Ion Transport Through Channels Formed in Lipid Bilayers by *Staphylococcus aureus* Alpha-Toxin

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Abstract. Staphylotoxin channel appears to be predominantly anion-selective with non-linear and asymmetric current-voltage characteristics (CVC) at neutral pH. Increased salt concentrations induce linearity and asymmetry of CVC and loss of selectivity. At lower pH both the channel conductivity and anion selectivity increase. Higher temperatures raise the channel conductivity in parallel with the changes in electrical conductivity of the salt solution, but do not change selectivity. Experimental dependences are described obtained by approximation of electrical diffusion and considering the interactions of penetrating ions with fixed charges at the entrances and the channel energy profile.

Key words: *Staphylococcus aureus* α-toxin — Ion channel — Simulation of electrical diffusion

Introduction

The cytolytic effect of staphylococcal alpha-toxin (ST) is known to be connected with its ability to form ion channels in lipid matrix of plasma membranes. In our previous paper we showed that the structure of the ST channel formed in artificial bilayer lipid membranes (BLM) is similar to that in erythrocyte membranes (Krasilnikov et al. 1988). This channel appears to be a water-filled pore composed of six toxin molecules, with a diameter of 2.5—3.0 nm and a length of up to 10 nm; it protrudes 4—5 nm from one membrane plane. The present article deals with the detailed analysis of ion transport through ST channels.

Materials and Methods

S. aureus alpha-toxin was isolated from a commercial preparation (Institute of Epidemiology and Microbiology, Moscow) according to Watanabe (1976). The toxin was proved homogeneous by

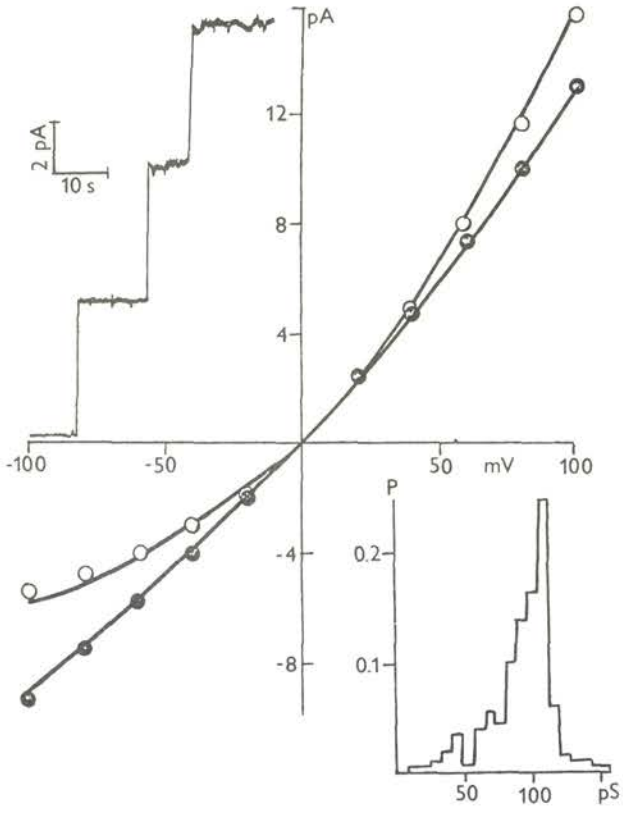

Fig. 1. Current-voltage characteristics of staphylotoxin channels in phosphatidylcholine (PC) (filled circles) and phosphatidylserine (PS) (open circles) BLM. Current steps, PC BLM, in the presence of 0.2 μg/ml ST. Inset: Distribution of ST channel conductance amplitudes in PC BLM. The total number of events is 180. Medium: 0.1 mol/l KCl, pH 7.5. The solid curves in this Figure and those in Figures 2—4 were calculated using equations (1)—(5).

SDS electrophoresis in PAG (m.w. 32 $\pm$ 2 kD). On isoelectric focusing the preparation contained one main component with isoelectric point at pH 8.4 and trace amounts of protein at pH 8.2.

Chromatographically pure phosphatidylcholine (PC) from egg yolk and phosphatidylserine (PS) from ox brain were prepared according to Bergelson et al. (1981).

BLM were formed in the usual way from 1—2 % lipid solution in *n*-octane in a thermostatic two-chamber Teflon cell. The "trans" compartment of the cell was considered the virtual ground and the potential sign was given with reference to it. Current was taken as positive for cation movement to this compartment. Unless specially mentioned, BLM were formed of PC at 25 °C. Tris citrate buffer, pH 8.4, 5—10 mmol/l was used. The channel conductance was measured at 50 mV. The toxin was usually added to the "trans" compartment.

