Vol. 171, No. 1, 1990 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
August 31, 1990 Pages 280-286

Cooperative mechanosensitive ion channels in *Escherichia coli*

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Received July 13, 1990

A patch-clamp investigation was carried out on giant *Escherichia coli* spheroplasts. The membrane exhibited stretch-induced as well as "spontaneous" activity, with similar characteristics, i.e., a large number of conductance values arising from the cooperative behavior of channels in functional clusters. It appears likely that the same molecular species are responsible for both stretch-induced and "spontaneous" current conduction; the channel multiplexes can either respond to membrane stretch or function in an activated state, presumably brought about by the previous application of the mechanical stimulus.

The recent application of the patch-clamp technique [1] to bacterial membranes has led to some interesting observations. *Escherichia coli* has been reported to possess a 1 nS (350 mM KCl) channel [2], as well as a stretch-insensitive, voltage-modulated channel with a conductance of about 91pS in 150 mM KCl [3]. The latter gives rise to functional clusters exhibiting cooperative gating. Berrier and colleagues [4] detected a large number of conductances, some still stretch-sensitive, in proteoliposomes containing purified *E.coli* membrane fractions. In studies on gram-positive bacteria, Zoratti and colleagues found that the membrane of *Streptococcus faecalis* [5,6] and *Bacillus subtilis* [7] exhibited a variety of stretch-activated conductances.

In this communication, we report our patch-clamp study on giant *E.coli* spheroplasts. It provides evidence that the 1 nS stretch-activated conductance actually arises from the cooperative gating in multiplexes of one or more channel types, which may increase their open probability in response to membrane stretch or may function apparently without an applied stimulus after they become activated.

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0006-291X/90 $1.50

MATERIALS AND METHODS

Giant spheroplasts (5-10 μm) were obtained from *E.coli* strain DH1 by the penicillin method [8]. After harvesting, the spheroplasts were suspended in 350mM KCl, 10mM $CaCl_2$, 1mM $MgCl_2$, 5mM HEPES/KOH, pH 7.2. In all experiments reported the patch pipettes (Hildenberg 11411 glass, uncoated) were filled with the same medium. The data reported here were obtained from excised membrane patches. Experiments were conducted as described in [7]. Ohmic behavior was experimentally verified for most conductances (not shown).

RESULTS

Voltage-clamped spheroplast membrane patches often exhibited either no measurable step currents or only sparse activity by pores with conductances of about 9, 24 and 40-58 (one or more channels) pS. In these cases application of suction (0-10 cm Hg) by mouth to the pipette interior almost invariably resulted in the activation of large-conductance stretch activated (SA) channels. Fig. 1 A-B presents typical records obtained under these circumstances. The activity in Fig. 1A is the phenomenon described by Martinac et al. [2], and it is due to the operation of seemingly independent

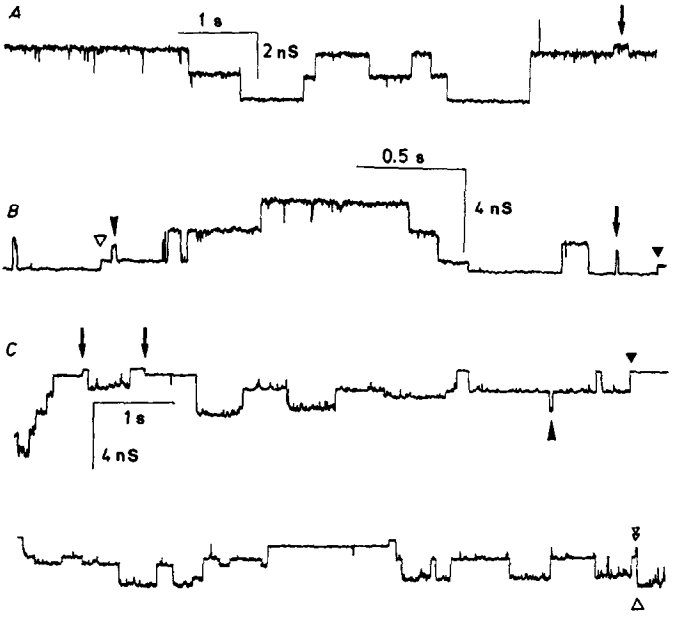

Fig. 1. *Typical stretch-induced (A and B) and spontaneous (C) channel activity.* A) V: +17 mV. Digitizing frequency: 5 KHz. Stretch induces the appearence of 0.95-1.1 nS steps. The arrow (↓) points to an approx. 4.6 pA (275 pS) event. B) V: +87 mV. Digitizing frequency: 10 KHz. Stretch-induced events. The largest steps fall in the range 1.2 - 1.3 nS. Arrows point to 36 pA (410 pS) (▽), 60 pA (690 pS) (▼), 87 pA (1 nS) (↓) and 31 pA (360 pS) (▼) events. C) V: -47 mV. Spontaneous activity. Continuous trace. Digitizing frequency: 5 KHz. Arrows point to approximately 16.4 pA (350 pS) (↓), 48 pA (1020 pS) (↑), 57 pA (1.2 nS) (▼), 26 pA (550 pS) (⇓) and 107 pA (2.28 nS) (△) steps. This last transition cannot be resolved into separate steps even at higher resolution (40 KHz digitizing frequency).

