Channel-Sizing Experiments in Multichannel Bilayers

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Abstract. The possibility of obtaining information about the radius of high and low conductance states of channels in multichannel membranes was tested experimentally In spite of the interference of non electrolytes on the numbers of channels that appeared in the membrane the non electrolyte exclusion method was successfully adapted to multichannel bilayers to estimate the radius of the larger opening of the low conductance state of the channel induced by *Staphylococcus aureus* alpha-toxin At the pH used, the channel transition to a low conductance state was accompanied by a decrease of the opening radius from 1 3 ± 0 2 nm to 0 9 ± 0 1 nm The determination criteria for maximum size of a channel opening when using the non electrolyte exclusion method is discussed

Key words: Alpha toxin — Planar bilayer — Ion channel size — Conductance states — Multichannel bilayer

Introduction

Staphylococcus aureus alpha-toxin (ST) is a single chain protein with molecular mass 33 kDa (Gray and Kehoe 1984) Its ability to form transmembrane ion channels was established more than 15 years ago (Krasilnikov et al 1980, 1981) Since that time, the properties of the channel incorporated into lipid bilayers as well as into cell membranes have been under intensive study (for review, see Bhakdi and Tranum Jensen 1991, Krasilnikov et al 1991) Most likely, the channel is oligomeric,

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containing 7 molecules of the toxin (Gouaux et al 1994) Different approaches were used to establish the apparent radius of the channel which was found to be in the 0 5 1 4 nm range in biological membranes and in the 0 5 1 3 nm range in lipid bilayers (Bhakdi et al 1984, Menestrina 1986 Krasilnikov et al 1988a, 1992 Sabirov et al 1991, 1993 Walev et al 1993, Jonas et al 1994 Bezrukov et al 1996)

Bilayer experiments (Krasilnikov et al 1988b, 1990a, Kasianowicz and Bezrukov 1995) have demonstrated that at neutral pH, ST forms ion channels which mainly show a high conductance state Decreasing the pH led to an increase in the sensitivity of the channel to voltage gradient As a result, transitions between a high and a low conductance state occur more frequently and can be easily observed (Krasilnikov et al 1988b, 1990a, Korchev et al 1995) The nature of the gate is unknown although it was surprisingly similar to the current fluctuation shown in ion channels present in biomembranes In some recently published studies (Krasilnikov et al 1988a, 1991 1992 Sabirov et al 1991, 1993) on individual ST-channels the apparent radius of the channel was estimated to be 1 3 nm The channel radius in the low conductance state was found to be considerably smaller ($\sim$ 0 6 nm Krasilnikov et al 1990b) A few years later Korchev et al (1995) examined the channel cross-section in high and low conductance states and found much smaller changes in the channel radius (from $\sim$ 0 8 nm to $\sim$ 0 64 nm) The declared value for the radius of the ST-channel in high conductance states was considerably smaller ($\sim$ 0 8 nm) than the early data (Krasilnikov et al 1988a 1991, 1992, Sabirov et al 1991 1993), although both groups of authors have used non-electrolytes (NE) as molecular probes to size the channel *A resolution of this discrepancy was one of the aims of the present study*

In the original description (Sabirov et al 1991, 1993, Krasilnikov et al 1992), the NE- exclusion method allows to size individual pores by a technique based on the decrease of conductivity induced by high concentrations of neutral NE applied to both sides of lipid bilayers small NE that enter the pore decrease the conductance whereas large NE that do not enter it do not affect the conductance The method is based on observations made on a number of separate single channel events, and then the data are averaged in order to permit a fair decision about the sizes of both the high and the low conductance states *In this respect, a bilayer containing many channels seems to be a more appropriate system to study, being less time consuming and especially appropriate for low conductance channels The examination of the use of multichannel bilayers to determine channel size was the second aim of our study* Lipid bilayers modified by *S aureus* α-toxin were chosen as the model This model has advantages as well as disadvantages Information about the channel size (Krasilnikov et al 1988a, 1990b, Korchev et al 1995) facilitates the study On the other hand, the influence of polymer non-electrolytes on the equilibrium between high- and low conductance states of the channel and on

the process of pore formation (opening) has also been demonstrated (Zimmerberg and Parsegian 1986, Bashford et al 1993 Korchev et al 1995), a fact that strongly complicates the study Hence, if there is a possibility to establish the channel size for this complicate case the approach could be applied to any other channel The present study was undertaken to examine this possibility

