

## Memory is a Property of an Ion Channels Pool: Ion Channels Formed by *Staphylococcus Aureus* Alpha-Toxin

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**Abstract.** The short-time depolarization effects on the integral conductance induced by *S. aureus* alpha-toxin (ST) in planar lipid bilayer membranes has been studied. Ion channels formed by ST were found to have several potential-induced nonconductance (closed) states. The transitions of ion channels between the states are only through one conductance state. The transition of ST-channels from closed to open state is induced by membrane depolarization. The amplitude current after a series of voltage pulses is a function of pulse number, and is effectively independent of the time interval between the neighbouring pulses. Therefore, a membrane which contains a pool of ion channels "remembers" its previous existence. A simple model can be used to explain this phenomenon.

**Key words:** Memory — Ion channel — Voltage gating — Planar lipid membranes — *Staphylococcus aureus* alpha-toxin

### Introduction

Memory is one of the most important properties of organisms. Extensive literature exists on activation/inactivation phenomena of ion currents in biological membranes, the relationship between these properties and memory has not been clearly understood as yet. To circumvent the complexities of cellular membrane proteins, numerous cytotoxic polypeptides, long enough to span a lipid bilayer, have been used as model systems. The alpha-toxin of *Staphylococcus aureus* (ST) has been one of these model agents. The properties of ST ion channel formed in artificial or natural membranes have been studied fairly well. This channel structure is very similar to that of some ion channels in natural

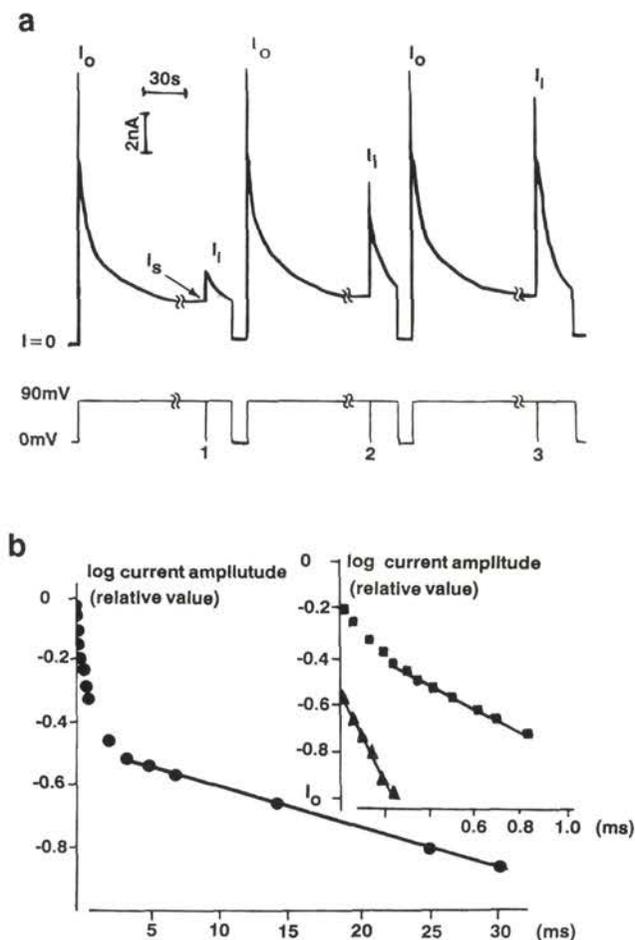
membranes. The ST channel is composed of 6 ST molecules. The ST channel is a larger pore (approx. 2.5–3.0 nm in diameter and 10 nm in length; Fussle et al. 1981; Bhakdi et al. 1984; Krasilnikov et al. 1988a). The voltage gating of this channel conductance has also been studied. Recently, it has been shown (Mene-strina 1986; Krasilnikov et al. 1988b) that the conductance of a multi-channel membrane sharply relaxes to a new less value after the stepwise increasing membrane potential. It results in the transition of ST channels from open to closed state. A single ST channel has several states of lower conductance (Krasilnikov et al. 1986; 1988b) similarly as ion channels in natural membranes (Colquhoun and Hawkes 1983). We could show that the application of a series of short depolarizing pulses to a membrane containing closed ST channels results in a decrease of the transmembrane current amplitude. Based on experiments performed over a wide range of pulse durations we could conclude that after depolarization, a redistribution of ST channel non-conductance states occurs. Our observations introduce a new aspect concerning voltage-induced activation-inactivation processes of ion channels in membranes: these processes might represent the basis of short-time cell memory.