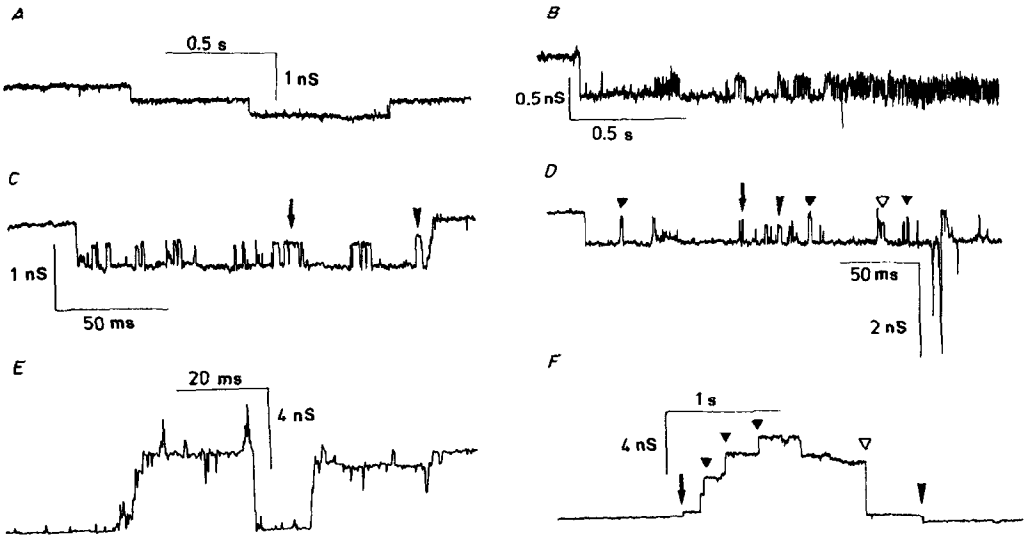

Fig. 2. *Membrane stretch-induced events.* Records A-E were obtained from the same patch. Digital sampling rate: 10 KHz unless otherwise specified. A) V: -23 mV. Current steps: 7.4 pA (320 pS). B) V: -43 mV. Initial opening step: 22.5 pA (520 pS); fast closures: approx. 11.6 pA (270 pS). C) V: -33.5 mV. Digital sampling: 20 KHz. Initial opening step: 32 pA (960 pS). Closures: approx. 16.4 pA (490 pS) ( ), 22 pA (660 pS) ( ). Notice the stepwise final closure. D) V: -33.5 mV. Digital sampling: 20 KHz. Initial opening step: 31.5 pA (940 pS). Closures (approx.): 27 pA (800 pS) (▼), 24 pA (720 pS) ( ), 17.5 pA (520 pS) ( ), 20 pA (610 pS) (▽). E) V: +67 mV. Digital sampling: 40 KHz. F) V: +27 mV. Digital sampling: 5 KHz. Current steps: 8.6 pA (320 pS) ( ); approx. 32 pA (1.15 nS) (▼); approx. 88 pA (3.3 nS) (▽); 13 pA (480 pS) ( ).

approximately 1 nS channels. Fig. 1B shows that conductances of other sizes appeared frequently.

In other instances, large-channel activity was evident from the outset of the experiment, even though no suction was applied after seal formation. Fig. 1C presents a typical record obtained in such a case. The activity observed is similar to that elicited by membrane stretch from the point of view of both the size of the conductances involved and of their general kinetic behavior. In other cases at the beginning of the experiment the channels were active only upon application of suction, but repeated stretch cycles resulted in their permanent activation to give the same behavior exemplified in Fig. 1C.

