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A patch-clamp study of *Bacillus subtilis*

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In patch-clamp experiments on giant protoplasts of the Gram-positive bacterium *Bacillus subtilis*, membrane stretch resulted in an initial transient collapse of the membrane resistance, after which stretch-activated, voltage modulated, high-conductance channels could be observed. The channel open probability increased exponentially with applied suction and positive voltage, as a result of variations of both the mean open and the mean closed times. The substate structure and other characteristics of the electrical activity suggested the presence of a family of pores exhibiting cooperative behavior. A role in osmotic protection is suggested. In the intact bacteria, the pores may be part of an unidentified envelope apparatus, having other functions as well.

Introduction

The patch-clamp technique [1] has proved invaluable for the study of membrane phenomena as different as exocitosis (e.g. Ref. 2) and cell-cell coupling via gap junctions (e.g., Ref. 3). In recent years it has been applied to the study of the permeability properties of dozens of membranes. In this paper we report on our investigation of the membrane of *Bacillus subtilis*, a Gram-positive bacterium.

The electrophysiology of procaryotes has been developing over the past few years, since the investigation of *Escherichia coli* spheroplasts by the Madison group [4]. That first study identified a stretch-activated, voltage-dependent, high-conductance channel. Subsequent experiments on proteoliposomes reconstituted from *E. coli* membrane fractions [5] and on spheroplasts [6] recorded the presence of several different conductances. The existence of two cellular membranes in this Gram-negative bacterium generated some doubts concerning the location of the channels [5,7], which seem now to have been resolved by the recognition that channels other than porins exist in both the outer and the inner membrane [8]. No ground for such ambiguities exists in the case of Gram-positive bacteria, which possess only one membrane.

While the presence of channels in the cytoplasmic membrane of procaryotes might have been doubted on chemiosmotic grounds, there was reason to believe that the membrane might contain tightly controlled channel-like structures. The presence of a rapid efflux pathway in osmotic downshock experiments [9,10] suggested the presence of membrane stretch-activated (SA) channels. Also, some transport systems have been suggested to possibly comprise a channel-forming domain. Examples include the protein transport machinery encoded by the *Sec* locus [11,12] and the periplasmic solute uptake systems [13,14]. These apparatuses have been studied mainly in Gram-negative bacteria, but counterparts exist in Gram-positive organisms as well [14,15]. Electrophysiological evidence for the involvement of a high-conductance 'channel' in protein transport in *E. coli* has recently been presented [16]. The periplasmic transport systems, on the other hand, belong to the family of 'cassette' transport ATPases, which also includes, inter alia, the cystic fibrosis transmembrane regulator (CFTR) and the multi-drug resistance (MDR) pump. Both the CFTR [17,18] and the MDR protein [19] have been found to form anion-selective channels.

As might have been expected on these basis, the *B. subtilis* membrane exhibited a variety of conductances, ranging from relatively small (< 50 pS in 350 mM KCl) to huge (above 6 nS). This paper is concerned mainly with the high-conductance channels. As detailed below, the smaller ones arise at least in part from the same channel system which originates the megaconductances. Some might instead represent independent molecular species having different properties and tasks, but their identification as such is made difficult by the presence and characteristics of the large-conductance

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Abbreviations: SA, stretch-activated; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

system. The literature contains reports of a low-conductance *B. subtilis* channel exhibiting some selectivity for divalent cations [20–22]. A study of *B. subtilis* channels by the planar lipid bilayer technique has provided evidence in agreement with our work (Alcayaga et al., in press).

Materials and Methods

Protoplast and proteoliposome preparation. Experiments were performed on 'giant' protoplasts, since the cell wall was expected to prevent seal formation, and normal-size protoplasts would have been too small. *Bacillus subtilis* cells were grown to an optical density of about 0.3 in 20 ml of a defined composition medium (CDM) [23], harvested and resuspended in 0.4 ml of 0.5 M sucrose, 10 mM $MgCl_2$, 50 mM TrisCl (pH 7.2), 1.5 mg/ml lysozyme (Sigma). After about 1 h of incubation at 37°C the resulting protoplasts were diluted in CDM + 0.25 M sucrose and allowed to grow to diameters of 2–4 μm. If stored at 4°C, they maintained viability, including the ability to revert to normal-size cells, for up to a week. It is unlikely that the lysozyme treatment resulted in proteolytic damage to the cells, thus accounting for the presence of channels: *E. coli* spheroplasts prepared either by the EDTA/lysozyme method or by penicillin treatment had undistinguishable channel activities [6].

Proteoliposomes were prepared by the freeze-thaw technique [24]. Liposomes (20 mg lipids/ml) prepared by sonication of a suspension of acetone-purified asolectin (Sigma type II-S) were mixed with 0.5–1 volumes of sonicated protoplasts (1 mg protein/ml) and freezed/thawed 3–4 consecutive times.

Patch-clamp experiments. A few drops of the protoplast suspensions were deposited onto the coverslip constituting the bottom of a homemade patch-clamp chamber held by the holder of a 'patch-clamp tower' (List), covered with experimental medium (symmetrical 350 mM KCl, 10 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM Hepes/K^+, pH 7.2 unless otherwise specified) and the protoplasts were allowed to become attached to the glass (approx. 10 min). The chamber (capacity: about 1 ml) was then extensively perfused with the medium to remove any debris. The success rate in attempts at seal formation was approx. 50%. The protoplasts at times disintegrated upon or after seal formation (see Results), leaving behind excised patches. Seals were often lost upon application of stretch or of voltages higher than about ± 50 mV, or collapsed spontaneously. Only about 25% of seals lasted long enough for recordings to be obtained.