Materials and Methods

S aureus α-toxin was donated by Dr K D Hungerer (Behringwerke Laboratories, Marburg, Germany) Pure phosphatidylcholine was prepared according to Bergelson et al (1981) or purchased from Sigma (St Louis USA) (Type V-E) Cholesterol was purchased from Sigma and used without any modification Glucose was purchased from Merck (Darmstadt, Germany) and sucrose from Reagen (Rio de Janeiro Brazil) Polyethylene glycol (PEG) 1000 and PEG 1450 (Sigma) PEG 2000, PEG 3000, PEG 4000 and PEG 6000 (Loba Chemie, Mumbai, India) were used as non-electrolytes (NEs) When necessary the non-electrolyte polymers were additionally purified by anion-exchange chromatography using strong alkaline anion exchangers (III or IV Merck) to remove anion groups containing contaminants which decrease the stability (life time) of bilayer lipid membranes and increase the probability of ion channel transitions from open to closed states Other chemicals were of analytical grade and were used without additional purification

Twice-distilled water was used to prepare all buffer solutions The standard solution used in the bilayer experiments contained 100 mmol/l KCl 5 mmol/l citric acid and the pH was adjusted with Tris to 4 0 In experiments carried out to determine channel size this solution also contained 17% or 20% (w/v) of an appropriate non-electrolyte In all cases the solutions on both sides of the bilayer were the same The hydrodynamic radii of non-electrolytes were obtained from recent viscometry studies (Sabirov et al 1991, 1993), and were as follows (nm) glycerol, $0\ 31 \pm 0\ 02$ glucose, $0\ 37 \pm 0\ 02$, PEG 1000, $0\ 94 \pm 0\ 03$ PEG 1450, $1\ 05 \pm 0\ 03$ PEG 2000 $1\ 22 \pm 0\ 03$, PEG 4000, $1\ 92 \pm 0\ 03$, PEG 6000, $2\ 5 \pm 0\ 03$ The conductivity of each buffer solution was measured with an HI 9033 (HANNA Instruments, Woonsocket, RI) multi-range conductivity meter at 25°C

Planar lipid bilayers were formed at room temperature (25 ± 2°C) by the technique of Montal and Mueller (1972) from a phosphatidylcholine-cholesterol mixture (1 1, w/w) Monolayers were spread from a 10 mg/ml solution of lipids in n-hexane, on the surface of two buffered salt solutions, separated by a 25 μm thick Teflon partition in a Teflon experimental chamber The orifice diameter was about 0 2 mm

Experiments were carried out under voltage-clamp The current through the bilayer was measured with Ag/AgCl electrodes connected via salt bridges (3% agar with 3 0 mol/l KCl) to the *cis*- and *trans*-compartments of the bilayer chamber

The *trans*-compartment was connected to virtual ground through an operational amplifier (K284UD1A) in the current-to-voltage configuration Voltage pulses were applied to the *cis*-compartment of the chamber The toxin was also added to the *cis*-compartment The solutions in both compartments were magnetically stirred The amplifier signal was monitored with a storage oscilloscope and recorded on a strip chart or tape recorder Basal conductance of non-modified bilayers was less then 4 pS More than 200 ion channels were recorded in each experimental condition (5 7 channels per membrane)

Protein concentration was determined with a Bradford reagent (Bio-Rad, California USA) using bovine serum albumin as the standard

