The Structure of *Staphylococcus aureus* α-Toxin-Induced Ionic Channel

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Abstract. Polyethylene glycols (PEG) with molecular weight $\leqslant 3000$ were shown to effectively protect human erythrocytes from osmotic lysis induced by α-staphylotoxin (ST). PEG with MW < 3000 do not change the conductivity of ion channels induced by ST in bilayer lipid membranes (BLM). Changing the bilayer from a pure phosphatidylcholine (PC) to a negatively charged phosphatidylserine (PS) film results in an asymmetry of the current-voltage characteristics. This is evidenced by the asymmetrical position of the ST-channel pore in bilayer membranes. The results obtained allow to conclude that the ST-channel is an interprotein pore filled with water (with an inner diameter of 2.5—3 nm and a length of ~10 nm). It is composed of six molecules of α-toxin from *Staphylococcus aureus*. The ST-channel incorporates into a membrane with only one mouth in contact with the polar lipid heads and the other one protruding 4.5—5 nm from the bilayer plane in water solution.

Key words: *Staphylococcus aureus* alpha-toxin — Ionic channel — Lipid bilayer

Introduction

The staphylococcal α-toxin is one of several cytolytic extracellular toxic proteins produced by *S. aureus*. The virulence of the microorganism is determined to a great extent by this toxin. The toxin seems to be a simple polypeptide chain with MW of 3300 D. Its amino acid composition has been proposed recently (Gray and Kehoe 1984) on the basis of the complete nucleotide sequence of its cloned gene. Earlier studies concluded that the biological effects of this toxin (hemolytic and dermal necrosis inducing effects, actions on neuronal cells and smooth muscles, etc.) are the result of physical damage to cell membranes (Bernheimer and Rudy 1986). Some authors believe that α-toxin induces the formation of

small transmembrane ion-permeable pores in target membranes (Füssle et al. 1981; Bhakdi et al. 1984). The toxin has been shown to increase the conductance of BLM by forming discrete ion channels (Krasilnikov et al. 1980; Menestrina 1986). The present paper describes experimental evidence of the molecular model of ST channel.

Materials and Methods

a-Toxin produced by *Staphylococcus aureus* strains 0—15 (IEM, Moscow) was purified according to Watanabe (1976). On SDS polyacrylamide gel electrophoresis the a-toxin was shown to contain only one band with MW $33 \pm 2 \text{kD}$. Isoelectric focusing of the a-toxin preparation over wide pH range (pH = 3—10) revealed the presence of the main component at pH = 8.4 and some trace material at pH = 8.2.

TLC-pure phosphatidylcholine and phosphatidylserine were obtained from fresh hen eggs and ox brain, respectively, according to the method described by Bergelson et al. (1981).

Optically black BLM were prepared with the common technic from 1—2% (w/v) lipid solution in *n*-octane in a thermostated Teflon cell filled with aqueous electrolyte solution (0.1 mol/1 KCl, 0.01 mol 1 Tris-citric acid, pH = 7.5, $t = 25\,^{\circ}\text{C}$). BLM were formed on hole ($d \sim 0.4\,\text{mm}$) in a partition used. The conductance of ST-induced single ion channels was measured as described elsewhere (Ermishkin et al. 1977). The *trans* compartment was connected to virtual ground. It was used to read the supplied voltage. The current was considered positive if cations were flowing into this compartment. Protein was added to the *cis* compartment. The cation transference number (t_+) for the membrane was calculated from the measured zero current potential (E_m) in a 0.12 (*cis*)/0.04 (*trans*) mol 1 KCl system by

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

where E_c and E_a are theoretical Nernst potentials for cations and anions in the system, respectively.

Hemolytic activity was determined on microtiter plates with 2% human erythrocyte suspension in citric-Tris buffered saline by a method similar to that described by Bhakdi et al. (1984).

Polyethylene glycols of different molecular weights (1000—6000 D, Ferak, Berlin and Lobo Chema) were used for the determination of the ionic channel diameter. The conductance of the buffer solutions was measured with a Radelkis OK 102/1 conductometer.