In selectivity determination experiments, an *I–V* curve was first determined in symmetrical standard medium. The chamber contents were then substituted with either KCl at a different concentration (to determine P_K/P_{Cl}) or either M^+Cl^- or K^+X^- at the same concentration of the ion in common with the pipette (to determine P_K/P_M or P_{Cl}/P_X). *I–V* curves were then determined again, and permeability ratios calculated from the reversal potential using the Goldman-Hodgkin-Katz equation or the general current equation of the constant field theory [25,26] taking $P_K/P_{Cl} = 1$ (as justified by the experimental results). The *I–V* curves of the conductances we examined were linear in the restricted voltage range used (not exceeding ± 50 mV).

Pipettes were drawn from Hilgenberg 11411 glass and polished to a tip o.d. of about 0.5 μm (resistance in the experimental medium was 3–5 MOhm). They were used without coating. Suction was applied through a side arm of the pipette holder, and measured by a mercury manometer. The patch chamber was grounded via an agar bridge. Voltage at the pipette electrode was controlled by a List EPC-7 patch clamp. The output signal was filtered through an 8-pole filter (Frequency Devices 902LPF; corner frequencies of 5–10 kHz) and recorded on videotape after encoding at 22 kHz by a Medical Systems PCM-2. The data were analyzed off-line using Axon's TL-1-125 interface and pClamp 5.5.1 program set. Voltages quoted are those of the bath electrode (the pipette electrode being conventionally assigned a value of zero). Inward currents (cations flowing from the pipette to the bath) are considered negative and plotted downwards.

Variability. While activation by stretch and voltage was always observed, the suction needed to elicit a given channel activity level varied from experiment to experiment. This variability is presumably due to the fact that the relevant parameter is not the transmembrane pressure gradient, but rather the tension exerted on the membrane, which is a function of the curvature of the membrane patch [27]. The latter presumably varied from patch to patch, and we made no attempt to control or measure it. Furthermore, the channels exhibited hysteresis with respect to modulation of the open probability by both suction and voltage, and sometimes their kinetic behavior changed during the experiment without evident cause. These problems, together with the tendency of the channels to run down (see Results), thwarted our attempts to perform some lengthy experiments, such as the determination of a three-dimensional surface relating open probability to voltage and applied suction.

Results

When a high-resistance seal was established on *B. subtilis* protoplasts, in the cell-attached configuration, no channel activity could be observed unless suction, i.e., membrane stretch, was applied. The few (approx. 5%) exceptions to this statement are discussed below.

In about 75% of cases, the application of increasing suction resulted in a sudden, saturating, short-lived burst of current, which we refer to as the 'initial catastrophe' (Fig. 1). In synchrony with this electrical event, the protoplast, as viewed using phase contrast optics, either disintegrated (approx. 40% of cases) or changed from dark to a paler shade of gray or to transparent, suggesting that the cytoplasmic contents had been partially or completely substituted by experimental medium. In either case, the resistance and capacitance of the pipette-enclosed membrane patch were the same before and after the catastrophe, suggesting that no leaks had developed and that the experimental configuration had not changed to 'whole cell'.

Initial catastrophes nearly always comprised a few stepwise current increases (Fig. 1): the size of these steps was comparable to that of the largest stretch-activated channels (see below). In the cases in which an initial catastrophe took place, no channel-like events were detected before it. We never observed more than one initial catastrophe with a given membrane patch. While it often happened that the high-resistance seal collapsed upon application of suction, an initial catastrophe appeared not to be the underlying cause, since the current increase due to the loss of the tight seal never exhibited a stepwise development. By the same token, initial catastrophes presumably were not due to a reversible collapse of the seal, i.e., the phenomenon concerned the membrane and not the membrane/glass interface. During any given experiment, a part of the protoplasts in the patch chamber became semi-transparent, probably because of spontaneous permeabilization events. In a handful of cases, we recorded spontaneous initial catastrophes, occurring without the application of suction. Fig. 1 shows examples of both stretch-induced (1A, C) and spontaneous (1B) initial catastrophes. Fig. 1C shows the only case in which the electronics were not saturated by the current burst. No initial catastrophes, and no channel-like events, were observed in nine control experiments with giant asolectin liposomes.