Results and Discussion

As published elsewhere (Krasilnikov et al 1998), the size of individual pores can be evaluated by measuring a parameter which represents the filling (F) of a channel with NE F can be calculated as follows

$$F = ((g_o - g_i)/g_i/((\chi_0 - \chi_i)/\chi_i) \quad (1)$$

where g_o is the single channel conductance in the presence of an impermeable non-electrolyte or the single channel conductance in a solution without non-electrolytes g_i is the single channel conductance in the presence of a solution containing NE with access to the channel interior on both sides χ_o is the conductivity of the solution without non-electrolytes or the conductivity of the virtual volume free of non-electrolytes in a solution with non-electrolytes χ_i is the conductivity of the solution containing a given NE

To apply the method for measuring the size of the high and low conductance states of a channel in a multichannel bilayer we must show the existence of a correlation between related parameters at the single- and multichannel levels This can be done by comparing the conductance distributions of the single ST-channel at high and low conductance states, on the one hand and the conductance levels obtained in a multichannel bilayer at a low- and high transmembrane potential (when ST-channels are previously in a high and low conductance state (Menestrina 1986 Krasilnikov et al 1988a, b, 1990a, b, Korchev et al 1995) on the other one

As expected when ST was added at low concentrations ($\sim 0\,1$ μg/ml) to the aqueous solution bathing a voltage-clamped bilayer the membrane conductance increased in discrete steps, indicating the incorporation of ionic channels into the lipid bilayer The membrane potential was fixed at 20 mV Under these conditions channel openings were detected as upward current deflections Downward steps, representing the closing of the channel, were very seldom observed at this low voltage The conductance values for the unitary events were calculated and plotted

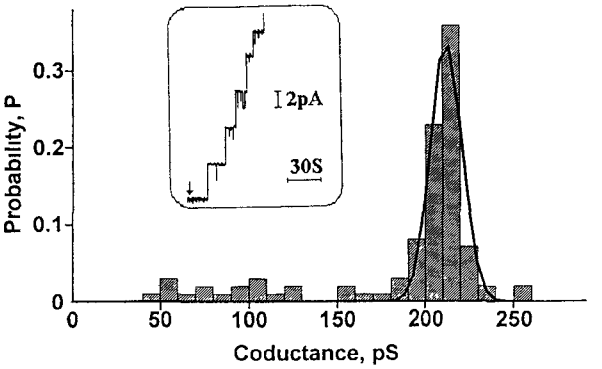

Figure 1. Amplitude histogram of conductance fluctuations for the high conductance state of the ST-channel The probability, P to observe conductance steps like the one shown in the current traces in the inset is represented Standard solution of pH 4 0 was used The record was discarded when any of the open channels temporarily closed ST was added to the *cis* compartment to a final concentration of $\sim$ 0 1 μg/ml The bilayer was clamped at 20 mV More than 200 ion channels were recorded (5 7 channels per membrane) Bin width was 10 pS All other conditions for the experiment are described in Materials and Methods The mean value for single channel conductance was obtained using Gaussian distribution for the main pool of this histogram (g_{high} = 212 $\pm$ 9 pS) A sample of an original single channel recording is shown in the inset The dashed line indicates zero current level the arrow indicates the addition of the toxin

in a cumulative histogram (Fig 1) A mean value of 212 $\pm$ 9 pS in 100 mmol/l KCl (pH 4 0) for single channel conductance (g_{high}) was obtained by fitting a Gaussian curve to the main pool of this histogram Hence we found that the high conductance state of the ST-channel is quite uniform in size, in agreement with data reported by others (Menestrina 1986, Krasilnikov et al 1988b, Krasilnikov and Sabirov 1989)

