac et al. in *E. coli* [6]. The channel they studied is also pressure sensitive, has a conductance of about 1 nS, and exhibits infrequent lower conductance substates. The current records presented appear to contain a few 2 nS transitions, and the authors mention that the channel might be composed of multiple subunits. Furthermore our preliminary observations indicate that the *S. faecalis* channel, like the *E. coli* one, is somewhat voltage-sensitive and that most of its substates conduct both cations and anions. It seems appropriate to speculate that these two channels may be similar, that both reside in the cytoplasmic membrane, and that they represent a widespread class of bacterial proteins, possibly involved in osmotic responses [6]. *E. coli* cells are known to rapidly lose a large fraction of their K^+ and other solutes when subjected to osmotic downshock [14]: operation of a large-conductance channel may well account for the phenomenon. Conceivably, these channels might be expressed at a higher level in protoplasts or spheroplasts, which lack the protection afforded by the cell wall. Roles other than osmotic shock protection may be easily envisioned [1,4,5,7]: a list would include tasks in both sensory transduction and transmembrane transport.

Acknowledgements: We thank the Fidia Research Laboratories, in particular Drs M. Fiori, F. Di Gregorio, G. Favaro and G. Cinetto, for allowing and helping us to carry

out this work in their laboratories. We also thank Professor A. Peres for introducing one of us (M.Z.) to cellular electrophysiology and for the loan of some equipment, Professor R. Fontana for providing the bacteria and the know-how necessary for the production of giant protoplasts, and Professor G.F. Azzone for support. He and Dr D. Pietrobon offered helpful comments.

REFERENCES

[1] Boulanger, P. and Letellier, L. (1988) J. Biol. Chem. 283, in press
[2] Benz, R., Schmid, A. and Hancock, R.E.W. (1985) J. Bacteriol. 162, 722–727.
[3] Konisky, J. (1982) Annu. Rev. Microbiol. 36, 125–144.
[4] Heller, K.B., Lin, E.C.C. and Wilson, T.H. (1980) J. Bacteriol. 144, 274–278.
[5] Nichols, W.W. (1987) Biochim. Biophys. Acta 895, 11–23.
[6] Martinac, B., Buechner, M., Delcour, A.H., Adler, J. and Kung, C. (1987) Proc. Natl. Acad. Sci. USA 84, 2297–2301.
[7] Saimi, Y., Martinac, B., Gustin, M.C., Culbertson, M.R., Adler, J. and Kung, C. (1988) Trends Biochem. Sci. 13, 304–309.
[8] Roth, G.S., Shockman, G.D. and Daneo-Moore, L. (1971) J. Bacteriol. 105, 710–717.
[9] Fox, J.A. (1987) J. Membr. Biol. 97, 1–8
[10] Engel, A., Massalski, A., Schindler, H., Dorset, D.L. and Rosenbush, J.P. (1985) Nature 317, 643–645.
[11] Mannella, C.A., Colombini, M. and Frank, J. (1983) Proc. Natl. Acad. Sci. USA 80, 2243–2247.
[12] Kazachenko, V.N. and Geletyuk, V.I. (1984) Biochim. Biophys. Acta 773, 132–142.
[13] Krouse, M.E., Schneider, G.T. and Gage, P.W. (1986) Nature 319, 58–60.
[14] Britten, R.J. and McClure, F.T. (1962) Bacteriol. Rev. 26, 229–335.