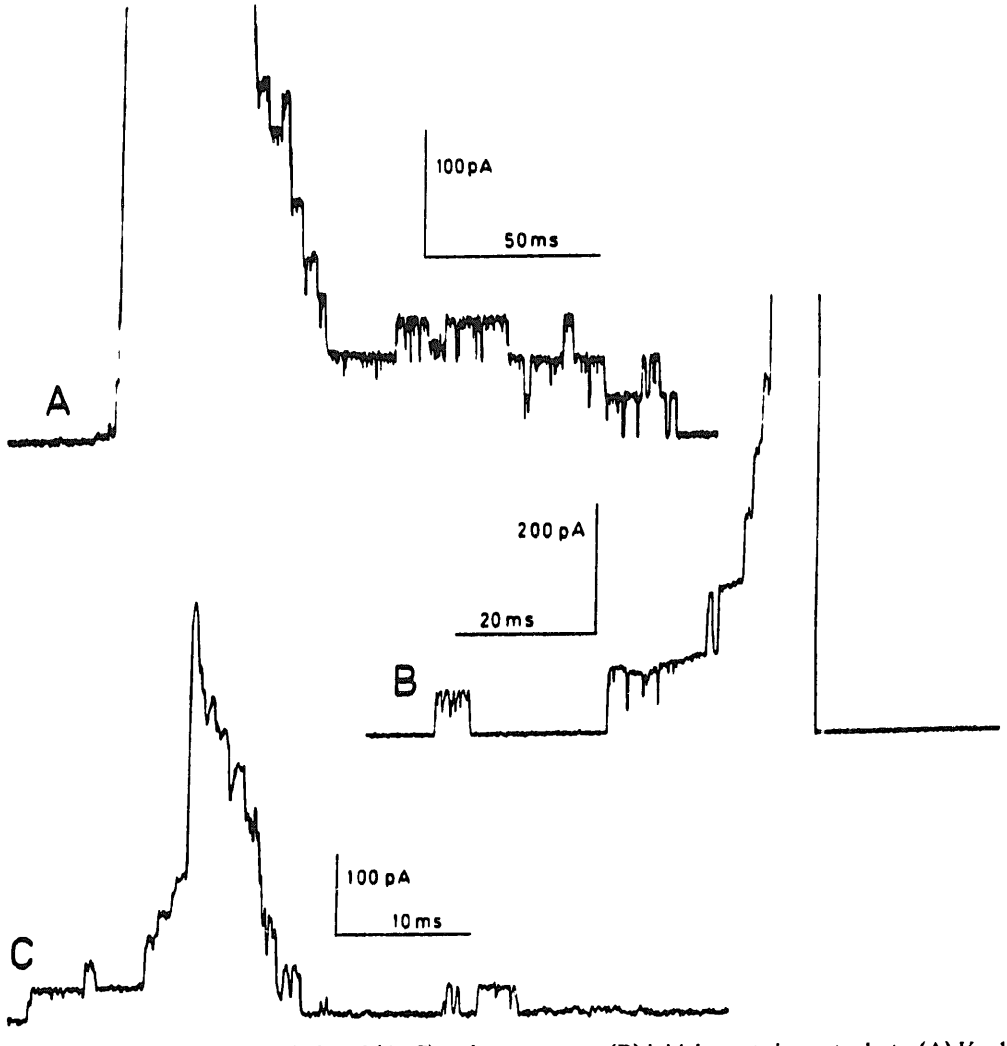

Fig. 1. Examples of initial catastrophes. Suction-induced (A, C) and spontaneous (B) initial events in protoplasts. (A) $V = 10$ mV. Step sizes: 27, 30, 44 pA. (B) $V = 35$ mV. Step sizes: 100 and 64 (first, isolated event) pA. (C) $V = 10$ mV. Step sizes: 31, 37, 57 pA. Peak current: 513 pA.

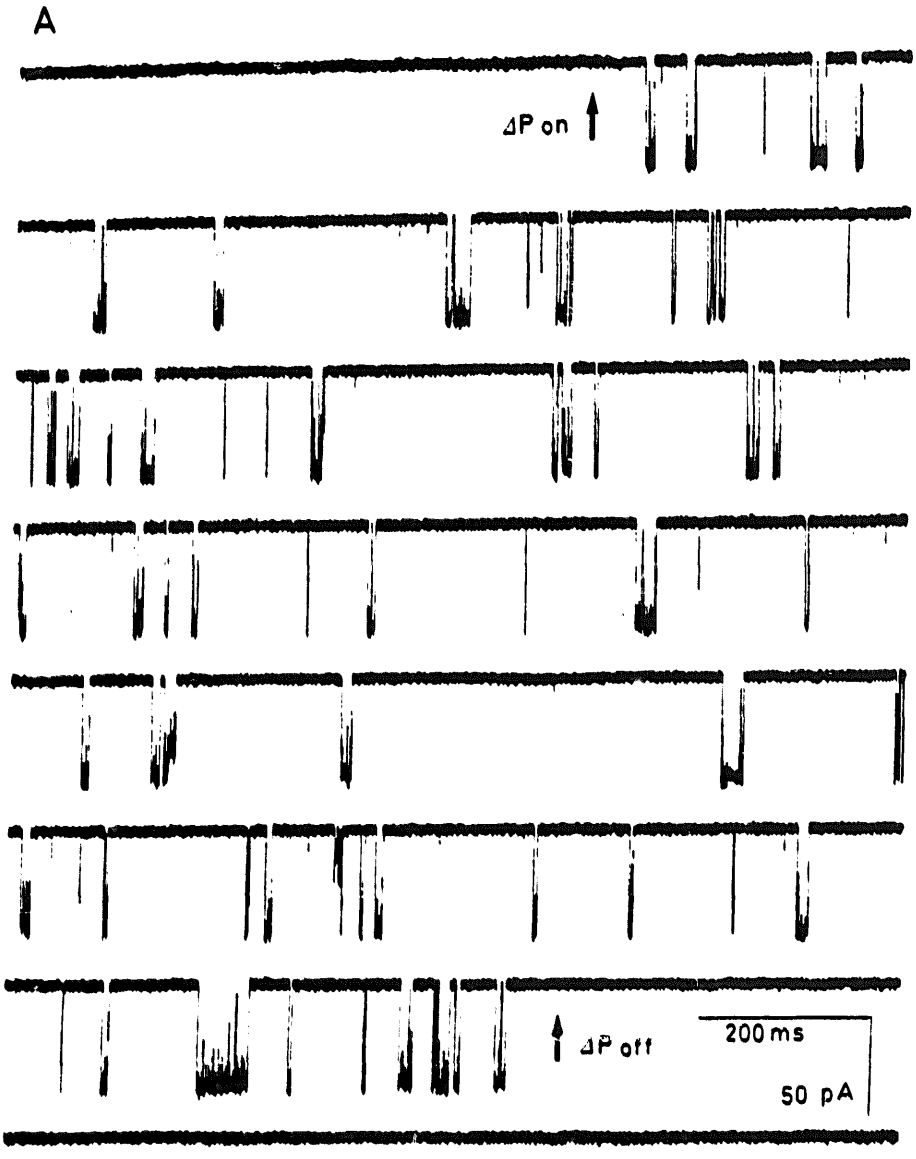

Fig. 2. Examples of stretch-elicited activity. (A) Suction-dependent activity of (a) 2.5 nS channel(s) in a proteoliposome. 15.5 cmHg of suction were applied and released when indicated. $V = -20$ mV. (B) Activity of 5 nS channels recorded from a protoplast, under 19 cmHg of applied suction. $V = 5$ mV. Substates are pointed out by arrows. (C) Examples of high-amplitude events recorded from one patch during a 5 min period. Applied suction: 30 cmHg. $V = 50$ mV. Conductances (from the left): 1.8, 2.1, 2.5, 2.7, 3.0, 3.9, 4.2, 4.5 and 7 (4.5 + 2.5) nS. The activity of the patch was low: the sum of the open probabilities for all channels was 0.0062, with a mean silent interval of 3.8 s. Therefore it is likely that the last event shown is an example of cooperative gating by two channels. (D) 'Noisy' activity by a 3.6 nS channel operating with high open probability at 5 cmHg of suction. Filter: 1 kHz. $V = 40$ mV.