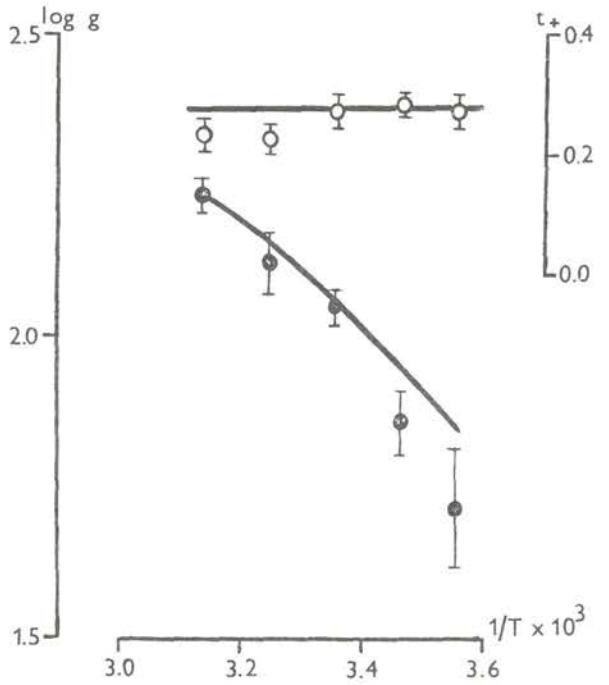

Fig. 2. The effect of temperature on conductance and cation-anion selectivity of ST channels. Symbols: ● — logarithm of ST channel conductance in 0.1 mol/l KCl solution, pH = 7.5; ○ — cation transference numbers calculated from the diffusion transmembrane potentials at 3-fold KCl concentration gradient (0.05/0.150 mol/l, pH = 7.5). Abscissa: inverse temperature values, K°.

The cation transference number (t_+) was calculated using zero current potential (E_m) observed in the presence of KCl concentration gradient according to

$$t_+ = (E_m - E_a)/(E_c - E_a)$$

where E_c and E_a are the respective theoretical Nernst potentials for cations and anions in this system.

The electrical conductance of aqueous solutions was measured by a conductometer type OK 102/1 (Radelkis, Hungary). Half of the specific electrical conductance measured divided by electrolyte concentration was taken as equivalent ion electrical conductance of KCl solutions.

Results and Discussion

The addition of alpha-staphylotoxin in a final concentration of 0.1—0.5 μg/ml to one BLM side resulted in a steplike increase of membrane conductance (Fig. 1). The histogram of a single channel conductance amplitude shows a

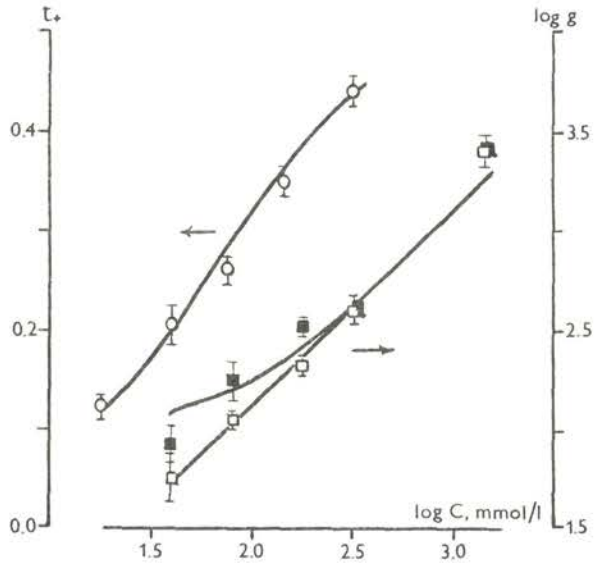

Fig. 3. The dependences of cation-anion selectivity and conductance of ST channels on KCl activity. Symbols: ○ — cation transference numbers calculated from the slope of zero current potential dependence at fixed KCl activity on one BLM side (abscissa) and varying KCl activity on the other side; pH of the medium was 7.5. □ — ST channel conductance at pH = 7.5; ■ — ST channel conductance at pH = 4.0.