To study the low conductance state, the bilayers contained only one ST-channel The experiment was started as described above and when a single channel appeared the transmembrane voltage was increased to 100 mV Under these conditions channel closures were detected as downward current deflections, but the current never dropped to zero value This remaining conductance was used to construct a histogram and to measure the average low conductance Since ST-channels in the low conductance state change to a high conductance state after applying zero potential (Menestrina 1986, Krasilnikov et al 1988b, 1990a b, Korchev et al 1995) we used this property to determine the variations in conductance by observing about 10 transitions for a given channel These conductance values for many individual channels were plotted in a cumulative histogram (Fig 2) A mean value (g_{low}) of 27 $\pm$ 13 pS in 100 mmol/l KCl (pH 4 0) was obtained for this low conductance level by fitting a Gaussian curve to the main pool of this histogram The

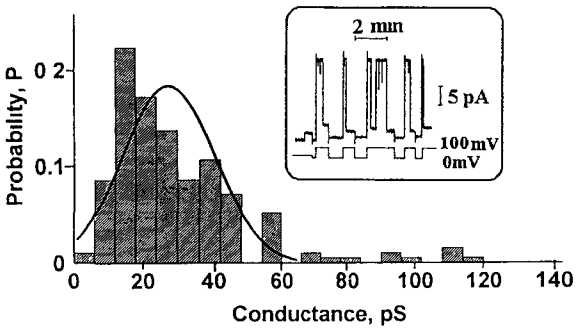

Figure 2 Amplitude histogram of conductance fluctuations for the low conductance states of the ST-channel The probability P to observe a low value of channel conductance is represented ST was added to the *cis* compartment to a final concentration of ~ 0 05 μg/ml Standard solution of pH 4 0 was used The analysis of the channel transitions to closed state conductances was done in bilayers containing only one channel The record was stopped when a second channel appeared Data obtained from more than 30 membranes are plotted Bin width was 6 pS For all other experimental conditions see Materials and Methods and the text The mean value of the low conductance state was obtained by plotting a Gaussian distribution for the main pool of this histogram (g_{low} = 27 ± 13 pS) An original recording is displayed in the inset The dashed line indicates zero current level At the beginning the bilayer was clamped at 20 mV When a channel appeared the potential was switched to 100 mV This forced the channel to go to a low conductance state In order to reopen the channel the transmembrane potential was transiently switched to 0 mV and then back again to 100 mV

distribution of the low conductance state was found to be much wider (in relation to its mean value) than that established for the high conductance state suggesting the existence of different low conductance states, as demonstrated previously (Krasilnikov et al 1990a) From this type of experiment, one can see that, under these conditions the mean value of the conductance of the low state is about 12% of that of the high state

In multichannel experiments we examined bilayer conductances at two different transmembrane potentials low (10 mV) and high (100 mV) for the high and low channel conductances respectively The experiment was carried out as follows after the bilayer was formed and its parameters stabilized, ST was added to the *cis* compartment at a relatively high concentration (~ 4 μg/ml) and voltage was clamped at 10 mV When the conductance of the bilayer reached approximately 10 nS the toxin-containing solution was replaced with fresh buffer The increase in bilayer conductance stopped within a few minutes and the final value (G_{high}) was determined at this point for ST channels in the high conductance state The transmembrane potential was then increased to 100 mV The conductance of the

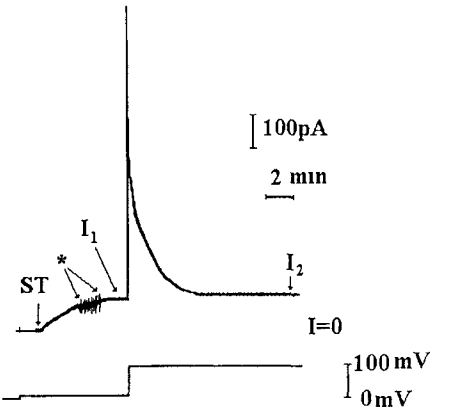

Figure 3 Time course of the current in response to the application of a voltage pulse to a lipid bilayer containing numerous ST-channels in the presence of standard buffer solution pH 4.0. The dashed line on the current trace indicates zero current. I_1 and I_2 are current levels used to calculate G_{high} and G_{low}. The voltage pulse protocol is shown below the current trace. The stepwise increase in transmembrane potential to 100 mV leads to the large instantaneous value of the current passed through the channels with a subsequent decrease toward a lower steady state value. At least five half times were allowed to pass before steady state conditions with G_{low} conductance were obtained. The asterisk with the arrows indicates the perfusion time interval of the *cis* compartment of the experimental cell with standard solution without the toxin. For other experimental conditions see Materials and Methods and the text.