Once the initial catastrophe had taken place (and not before), stretch-induced channel activity could be elicited by suction. As mentioned, in about 25% of cases activity could be observed even though no initial catastrophe had been recorded. We presume that in these cases the catastrophe had actually taken place while suction was being applied to establish the high-resistance seal.

In 18 experiments on proteoliposomes, both stretch-dependent and spontaneous activity could be observed, while we never recorded an initial catastrophe. The channel activity was practically indistinguishable from that observed in protoplasts, except that spontaneous activity occurred in 50% of cases (vs. 5% in protoplasts), displaying both high (> 1 nS), fast conductances and lower ones. The latter seemed to constitute a larger fraction of the total activity than in the case of protoplasts.

The conductance of the most commonly observed SA channel fell in the range 2.5–3.4 nS. Representative recordings, taken from an experiment on a proteoliposome, are shown in Fig. 2A. Quite commonly, its 'double', with a conductance of 5–6.5 nS was encountered. (Fig. 2B). Less frequently, intermediate and lower conductance values characterized the activity. Fig. 2C present a collection of high-conductance events

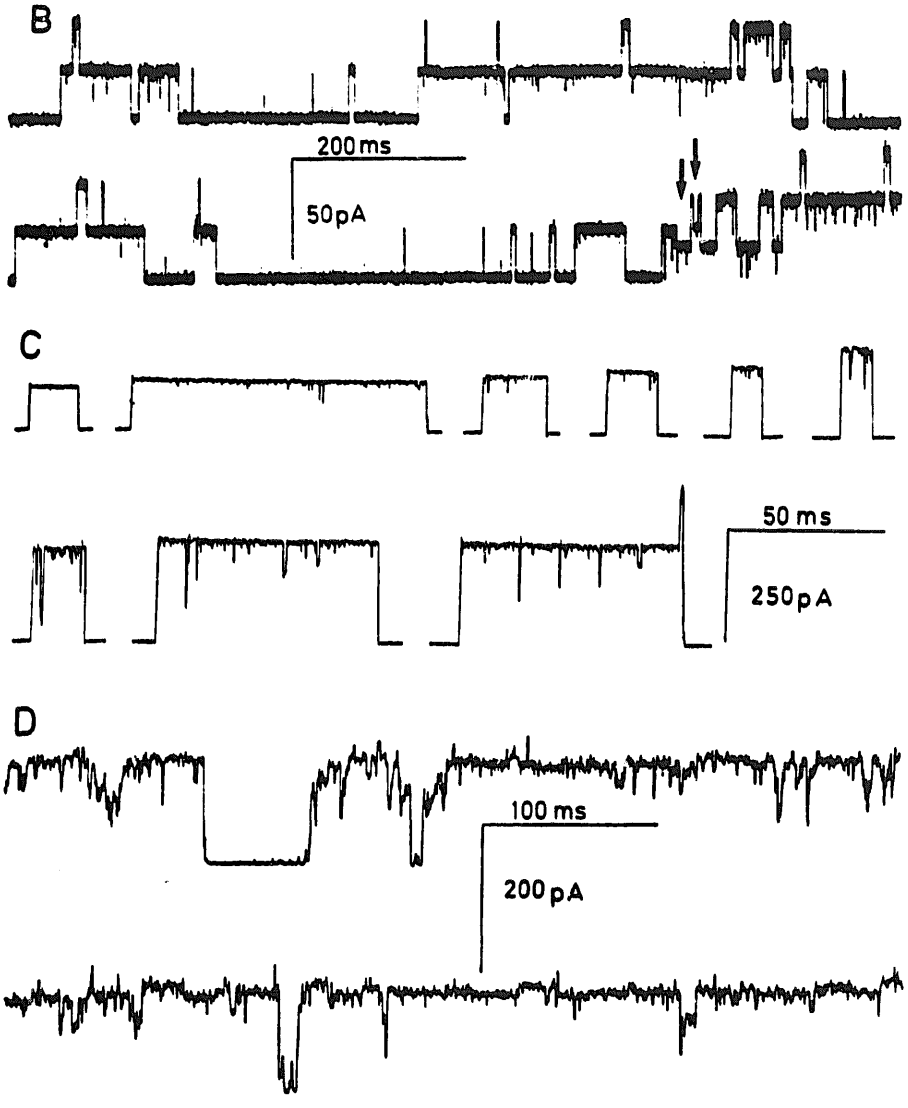

Fig. 2. (continued).

from one patch, to emphasize the variety of different conductances which could at times be encountered. Channel activity did not always result in relatively 'clean' current steps as in the examples of Fig. 2A–C. On the contrary, 'open channel noise' was often considerable, as in Fig. 2D.