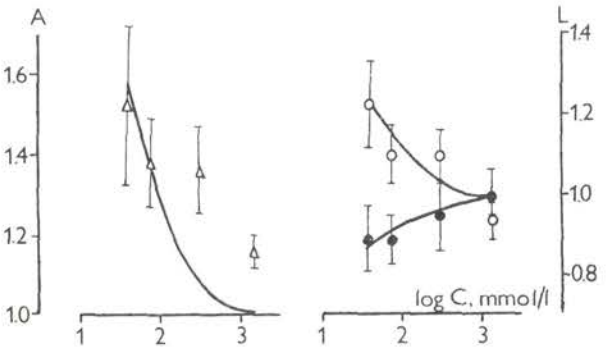

Fig. 4. The dependence of ST channel CVC parameters on KCl activity in the medium at pH = 7.5. *Left:* CVC asymmetry (△). *Right:* CVC non-linearity (*L*) at positive (○) and negative (●) potentials. For details see the text.

marked peak at 110 ps (Fig. 1). This was exactly the pool used for further analysis.

In order to understand the nature of the forces that determine the transport of ions through ST channels we studied the effects of temperature, electrolyte concentration and pH of aqueous solution on the conductance, cation-anion selectivity and current-voltage characteristics (CVC).

An increase in temperature of the aqueous solution results in an increase of ST channel conductance (Fig. 2). Activation energy (E_{ae}) of ion transport through ST channel is 15.6 ± 1.4 kJ/mol in the temperature range 25—45 °C. This is close to E_{ae} value for the electrical conductance of aqueous solution (14.2 ± 0.5 kJ/mol) (Dobos 1980).

The channel conductance undergoes linear increase if KCl concentration grows (Fig. 3). This is in support to our earlier conclusion that the ions in the channel are in the same environment as they are in aqueous solutions (Krasilnikov et al. 1988), i.e. the ST channel appears to be a waterfilled pore.

It should be noted that at temperatures ($t°$) < 25 °C the dependence of ST channel conductance (g) on $t°$ (Fig. 3) differs statistically significantly from the Arrhenius plot. This may reflect changes in lipid bilayer structure and/or channel geometry.

Non-linearity and asymmetry of ST channel CVC suggest an inhomogeneity of its energy profile. The relationship between the current through a single channel at +100 mV and its value at −100 mV (A) is taken as a quantitative measure of CVC asymmetry, and the non-linearity measure corresponds to the relationship of the current value measured at ±100 mV and the rated one, obtained by linear extrapolation of the current value at ±20 mV (L). CVCs were found to be asymmetric ($A = 1.5 \pm 0.2$) and non-linear at 40.3 mmol/l KCl, i.e. they are hypolinear at negative ($L_- = 0.89 \pm 0.08$) and hyperlinear at positive potentials ($L_+ = 1.22 \pm 0.11$). CVCs become linear and asymmetric when KCl concentration increases (Fig. 4). In the case the initially anion-selective channel ($t_+ = 0.19$ in 17 mmol/l KCl) becomes non-selective (Fig. 3). These data suggest the electrostatic nature of the interactions that determine the energy profile of ST channel.

This is further supported by our data on the effect of pH on CVC shape. At pH below 6.0 the CVC asymmetry undergoes marked changes, i.e. the channel becomes more conductive at minus potentials that at plus potentials (Fig. 5). This points to the crucial role played by the group with pK ~ 6.5 in the determination of CVC shape.

Lower pH values induce changes in other characteristics of open ST channels: both the channel conductance and ion selectivity increase (Fig. 5).

ST channel appears to be a water-filled pore that carries a certain number of asymmetrically located charges that interact electrostatically with the pene-

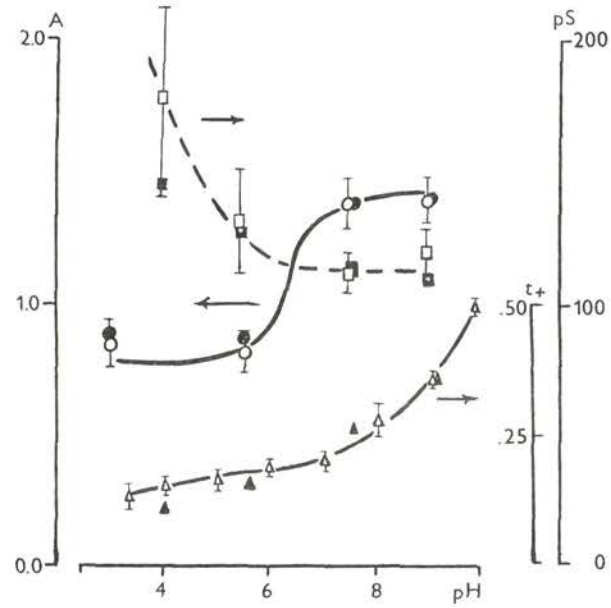

Fig. 5. Effect of pH of the medium on staphylotoxin channel properties. Symbols: □ — channel conductance in 0.1 mol/l KCl solution; ○ — channel asymmetry in the same medium; △ — cation transference numbers calculated from the zero current potential at a 3-fold KCl concentration gradient (0.04/0.12 mol/l). Filled symbols stand for the corresponding values calculated by the equations (1)–(5) (see the text). The curves were drawn by eye.

trating ions and determine CVC, conductance and selectivity of the channel.