low state G_{low} was determined when the current reached a steady level after the initial transient (Fig. 3). As a result the G_{low}/G_{high} ratio of about 0.12 was found. This is equal to the value of g_{low}/g_{high} obtained from single channel experiments. This finding indicates that channels in a multichannel bilayer behave in the same manner as in single channel bilayers.

With this in mind, one can apply the NE- exclusion method to estimate the size of the channel using multichannel experiments. In this case, Equation (1) which determines the filling of the channel with NE in the high conductance as well as in the low conductance states should be written in a slightly different form

$$F = ((G_o - G_i)/G_i)/((\chi_0 - \chi_i)/\chi_i) \tag{2}$$

where G_o (G_{high} or G_{low} for high and low conductance state, respectively) is the bilayer conductance in the presence of an impermeant NE or without any NE at either channel opening, G_i is G^{NE}_{high} or G^{NE}_{low}, and is the bilayer conductance in the presence of a solution containing NE with access to the channel interior on both sides. The conductance of non-modified bilayers ($\sim$ 4 pS) was negligible in

comparison with that of the modified bilayers, and was not taken into account χ_o and χ_i have the same meaning as in Equation (1)

Equation (2) is only valid if there is no marked influence of NE on the equilibrium between high- and low conductance states and on the opening process and the number of channels in the bilayer (N) is kept constant during the experiment Unfortunately this is not the case for ST induced channels, since the addition of some NE can change the number of functional ST-channels in the membrane This phenomenon, noted by Bashford et al (1993), taken together with recent observations of an increase in the numbers of operated ST-channels induced by a pH shift from any value to 5 6 (which is the pH value at which maximal rate of ST channel formation is observed (Krasilnikov et al 1986, 1991)), suggests the existence of a pre-formed, but not functional ST channel pool in the membrane This fact precluded the direct use of Equation (2) to determine the size of the high and low conductance states of ST channels in a multichannel bilayer simultaneously because at least two parameters (g_i and N) are unknown Thus, we were forced to first estimate g_i for one of the ST-channel states The high conductance state of the channel was chosen Values of g_i obtained in the presence of different NE in the bathing bilayer solution are shown in Table 1 These data can be used to determine the size of ST channel in the high conductance state by calculating F (using Equation (1)) and plotting this F against the hydrodynamic radii (r) of the NE (Fig 4) The radius of the channel can be considered to be equal to the hydrodynamic radius (r) of the NE corresponding to the point of transition from the falling part to the lower quasi-horizontal branch of the relationship of F

Table 1 High conductance state of ST channel in the presence of non electrolytes in the bathing solutions

	Non electrolyte	g_{high}^{pH4} (pS)	g_{high}^{pH6} (pS)	χ (mS/cm)
1	None	212 ± 9	130 ± 11	12 6
3	Glycerol	124 ± 11		7 8
4	Glucose	113 ± 8		7 5
5	Sucrose	112 ± 10	74 ± 10	7 6
10	PEG 1000	134 ± 20	97 ± 15	6 6
11	PEG 1500	181 ± 22*	103 ± 18	6 5
12	PEG 2000	213 ± 26*	136 ± 17#	6 5
13	PEG 4000	238 ± 28*	147 ± 20#	6 5

* and # mark differences at $P > 0\ 02$ (t test) In all other cases, $P < 0\ 005$ Standard solution of pH 4 0 and pH 6 0 was used All non electrolytes were used at 20% (w/v) concentration χ (mS/cm) is the conductivity of used solutions For other conditions, see the text