The relationship between $\bar{P}_0$, the channel open probability, and ΔP, the applied suction, was of the Boltzmann type, i.e.

$$\ln[\bar{P}_0/(A-\bar{P}_0)] = a * \Delta P + b$$

where A, a and b are fitting parameters. Fig. 3 shows data from one representative experiment. The behavior is strongly reminescent of those observed with *S. faecalis* (Szabó et al., submitted) and *E. coli* [4] membranes. While we have determined the $\bar{P}_0$ vs. ΔP relationship only for a few cases, the available data suggest that the various high-conductance SA channels behave similarly. In six experiments on conductances between 1 and 4 nS the increase in suction needed for an e-fold variation of $\bar{P}_0$ always fell within the range 0.7–1.0 cmHg. As mentioned, some variability was to be expected since the radius of curvature of the patch membrane is one of the elements intervening to determine the relationship between open probability and suction [29].

The stretch sensitivity of these channels suggested that they might be involved in the efflux of K^+ and other ions from the cells upon osmotic downshock. Fig. 4 shows that an osmotic shock could elicit high-conductance channel activity from a patch of *B. subtilis* membrane held by the patch-clamp pipette, imitating the effects of suction. This observation might be relevant to phenomena taking place in vivo, suggesting that efflux of cytoplasmic solutes from osmotically shocked whole bacteria might take place through these channels. This possibility has recently been strengthened by the observation that Gd^{3+}, the only available inhibitor of stretch-activated channels, inhibits both SA channel

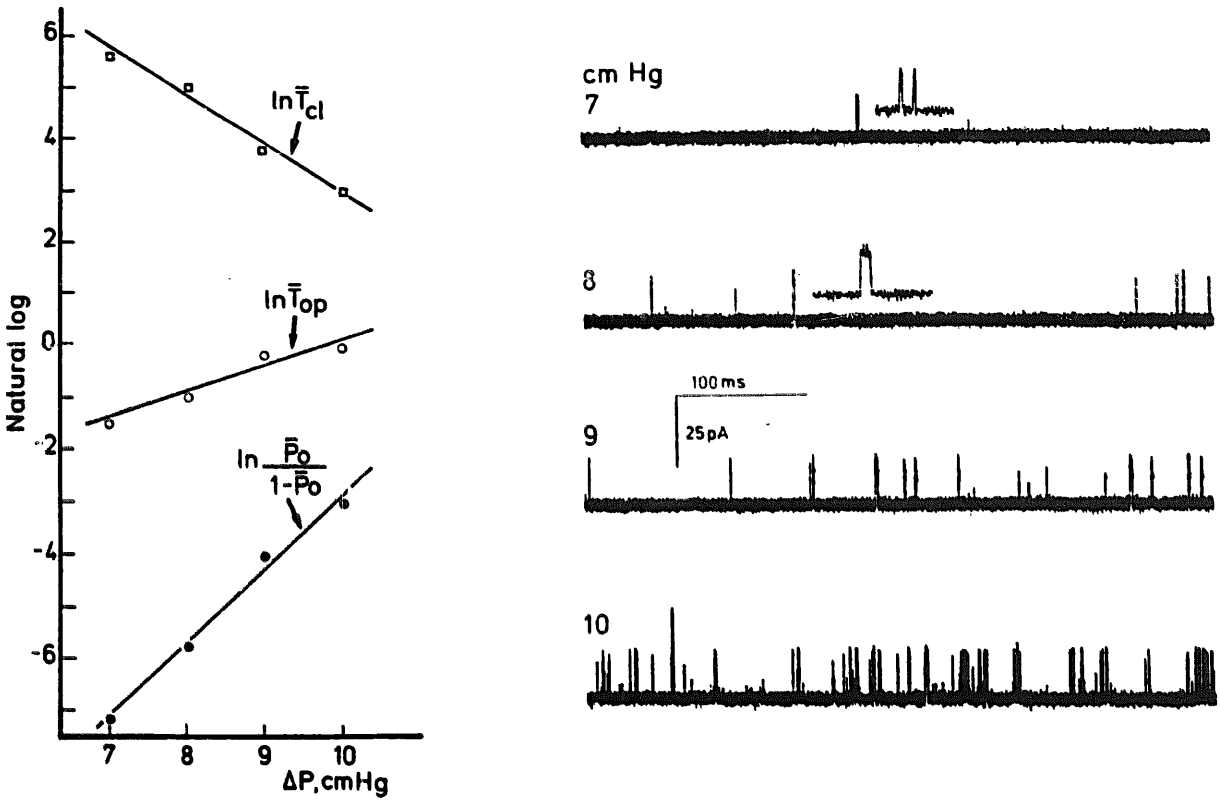

Fig. 3. Dependence of channel activity on applied suction. (Left side) Logarithmic plots of channel activity and of the mean open and closed (silent) times vs. applied suction. The lines were fitted by linear regression. The number of channels in the patch is unknown: $\bar{P}_0$ and $\bar{T}_{cl}$ values were calculated as if only one had been present. The slopes of the lines were 0.71 ($\ln[\bar{P}_0/(1-\bar{P}_0)]$), 2.08 ($\ln\bar{T}_{op}$) and 1.07 ($\ln\bar{T}_{cl}$) cmHg^{-1}. $V = 5.0$ mV. Conductance: 3 nS. (Right side) Traces showing examples of channel activity at the various applied suction values. (Insets) The closest event plotted on a 10-fold expanded time scale.