We attempted describing the ion transport through ST channel by assuming ion diffuson and migration in an asymmetrically charged water pore and considering the interactions of penetrating ions with fixed charges at the channel entrance. A similar approach proved unsuccessful in describing CVC of hemocyanin channels (Menestrina and Antolini 1982). We believe that the reason for this failure is to be searched in the fact that the above authors ignored the energy profile of the charge-carrying water pore.

For an arbitrary profile of potential energy in a membrane and equilibrium at the channel entrances the partial current density is determined according to Markin and Chizmadzhev (1974) by:

$$I_i = Z_i \lambda_i \frac{RT}{F} \frac{C_1^i \exp(Z_i \psi) - C_2^i}{\int_0^{\delta} \left[\exp\left(Z_i \psi \left(\frac{\delta - X}{\delta}\right) + W_i\right) \right] dx} \tag{1}$$

where Z_i is the charge of ion i; λ_i is the equivalent electrical conductance; δ is

the channel length; X is the channel length coordinate which is zero at the left channel entrance ("trans") and δ at the right entrance (see Fig. 7); W_i is the potential energy of the ion in the pore; kT; $\psi = \psi_0 + \varphi_1 - \varphi_2$ is the intermembrane of potentials; kT/e; C_1 and C_2 are equilibrium ion activities at the entrances (1 — left) ("trans") and (2 — right), connected with ion activity in water solution (C_0) by the Boltzman relationship

$$C^i_{1,2} = C^i_0 \exp(Z_i \varphi_{1,2}) \tag{2}$$

where

$$\varphi_{1,2} = \frac{eQ_{1,2}}{4\pi\varepsilon\varepsilon_0 r} \exp(-r/L_D) \tag{3}$$

is the equilibrium potential generated by the charges at the channel entrances; $Q_{1,2}$ is the total charge at entrances 1 and 2; r is the channel radius; $L_D = 1/F\sqrt{\frac{\varepsilon\varepsilon_0 RT}{2C_0}}$ is Debye length; e, π, ε, ε_0, T, R, F, k have their usual meaning.

Considering the interactions of ions with charges at the channel entrances, the potential energy profile may be expressed as

$$W_i = Z_i\varphi_1 \exp(-x/L_D) + Z_i\varphi_2 \exp\left(-\frac{X-\delta}{L_D}\right) + E \tag{4}$$

E must be included due to several reasons. First, ions in the channel are subject to an action of image forces (Markin and Chizmadzhev 1974). Moreover, hindrance to the ion movement due to friction forces should also be considered (Antonov 1982). The principal peculiarity of the latter forces is their independence on the charge sign of the penetrating ions. For simplicity X-independent E was taken for the first approximation.

The total current is the product of the sum of partial currents and the channel cross section square:

$$I = \pi r^2 \Sigma I_i \tag{5}$$

To compare the rated and measured values of currents flowing through ST channel the values of 1.3 nm and 10 nm were taken for the radius and length, respectively. In this case Q_1, Q_2 and E are unknown parameters. Numerical analysis of the model showed that t_+ is determined only by the sum of charges at the entrances ($Q_1 + Q_2$) and that it is independent of E. To obtain the parameters of interest we used the experimental dependences of t_+ on log C and A on log C, as this allows to exclude E and to reduce the number of unknown variables to two. Parametrization was carried out by the least square method using minimum random search algorithm (Eler 1972). The computations were

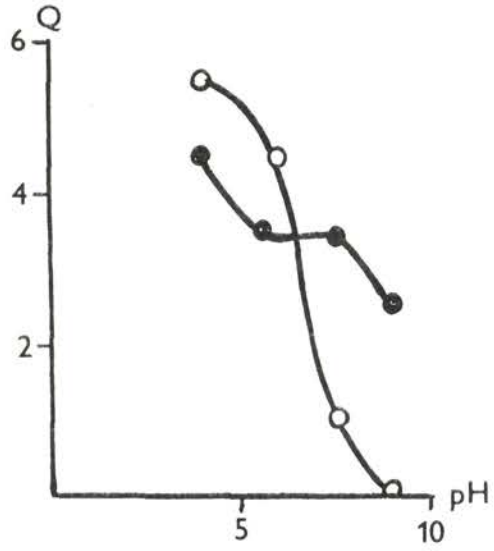

Fig. 6. Rated values of the total charge in the channel mouths at various pH. Symbols: ● — Q_1; ○ — Q_2. For details see the text.

performed using an Iskra 226.6 microcomputer. Confidence intervals for the parameters were found using the Monte-Carlo method (Bard 1979). The integral in (1) was obtained numerically by the trapezium formula. The best approximation was obtained at $Q_1 = 3.46 \pm 0.04$ and $Q_2 = 1.05 \pm 0.014$.