Figs. 2 and 3 present anthologies of records obtained with and without membrane stretch respectively. They are meant to illustrate the variety of conductance sizes observed and the frequent observation of a substate structure involving most of the conductances themselves. A more complete comparative catalogue is presented by Table I. The most commonly encountered events measured about 1 nS. Two events of this class are shown in Figs. 2C and 2D: they present brief partial closures, due to

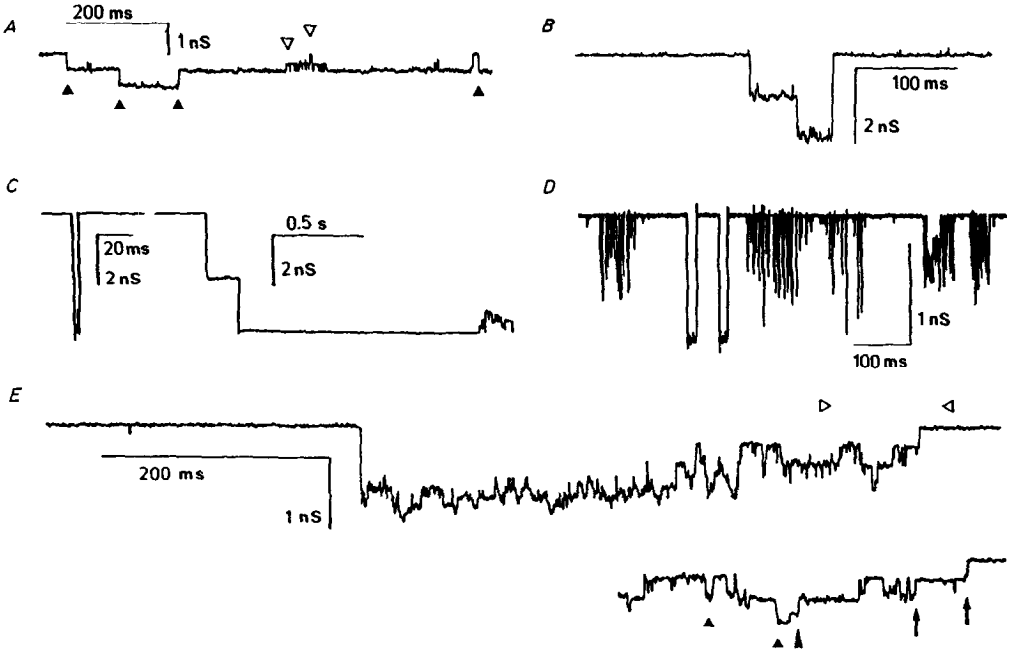

Fig. 3. *Traces recorded without applying a transmembrane pressure gradient (spontaneous activity).* Digital sampling frequency: 10KHz. A) V: -23 mV. Current steps: 12 pA (520 pS) (▲) and 5.7 pA (250 pS) (▽). B) V: -41 mV. Current levels: 47, 93 pA (1.15, 2.3 nS). C) V: -63 mV. Major step sizes (from left): 306, 168, 136 pA (4.9, 2.7, 2.2 nS). Events at left and right were separated by about 0.7 seconds of inactivity. Notice the partial closures at extreme right. D) V: -63 mV. Major steps: 80 pA (1.27 nS). E) Major opening: 81 pA (1.2 nS). The segment between the open triangles (▷ ◁) is shown below with an expanded time scale (4X; digitized at 20 KHz). Arrows point to steps of about 22.6 pA (340 pS) (▲); 14.8 pA (220 pS) (▲); 19 pA (285 pS)(▲).

transitions to conductance substates. The events must be attributed to what functionally was a single channel: several independent channels would have had a low probability of opening together in an initial step, especially since a steady pressure gradient was being applied across the membrane. Remarkably, the size of the closures coincided with the size of self-standing transitions. For example, the 490 and 520 pS partial closures of Figs. 2C and 2D find a match in the 480 pS transition of Fig. 2F and in the approx. 520 pS event of Fig. 2B, which, in turn, shows frequent, rapid half-amplitude closures. The observations leave no doubt that the 1 nS conductances possess a complex substate structure. Particularly tale-telling are stepwise transitions such as the final closure in Fig. 2C.

In turn, the 1 nS channels may be organized into aggregates capable of exhibiting cooperative behavior, exemplified by the events shown in Fig. 2E and 2F. In the former, four very closely spaced but distinct steps lead to current conduction at approx. 4.4 nS. Closure takes place instead in a single-step transition. In Fig. 2F, a series of approx. 1.1 nS openings is followed by a closure due to the simultaneous gating of three of these

TABLE I
Conductance values consistently observed in *E.coli* spheroplasts

a) +Stretch pS	b) -Stretch pS	c) Substates pS
155±30	150±35	140±25
240±20	225±25	220±30
350±25	350±30	330±30
410±30	425±25	420±30
525±25	550±50	520±30
640±30	650±50	620±30
760±10	750±10	740±30
850±25	850±30	820±50
1000±60	1050±60	
1250±70	1200±60	

The values are meant to be indicative of ranges of conductance values observed in symmetrical 350 mM KCl. Values below 120 pS have not been included.
a) Conductance steps appearing upon application of stretch (suction) to otherwise silent patches, and disappearing upon release of suction. b) Conductances appearing "spontaneously" from the beginning of the experiment or persisting after repeated application of suction. c) Closure-and-reopening transitions from "1 nS" stretch-induced single-channel events, as exemplified in Fig. 2C-D.

conductances. A few times we also observed events which began or ended with a fast spike to a high conductance level, as exemplified by Fig. 2D.