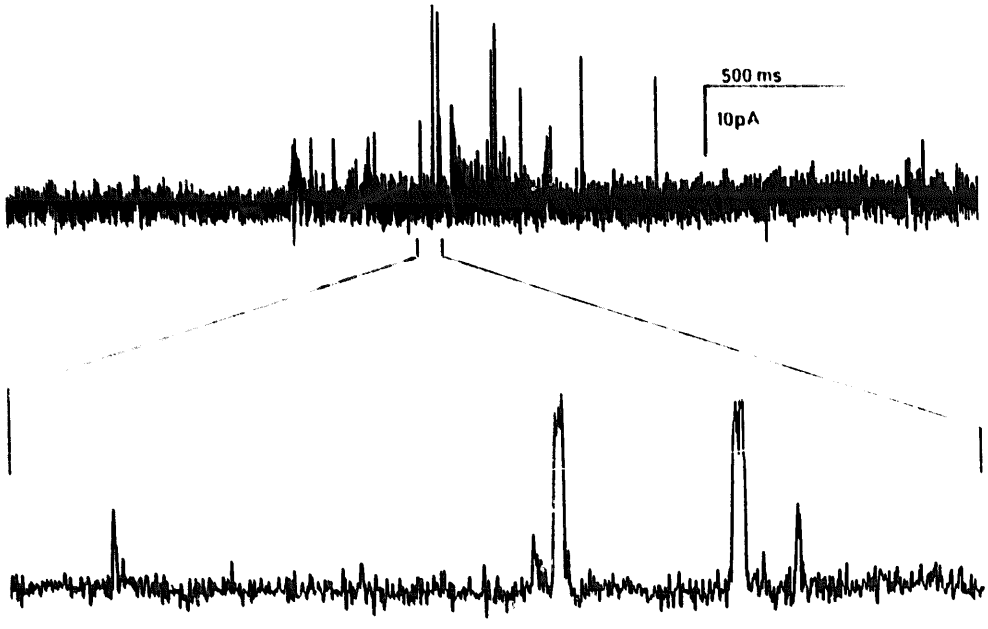

Fig. 4. Osmotic shock-induced channel gating. Application of suction to a protoplast induced an initial catastrophe followed by strictly suction-dependent activity by 5 nS channel(s). About 90% (approx. 0.8 ml) of the medium in the patch chamber was withdrawn and substituted by 0.8 ml of ice-cold distilled water, added by hand with a pipette. The beginning of the addition coincided approximately with the beginning of the trace segment shown. Many of the current steps following the addition can be attributed to channel gating: two are shown in the lower trace (time scale expanded 35-fold). The calculated conductance of the longer-lasting events was close to 2.5 nS, due to the 10-fold dilution of the medium on one side of the membrane. Control experiments showed that the activity was not caused by the dilution of Ca^{2+}. $V = 10$ mV.

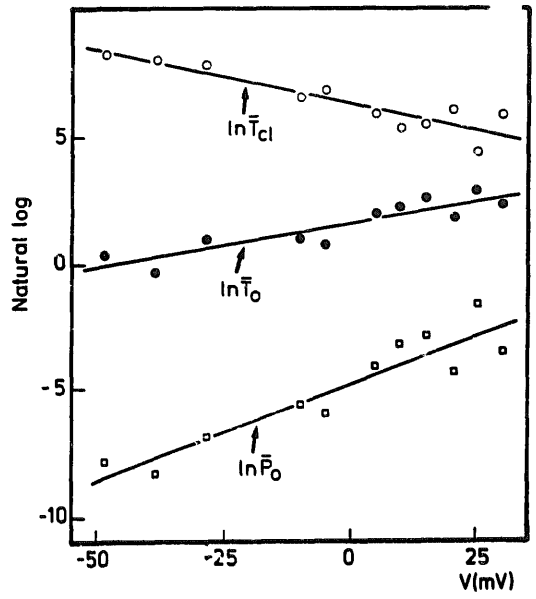

Fig. 5. Voltage dependence. Plot of the natural logarithm of the mean closed (silent) and open times (expressed in milliseconds) and of the parameter $\bar{P}_0/(1-\bar{P}_0)$ vs. voltage, for (a) 2.8 nS channel(s) gating spontaneously in an excised patch. The number of active channels in the patch was undetermined: the values presented were calculated assuming only one was present. The slopes of the lines (fitted by linear regression) correspond to an e-fold change every 24 ($\bar{T}_{cl}$), 29 ($\bar{T}_{op}$) and 13 mV ($\bar{P}_0/(1-\bar{P}_0)$).

activity in patch-clamp experiments, and solute efflux from whole cells, with similar characteristics [28,29].

The giant channels are also modulated by the transmembrane voltage: the open probability increased as potentials became more positive, due to both an increase in the mean open time and a decrease in the mean closed time. This behavior is illustrated by the experiment presented in Fig. 5. In other experiments,

TABLE I

Selectivity of some stretch-activated conductances

See the experimental section for the method used.

Ionic couple [a] (A/B)	Conductance [b] (nS)	Rev. potential [b] (mV)	Permeability ratio (P_A/P_B)
K^+/Cl^-	1.7	0.6	1.0 [c]
K^+/Cl^-	0.9	2.7	1.2 [c]
K^+/Cs^+	1.4	−2.6	0.8 [d]
$Cl^-/iBuO^-$	3.0	0.4	1.0 [d]
$Cl^-/iBuO^-$	1.8	−0.6	1.0 [d]
Cl^-/SO_4^{2-}	3.7	−1.5	1.2 [e]
Cl^-/SO_4^{2-}	2.3	−3.6	1.4 [e]

[a] Ions being compared. A: in the pipette; B: in the bath. The counterion was K^+ or Cl^-. In the experiments relating to P_K/P_{Cl}, [KCl] was 350 mM in the pipette and 100 mM in the bath.

[b] As determined by linear regression under asymmetric conditions.

[c] From the GHK equation.

[d] From the GHK equation, taking $P_K/P_{Cl}=1$.

[e] From the current equation of the constant field theory, taking $P_K/P_{Cl}=1$.