One of the criteria proving a model is correct appears to be the predicting ability. The given set of parameters describes well the dependences of g on T, t_+ on T, L on log C and CVC, and the dependence of g on log C (at $E = 1.95$). This is evident from the comparison of the rated with the experimental curves (Figs. 1—4).

The addition of 4.5 negative charges to Q_2, while passing to negatively charged bilayers from PS, was sufficient to explain both the growth of t_+ up to 0.53 and the increase in CVC asymmetry ($A = 2.8 \pm 0.6$) as well as its nonlinearity ($L_- = 0.63 \pm 0.06$ and $L_+ = 1.4 \pm 0.1$) compared to the same channel parameters in BLM of PC (Fig. 1). This coincides well with asymmetric channel position in the bilayer plane and also with the above assumption of only one of the mouths (corresponding to Q_2) being in contact with the polar lipid heads. A slightly lower value of E (1.85) may appear reflecting the difference between dielectric permeabilities of the hydrophobic zones of PS and PC bilayers.

The correspondence of the theoretical model to experimental results obtained at various pH was checked as follows. At each given pH the values of Q_1

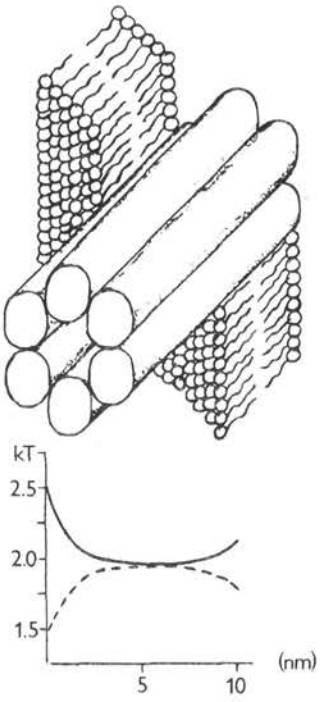

Fig. 7. Proposed structure of the staphylococcal toxin channel. Left mouth—charged residues of carboxyl-containing amino acids (glutamic and aspartic amino acids) and lysines, right mouth—histidine residues. $Q_1 = 3.46 \pm 0.04$ and $Q_2 = 1.05 \pm 0.014$ at pH = 7.5. Bottom: energy profile of the channel corresponding to the given charge locations. *Abscissa:* distance from the left entrance in nm; *Ordinate:* energy, kT. The mouth with the histidine residues is the only one in contact with the polar lipid heads.

and Q_2 (at constant E) were chosen so as to have co-ordinated values of channel conductance, CVC asymmetry and cation-anion selectivity. This approach introduces strict limitations as to the choice of parameters. For example, passing from pH = 7.5 to pH = 4.0 the increase in anion selectivity, i.e. the increase in total number of positive charges, is accompanied by a change in CVC asymmetry as well as by an increased channel conductance. In the model this corresponds to the growth of Q_1 by 1.4 and Q_2 by 4.45 elementary charges.

The results of this experiment are shown in Fig. 6. The shapes of the curves for the dependences of Q_1 and Q_2 on pH suggest the presence of two types of groups with pK $\sim$ 4 and $\sim$ 9 at one of the entrances (corresponding to Q_1) and of a further group with pK $\sim$ 6.5 at the other entrance (that is in contact with lipid polar groups). Judging by pK values one may suggest that Q_1 is determined mainly by the charges of carboxyls of dicarbon amino acid residues and by those

of ε-amino groups of lysine residues, while Q_2-mainly by the charges of histidine residues.

The rated values for conductance, CVC asymmetry and cation-anion selectivity of ST channels (filled circles in Fig. 5) agree sufficient well with the experimental measurements. These parameters can be successfully used to describe the non-linear dependence of ST channel conductance on KCl activity at pH 4.0 (Fig. 3).

Thus, by considering simple electrostatic interactions of passing ions with charges at the channel entrances (based on the electrical diffusion approach) one may describe with good approximation the properties of an open staphylotoxin channel. The results of our study allow to assume the structure of the staphylotoxin channel and its potential energy profile as shown in Fig. 7.

A similar approach may be applied also to describe the properties of other channels in artificial and natural membranes.

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