Results and Discussion

To understand the mechanism of the cytolytic action of various toxins, protective effects of various non-electrolytes with different molecular weights (Bhakdi et al. 1984; Weiner et al. 1985) were studied. This approach is based on the assumption that compounds unable to penetrate membrane pores (leaks) can prevent colloid-osmotic lysis and balance osmotic properties of intracellular impermeant substances. Dextran 4 is a known protector against toxin-induced hemolysis (Bhakdi et al. 1983).
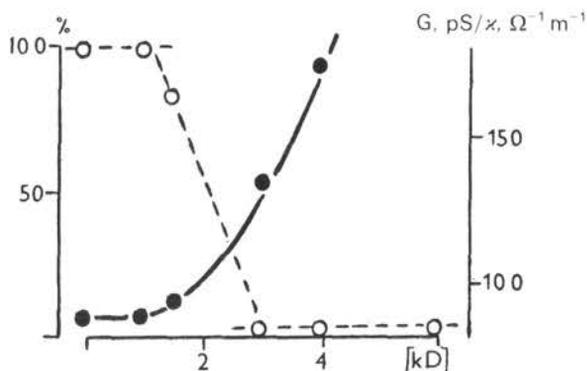
Fig. 1. Effect of polyethylene glycols on hemolysis of human erythrocyte induced by *Staphylococcus aureus* α-toxin (empty circles, left) and on the conductivity of single ST-channels normalized to electric conductivity of aqueous solution (filled circles, right). PEG concentration was 0.01 mol/l for hemolysis and 0.05 mol/l for bilayer experiments.

PEG-1000 and PEG-1500 were shown to leave hemolysis intensity unchanged (Fig. 1), whereas PEG-3000—6000 almost completely inhibited the toxin-induced lysis. The transfer of cells that seem intact to toxin and PEG-free medium results in a rapid lysis, very similar to that observed in absence of PEG. Thus, ST molecules obviously form pores both in the presence of PEG 3000 —6000 and in PEG-free medium. The calculated molecular weight of a PEG molecule that may have a 50% protection effects against lysis was 2000—2500 D.

α-toxin is known to induce ion channel formation in BLM (ST-channels). The conductivity distribution function of single channels in 0.1 mol/l KCl at 50 mV (pH = 7.5) is shown in the inset in Fig. 2. Although the channel conductivity varied significantly the great majority of events are induced in the well-pronounced peak at 110 pS which is the subject of our analysis.

The addition of urea (8 mol/l), PEG-1000 or PEG-1500 (0.05—0.2 mol/l) to 0.1 mol/l KCl bathing solution resulted in lowered ST-channel conductance. The decrease was nearly proportional to that of the bulk solution conductance. Thus, ST-channel seems to be a waterfilled pore and the movement of permeating ions and nonelectrolytes through it is similar to that in the bulk solution. After the addition of PEG 3000—6000, the ST-channel conductance was the same as in the initial solution. This may probably be explained by the fact that the diameter of the ST-channel is smaller than the effective diameter of PEG 3000—6000 molecules, which do not pass it. In this case, the microenvironment of permeable ions in the ST-channel and that in the aqueous solution of the
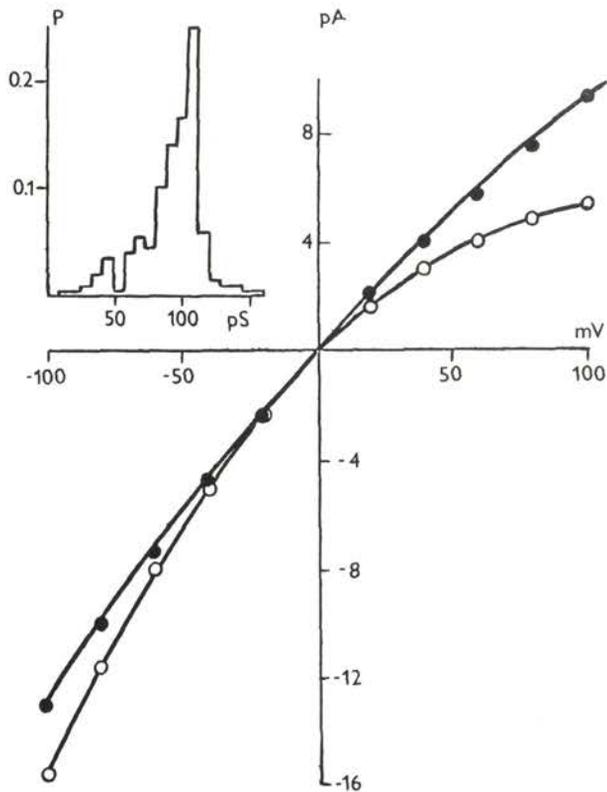
Fig. 2. Current-voltage characteristics of ST-channels in BLM. a) Phosphatidylcholine (filled circles); b) Phosphatidylserine (empty circles). Inset: the distribution of channel conductivity amplitudes in PC BLM. Experimental conditions: clamp voltage 50 mV; total number of events, 180 in 0.1 mol/l KCl (pH = 7.5); bin width 8 pS.