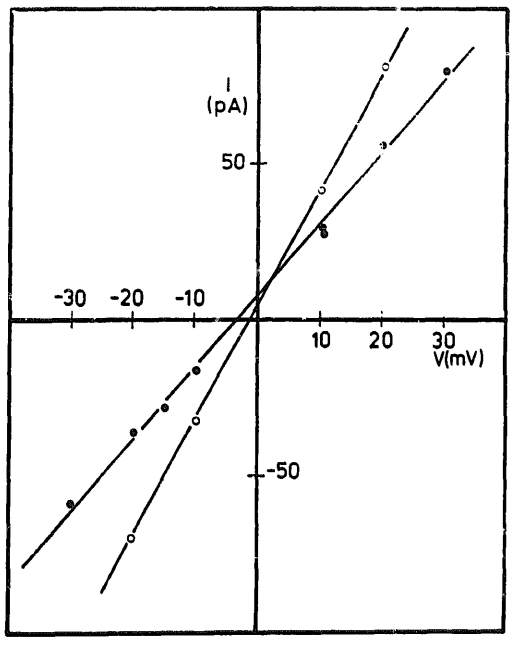

Fig. 6. Lack of selectivity between Cl^- and SO_4^{2-}. $I-V$ curves plotting the current level of single-channel events induced by stretching an excised membrane patch bathed in 350 mM KCl (pipette side) and 175 mM K_2SO_4 (bath side).

conductances above 1 nS appeared to possess essentially the same voltage dependence, with e-fold changes in activity every 9 to 13 mV of change in voltage. Variability over such a range may be ascribed at least in part to the amply precedented, but ill-understood, phenomenon of hysteresis. Channel instability (see Ma-

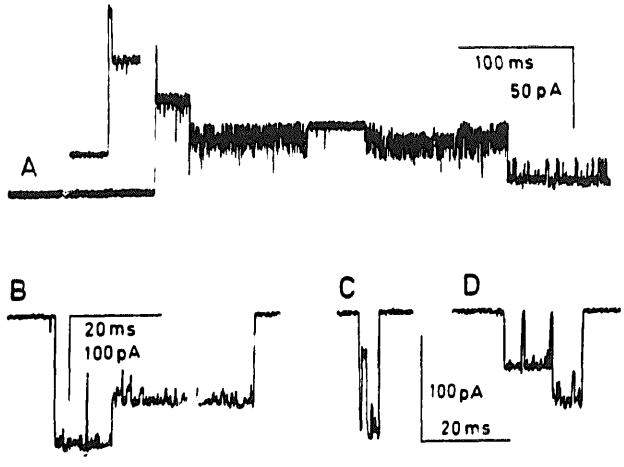

Fig. 7. Examples of subconductance levels. Stretch-elicited activity. (A) Applied voltage: 20 mV. The channel, or channel assembly, gates initially to a conductance of 4.5 nS (90 pA), stepping down almost immediately to a conductance of 3 nS (60 pA) (inset: 4× time scale). After a few more milliseconds the channel begins to flicker between 2 nS (41 pA) and approx. 1 nS levels. Eventually, it enters a 0.35 nS (7 pA) state, with frequent brief transitions to the 1 nS level. (B–D) Some substates exhibited by 3 nS events (all taken from the same experiment). (B) $V=-52$ mV. Substate levels: 2 and approx. 1.6 nS. (C) $V=-41$ mV. Substate: 1 nS. (D) The highest conductance event exhibits a substate (1.7 nS) closely matching the conductance level of the preceding events.

terials and Methods) prevented the determination of a surface relating open probability to both applied suction and voltage. Note that the voltage dependence of the channels is such that physiological transmembrane potentials would act to keep them closed.

Determining the selectivity of all the conductances observed by the patch-clamp technique would be a very difficult task. We have obtained data concerning a few ionic couples and a few high-conductance channels, as summarized in Table I, which show that the pores discriminate poorly among some common ions. A permeability ratio greater than a factor of 2 can be excluded even when comparing chloride and relatively bulky anions like isobutyrate and sulfate. Fig. 6 presents, as an example, the *I–V* curves determined for the latter comparison.

An evident characteristic of the channels was the presence of conductance substates, sometimes with values corresponding to a simple fraction of the maximum. Fig. 7 presents a few examples (others can be observed in Ref. 30). In many cases the conductance of a substate level briefly visited during an event matched the conductance of other, separate events, as exemplified in Fig. 7D.

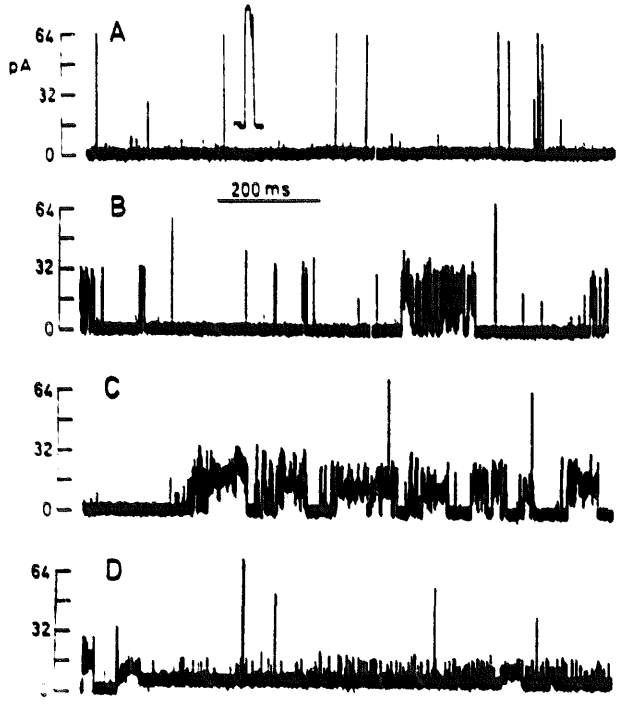

Fig. 8. An example of channel rundown. The patch initially contained a few 3.2 nS conductances gating rapidly (inset: 20× time scale) under 7 cm^2/g of suction (A). One of the channels suddenly changed its characteristics, giving rise to a flickering, approx. 1.6 nS conductance level (B). After a few seconds, another, approx. 0.8 nS level could be seen within the bursting events (C). Eventually the rundown resulted in a nearly always open 0.25 nS conductance, with fast events gating between this level and approx. 0.8 nS. Throughout the process, other 3.2 nS channels in the patch continued to operate.