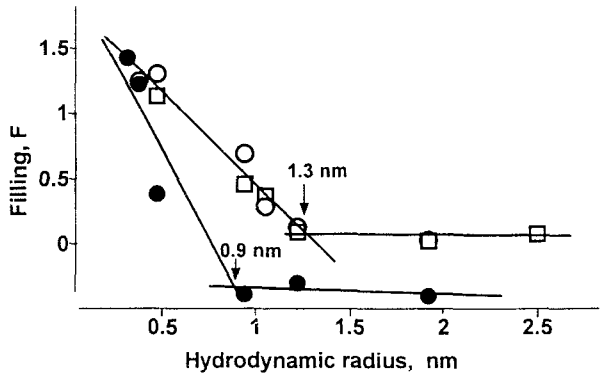

Figure 4. Dependence of parameter F on the hydrodynamic radius of the non-electrolyte for high (single channel level) and low (multichannel level) conductance state of the ST-channel. Symbols (○ and □) indicate the filling of the high conductance state of the ST channel with NE in single channel experiments at pH 4.0 and pH 6.0. For experimental conditions, see legends to Figures 1 and 2, and footnotes to Table 1.
Symbol (●) indicates the filling of low conductance states of the ST-channel with NE in multichannel experiments (pH 4.0). As described in text, data analogous to those presented in Figure 5 (G_{high}, G_{low}, G^{NE}_{high} and G^{NE}_{low}, together with values of conductances of the high conductance state of the single channel and the numbers of apparent channels in related conditions (Table 1 and Table 2), were used to calculate the apparent values of single channel conductance in the low conductance state and equation (1) was used to evaluate parameter F.
Lines are best fits to the experimental points. The horizontal line for the low conductance state of the ST-channel connects the points measured in the presence of PEG 1000, PEG 2000 and PEG 4000. The horizontal line for the high conductance state of the ST-channel connects the points measured in the presence of PEG 2000, PEG 4000 and PEG 6000. The points for radii ranging from 0.31–0.37 to 0.94–1.22 nm were used to draw another linear regression. The arrows indicate the maximal values of the radius of the ST-channel in the high and low conductance state. For types and sizes of used NEs, see Materials and Methods section. For other experimental conditions, see the text.

against r. Molecules with this and larger radii do not enter the channel and do not affect conductance. One can see that the value obtained for the channel radius was slightly larger than the hydrodynamic radius of the molecule PEG 2000 (1.22 nm), and equal to ~ 1.3 nm. The same values were found at pH 6.0 and 4.0 (Fig. 4). A similar radius was obtained earlier at pH 7.5 (Krasilnikov et al. 1988a, 1991, 1992; Sabirov et al. 1991, 1993), despite that fact that the conductance at acid pH considerably exceeds that at neutral pH (Krasilnikov et al. 1986, 1989, 1991; Kasianowicz and Bezrukov 1995).