PEG-free electrolyte are identical. This is supported by the similarity of the activation energy of electrical conductance of the aqueous solution of electrolyte (14.2 ± 0.05 kJ/mol) and that of the ST channel conductance (15.6 ± 1.4 kJ/ mol). The plot of normalized ST channel conductance and molecular weight of PEG is clearly biphasic. The curve slope changes around PEG 2000—2500. This corresponds to molecular weight of PEG responsible for half-maximum inhibition of the hemolytic action of ST. The results obtained suggest the identity of ST-induced channels in BLM and those in erythrocyte membranes. This also means that the effective radii of the PEG 2000—2500 molecules are equal or similar to those of the ST-channel water pores.

Viscometric radii of PEG-1000 and PEG-1500 molecules are known to be

1.0 and 1.2 nm, respectively (Weiner et al. 1985), and the molecule should be spherical in shape. If the molecules of all other PEG used are spherical, then the calculated radii of PEG-2000—2500 molecules are 1.26—13.6 nm. Most probably, the same size is also characteristic of water pores of the ST-channel. It should be noted that this value differs from that suggested by Bhakdi et al. (1984), while being in agreement with the reports of some other investigators (Freer et al. 1968; Füssle et al. 1981).

Earlier electron optic investigations (Arbuthott et al. 1973; Füssle et al. 1981) showed that α-toxin from *S. aureus* forms ring structures on membrane targets. The outer and the inner diameter of these rings was 8.5—10 nm and ~ 2.5 nm respectively. Biochemical analysis of these ring structures showed them to have MW ~ 200 kD. They are made of six ST molecules (MW 33 kD). Thus, there is a striking similarity between the diameter of water pores in ST-channel found in our experiments and the inner diameter of ST-induced ring structure on membrane surface measured electronoptically by Füssle et al. (1981).

From the point of view of simple geometry each of the six staphylotoxin molecules that form a ring structure with an inner diameter of 2.5—3 nm cannot be spherical; rather it should have a prolonged cylindrical shape. Suppose a protein density of 1 g/cm^3 and the cross section diameter of such a cylinder equal to that of the water pore of ST-channel. Then, the calculated length is 9.5—10 nm. This exceeds the thickness of the PC bilayer which is 5—6 nm (Passechnik 1982). Hence, ST-channel should protrude 2 nm symmetrically from the membrane or 4 nm on one side. To decide between these two possibilities, the relationship of the cation transference number (t_+) for ST-channel and BLM composition should be accounted for. A change of BLM from pure PC to negatively charged PS results in an increase in t_+ from 0.23 to 0.53 (the KCl gradient is 0.04/0.12 mol/l pH = 7.5). Thus, the channel mouth is situated close enough to the membrane surface. This is suggested by the dependence of the asymmetry of voltage-current characteristics of single ST-channels in the presence of 0.1 mol/l symmetric electrolyte solution on the bilayer composition (Fig. 2.). In addition, the data point to different numbers of polar heads of lipid molecules in contact with the ST-channel mouths. In the limited case lipid molecules are close to only one of the mouths and the ST-channel thus must ~ 4 nm over the membrane surface. Our results are in agreement with those electron optic studies of ST-induced ring structures in membrane vesicles: Füssle et al. (1981) found protein protruding 4—5 nm from the membrane surface.

This is the assymmetric case of ST-channel localization in BLM. The same authors reported the bilayer thickness to be nearly 5 nm, the pore length may be ~ 10 nm.

Based on the above results, the following ST-channel structure may be proposed:

1) It consists of six molecules of α-toxin.

2) It is incorporated asymmetrically into the membrane with only one of its mouths being in contact with polar heads of the lipid bilayer.

3) The water pore appears to be an interprotein channel with a diameter and length of 2.5—2.8 nm and 9.5—10 nm, respectively.

In conclusion, it should be mentioned that the calculated value of the conductivity of the cylindrical part of aqueous solution of 0.1 mol/l KCl 2.5 nm in diameter, 10 nm in length, ($t = 25\,°C$) is 630 pS. This is several times larger than the respective value of ST-channel conductance. Hence ion transport occurs in the area of the water pore of ST-channel. The anion selectivity of the ST-channel (Krasilnikov et al. 1986b), asymmetry and non-linearity of the current-voltage characteristics on non-charged PC bilayers all speak for an inhomogeneity of the channels energy profile. An analysis of this ST-channel energy profile will be the subject of our further studies.

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