An annoying, but possibly informative characteristic was the occurrence of rundown (Fig. 8). Upon repeated or protracted application of membrane stretch, or simply upon aging, the high-conductance channels transformed themselves into lower-conductance ones, with different characteristics. In some cases the daughter channels had conductances approximately corresponding to a fraction of the mother conductance, e.g. 1/2 and 1/4, as in Fig. 8. In most cases, this decay was preceded or accompanied by the loss of stretch sensitivity, i.e., the channels became active even in the absence of applied stretch. While in some cases this may have been due to a residual membrane tension remaining after the application of suction, in many other cases the spontaneous activity continued for at least several minutes. Any membrane tension would have been expected to relax over such times [31].

Rundown of the megachannels during seal formation, with a partial or complete loss of stretch sensitivity, is likely to account for the spontaneous activity observed immediately after seal establishment in about 5% of cases (with protoplasts). This hypothesis is strengthened by the following observations (not shown): 'spontaneous' activity was more likely to be found if formation of the high-resistance seal had been difficult, requiring the repeated application of suction; the voltage dependence of the spontaneous conductances was at least qualitatively the same as that of the SA channels; often, suction increased the activity of spontaneously gating channels; lanthanides inhibited both spontaneous and SA channels (Szabó et al., in preparation). The occurrence and characteristics of rundown, together with the presence of substates, the observation of conductance values which correspond to the sum of others, the common requirement for the initial catastrophe, the similar voltage and stretch dependence of the various conductances, make it likely that the huge, stretch-activated conductances arise from the cooperative gating of similar channels or channel subunits. Conversely, many of the lower-conductance channels may well be generated by megachannel components existing as single units or lower-stoichiometry assemblies. Whether the megaconductances arise from the simultaneous gating of 'parallel' channels, or represent the operation of a multi-subunit single channel (e.g., a 'barrel stave' model) or still some other arrangement cannot be decided at present. Dani and Fox [32] have recently suggested caution in drawing conclusions on questions such as these, pointing out that conformational changes of a single pore might give rise to regularly-spaced subconductance levels.

Discussion

The observations summarized above leave no doubt that stretch- and voltage-modulated channels can be

observed in the membrane of *B. subtilis* protoplasts. The first question to come to the reader's mind probably concerns the status and role of these channels in vivo. Two observations are of direct relevance to this point, namely that no channel activity could be observed unless an initial catastrophe had taken place, and that this initial electrical event often displayed a well-resolved stepwise structure. The tentative interpretation we offer of these facts is that the channels constitute part of a membrane complex or structure, which comes apart upon application of a sufficient amount of stretch. The process may generate a number of species, most likely 2.5–3.0 nS channels, but also its 'dimer' or others. Whether the activation of the channels is the consequence of this putative structural collapse, or (also) of the loss or dilution of cytosolic regulatory components by washout of the cytoplasm remains to be ascertained. Be this as it may, it seems obvious that these high-conductance channels are tightly regulated in wholesome cells, where they might open only when performing a specialized task, or possibly under duress, in particular when subjected to an osmotic downshock [28,29]. The presence of the cell wall might well mean that the threshold for channel activation is higher in vivo. Indeed, wild cells are liable to be subjected to osmotic gradients much more severe than the pressure differences we applied.

The high conductance of these pores suggests a comparison with the porins of Gram-negative bacteria. The latter are generally envisioned as a general-purpose filter designed to allow the supply of nutrients to the cell, while at the same time excluding dangerous compounds, such as bile acids. Clearly this character cannot be attributed to the pores described above. While most electrophysiological work on bacterial porins to date has been performed in the lipid planar bilayer experimental system, after isolation of the porins themselves, the most appropriate biophysical comparison might be with porins in proteoliposomes formed by the fusion of outer membrane and phospholipid vesicles. The recent patch-clamp experiments on OmpC and OmpF proteins in proteoliposomes indicates that these pores often visit substate levels, exhibit cooperative behavior, which may extend to large arrays, and tend to close at high voltages of either sign [29,33–35]. In the reconstituted system, OmpC was not stretch-activated (Ref. 29; Martinac, B. and Ghazi, A., personal communications), while the channels described in this paper often maintain stretch sensitivity in proteoliposomes (Fig. 2A). Both similarities and differences therefore exist, the latter probably outweighing the former. On the other hand, the channels described here have much in common with those observed in *E. coli* [4–6], and particularly with those of *S. faecalis* [36].

The function of the channels and their origin can only be speculated about. The most straightforward possibility is that of a fast-responding apparatus enabling the cells to better handle osmotic shocks [28], but other possibilities exist. As mentioned in the introduction, one is that of the periplasmic transport systems, which is given credibility by the recent results with the CFTR and the MDR protein [17–19]. However, it seems that this class of molecules gives rise to low-conductance, anion-selective channels, thus providing candidates for some of the low conductances, but probably not for the highest ones. A possibility we are examining is that the SA channels might represent an unphysiological mode of operation of the *MotA*-encoded [37] proton channels which ring the base of bacterial flagella and serve to power the motility apparatus, or that they might be otherwise derived from the complex flagellar structure [38,39]. Another is that these channels might arise from structures responsible for the uptake or release of macromolecules, e.g., transforming DNA. As mentioned, the most likely possibility seems to be that the channels described here might be analogous to those observed by Simon and Blobel [16] in *E. coli* membranes, where they might be part of the protein-conducting machinery.

The exploration of these and other possibilities, and a study of the possible modulation of these channels by soluble agents, constitute our next targets of investigation.

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