As shown elsewhere (Krasilnikov et al. 1998), this method allows the maximal

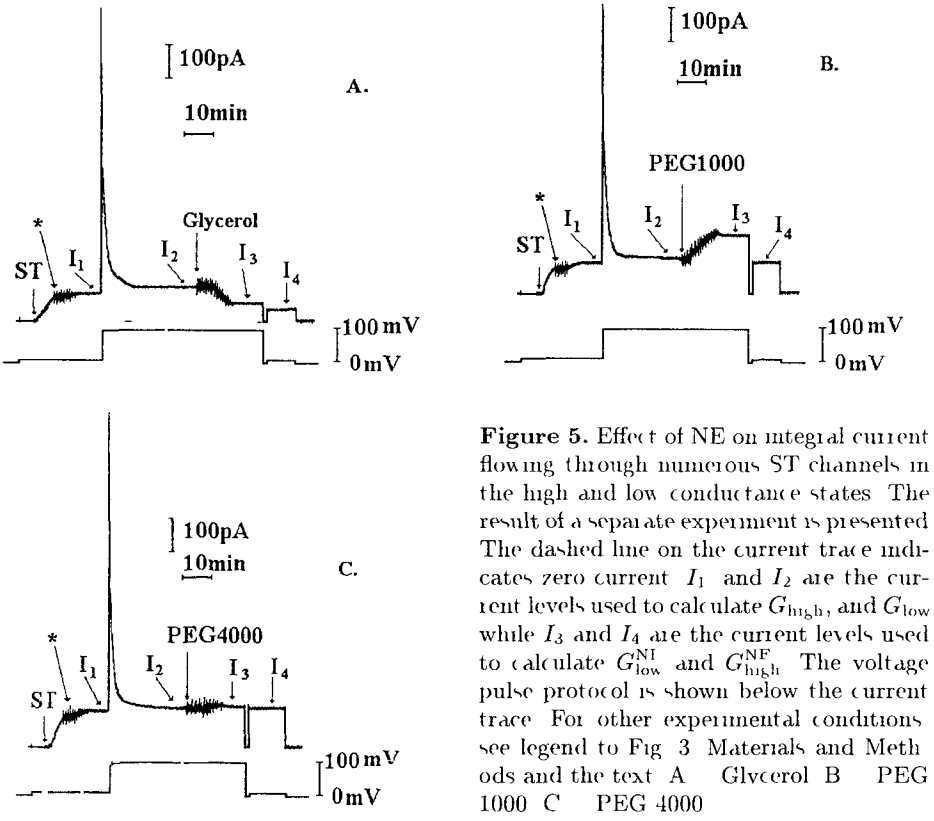

Figure 5. Effect of NE on integral current flowing through numerous ST channels in the high and low conductance states The result of a separate experiment is presented The dashed line on the current trace indicates zero current I_1 and I_2 are the current levels used to calculate G_{high}, and G_{low} while I_3 and I_4 are the current levels used to calculate G_{low}^{NI} and G_{high}^{NF} The voltage pulse protocol is shown below the current trace For other experimental conditions see legend to Fig 3 Materials and Methods and the text A Glycerol B PEG 1000 C PEG 4000

size of a channel opening to be established Consequently, the radius of the largest opening of ST-channels in the high conductance state is quite stable, does not depend on pH, and is close to 1 3 nm The data obtained are in excellent agreement with the recently established molecular architecture of the ST-channel (Song et al 1996) and are different from those published by Korchev et al (1995)

The data about q_i for the high conductance state allowed us to estimate the size of the low conductance state of the ST-channel in multichannel bilayers The experiment was started as described above (Fig 3) and the bilayer conductances (G_{high}, at 10 mV and G_{low} at 100 mV) for ST-channels in high and low conductance states were also determined Then, keeping the 100 mV potential, the solutions on both sides of the modified lipid bilayer were replaced with fresh (ST-free) basic solution with 17% (w/v) NE The result obtained using glycerol, PEG 1000 and PEG 1000 is presented in Fig 5 The conductance of the bilayer (G_{low}) was changed by substituting a new value (G_{low}^{NI}, determined by ST-channels in a low conductance state in the presence of NE) Two effects of NE may participate in this change 1)

an alteration in the number of functioning ion channels, and 2) a decrease in the conductance of the low conductance state of the ion channel. Only the latter effect should depend on the radius of the low conductance state of the ion channels. To correct for such effect we must subtract the influence of the non-electrolyte on the number of functional channels. Thus, we decreased the value of the fixed potential from 100 mV to zero for a few seconds and then fixed it at 10 mV. This protocol of voltage pulses is sufficient for complete transition of the ST-channels to their high conductance state (Menestrina 1986; Krasilnikov et al. 1988b, 1990a; Korchev et al. 1995). As a result, we determined the sum of conductances for all ST-channels (at their high conductance state) in the presence of NE (G^{NE}_{high}). Using the values of the single ion channel conductances in basic solution without (g_{high}) and with NE (g^{NE}_{high}) (which was established during the single ion channel radius determination) we could calculate the number of channels under these conditions (N^1 and N^2) as follows: $N^1 = G_{high}/g_{high}$ and $N^2 = G^{NE}_{high}/g^{NE}_{high}$. The results are presented in Table 2. One can see that addition to solutions bathing the multichannel bilayer of NE actually changes the number of functional channels. Knowing the number of channels allows us to find the mean value of the conductance of a single ST-channel in low conductance states without ($g_{low} = G_{low}/N^1$) and in the presence of NE in the solution bathing the membrane ($g^{NE}_{low} = G^{NE}low/N^2$). The results of this and many similar experiments with PEG 1000 as well as with other NEs were used to obtain reliable data for g_{low} and g^{NE} low under these conditions.