The recordings obtained without the application of membrane stretch presented the same picture. The sizes of the current steps closely matched those observed while applying stretch to otherwise inactive patches (Table I). A substate structure was also evident: as an example, Fig. 3A shows approx. 250 pS closures from a 520 pS conductance (compare Fig. 2B; see also Fig. 3C, extreme right). The composite nature of the 1 nS channels is again clearly evident in events such as the one in Fig. 3E : a one-step opening is followed by a protracted stepwise closure (see also Fig. 3C).

In the range 1.5-5.5 nS we measured no less than 12 distinct "spontaneous" single-step conductance values. It seems unlikely that the membranes possess so many independent huge pores. Rather, cooperative gating of channels appears again to be the most economical explanation. This concept is supported by observations such as those presented in Fig. 3B and 3C. In the former, two "1 nS" channels open separately but close almost together. In Fig. 3C, the size of a 4.9 nS single, fast event matches exactly the sum of two consecutive steps which occurred a short time later.

The larger-amplitude events were favored by higher transmembrane voltages and were often characterized by fast kinetics, sometimes giving rise to heavy flickering (see Fig. 3D).

DISCUSSION

The Results section presents evidence in favor of a composite structure of both the stretch-activated and the "spontaneous" channels. This, the match between the conductance values observed in the two cases (Table I), and the fact that "spontaneous" activity could be induced by repeated stretch cycles strongly support the notion that the activity in the two cases was due to the same channels. The variety and size of the apparently self-standing conductances, the match with the sizes of closures to substates, and a degree of regularity in stepwise transitions such as those of Fig. 3E suggest the following model: the bacterial membrane contains basic conductance units which normally combine to give functional "clusters" operating as single, stretch-activated channels. The most commonly formed "clusters" have a maximal conductance of about 1 nS, but other complexes, with different stoichiometries, may also form and conduct current. In particular, cooperative behavior may extend to "clusters of clusters" (See Fig.s 2E-F and 3B-C). Depending on ill-understood factors, including repeated or lengthy membrane-stretch, the channel may become capable to gate even when no stretch is applied. The possibility of a modular structure of the "1 nS" channel had been mentioned by Martinac et al. [2].

The size of the conductance "module" might tentatively be placed in the interval 110-130 pS. This range is close to one half of the value expected for the stretch-insensitive channel of Delcour et al. [3] in our medium. Those authors mentioned the occurrence of a "1/2 unit" conductance in their records. The possibility ought therefore to be considered that the "stretch-insensitive" activity described by Delcour et al. may coincide with the one described in this report, arising actually from "activated" SA channels. At present the most economical way to account for the numerous, closely spaced conductance values is to assume that the "modules" are capable of passing current in various conformational states having different conductances.

An identification of the SA channels with the major porins of *E.coli* seems unlikely [9]: porins have not been reported to be stretch-activated and they generally close only incompletely [10]. On the other hand, *E.coli* porins have been reported [11,12] to exist as triplets exhibiting a pronounced cooperative behavior, which can extend to clusters of triplets. These features point to a possible structural similarity between the SA channel and porins.

Which membrane the channels described here belong to is doubtful. The Madison group has provided evidence for a localization on the outer

membrane [9], while Berrier et al. [4] have assigned many of the conductances to the inner membrane fraction. Very similar activity has been found in gram-positive bacteria like *S. faecalis* and *B. subtilis* [5-7]. A possibility is that in gram-negative organisms these channels reside at the contact sites.

Inner mitochodrial membranes [13,14] and endoplasmic reticulum membranes [15] have been recently been reported to host similar, substate-rich high-conductance channels. The similarity of these widely distributed pores may well be expected to reflect similarities in their strucuture as well as in their function. The latter remain to be defined.

Acknowledgments

We warmly thank Prof. G.F. Azzone for his support and for useful comments, Prof. A. Peres for the use of some equipment, Dr.s A. Ghazi and B. Martinac for useful discussions and for providing manuscripts prior to publication, and Mr.s Righetti and Santato for technical assistance. We also thank the European Economic Community for support under the program "Stimulation-Science".

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