Table 2. Influence of non electrolytes on the numbers of ST channels opened in lipid bilayers

	Non-electrolyte	N^2
1	Glycerol	82.4 ± 18.3
2	Glucose	68.9 ± 10.2
3	Sucrose	75.0 ± 42.2
4	PEG 1000	187.0 ± 69.5
5	PEG 1500	144.2 ± 36.8
6	PEG 2000	103.9 ± 11.9
7	PEG 4000	65.7 ± 23.5

N^2 are numbers of channels after the addition of the NE to the bathing solution (expressed as % of those in pure buffer). For other conditions, see the text.

These findings permit us to use Equation (1) to calculate parameter F and to determine the channel radius of low conductance ST-channel from F against the hydrodynamic radius of NE dependence. The data are shown in Fig. 4 along with

those for the open state of the ion channel One can see that the maximal radius of the ST-channel openings decreases considerably upon transition from the high to the low conductance state (from 1 3 nm to 0 9 nm)

Using crystallographic data Song et al (1996) demonstrated that the two channel openings have almost the same radius On the other hand the NE-exclusion method provides information about the decrease in the maximal size of the channel openings during the channel transition to low conductance state Hence, we can suggest that both openings of the channel are involved (decrease in size) in the voltage-induced channel transition from high to low conductance state During this process the maximum cross-sectional area of the ion channel openings decreases more than two times from $\sim 5\,4$ nm^2 to $2\,5$ nm^2 The discrepancy between our data and those reported by others (Korchev et al 1995) who obtained a smaller difference (1 6 times) is certainly a result of differences in the evaluation of the channel radius from the relationship of F and r The size of the smaller NE which do not enter the channel at all, must be considered to be equal to the maximal size of the channel openings This criterion is in good agreement with the recently established molecular architecture of the ST channel (Song et al 1996) and was used in the present study whereas Korchev et al (1995) took a 50% "cutoff" size of NE as the size of the channel This latter assumption has no clear physical meaning By applying our criterion to Korchev's data one can obtain $\sim 1\,3$ nm and 0 9 nm as radii of the channel in the high and low conductance state, respectively This agreement suggests that the multichannel approach may validly be used for channel size determination (as used for the low conductance state of the ST channel in the present study) Using these data the calculated change in the maximum cross section of the ST-channel water pore during its transition from high to low conductance state is considerable, but still less than expected from the change in the channel conductance It should be pointed out, however, that channel cross-section might correlate with conductance for very large channels only (> 10 nm radius Pasternak et al 1993) For narrower channels (to which the ST channel belongs) the theoretical conductance data could differ from experimental conductances by a factor of 5 (Smart et al 1997) For these channels conductance should much more depend on the molecular nature of the surface, i e , charges and dipoles situated at the entrances and along the walls of the channel It should also be mentioned that image (Markin and Chizmadjev 1974) and friction (Antonov 1982) forces can also interfere with the cross section/conductance relation These explain the deviation of conductance from expected values This also accounts for the changes in selectivity and in conductance at high conductance state observed with a shift in bathing solution pH in using ST-channels (Menestrina 1986, Krasilnikov et al 1986 Krasilnikov and Sabirov 1989, Krasilnikov et al 1991, Bezrukov and Kasianowicz 1993)

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