## Materials and Methods

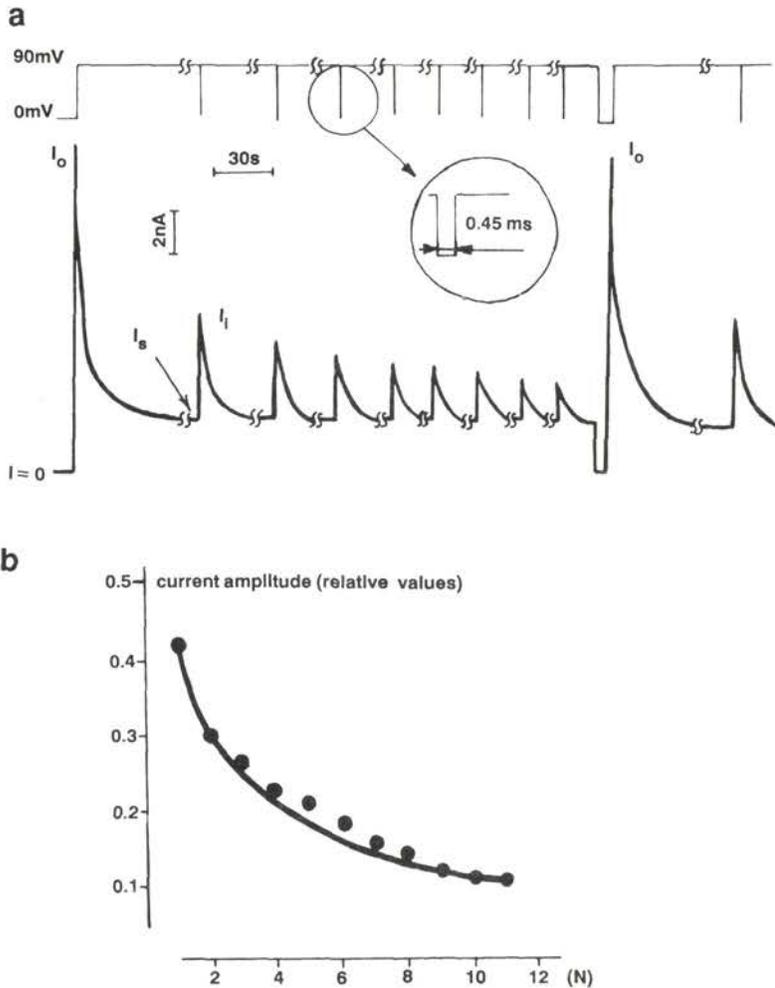
*Staphylococcus aureus* alpha-toxin (ST) was a kind gift of Dr. K. D. Hungerer of the Behringwerke Laboratories (Marburg, FRG). It was further purified by preparative isoelectric focusing. Planar phospholipid bilayer membranes (BLM) were formed at  $25 \pm 2^\circ\text{C}$  by the technique of Mueller et al. (1963) or by the opposition of two monolayers according to Montal and Muller (1972). The volume of each compartment was 2 ml, and they were filled with an appropriate salt solution. The membranes were prepared of a mixture of egg phosphatidylcholine (TLC-pure, Bergelson et al. 1981) with cholesterol (Sigma) in a 1:1 molar ratio, or of pure phosphatidylcholine. All other chemicals were reagent grade. When membrane formation was completed time was allowed for stabilization, and ST from a stock aqueous solution was added to one compartment (*trans* side), to a final concentration sufficient to produce a steady-state conductance of  $10^{-5} \text{ S/cm}^2$  (with a transmembrane potential value of 3–5 mV). The aqueous solution contained 100 mmol/l KCl, 5 mmol/l citric acid-*tris* buffer. The *cis* solution was taken as the virtual ground, the voltage signs are referred to it. Usually, positive voltage was used and all results reported herein were obtained with this voltage. However, qualitatively similar results were obtained with negative voltages.

## Results and Discussion

Multi-channel BLM were used to study the transition kinetics of ST channels from closed to open state. After a high steady-state conductance level had been achieved, the transmembrane potential was stepwise increased from 3–5 mV to 90 mV (Fig. 1). At high voltage, current  $I_0$  reaches a large instantaneous value with a subsequent decrease toward a lower steady-state value ( $I_s \approx 0.1 I_0$ ). For



**Fig. 1.** The time course of current in response to the application of voltage pulses to a planar lipid membrane, containing numerous alpha-toxin channels in the presence of 100 mmol/l KCl, 5 mmol/l citric acid-tris buffer, pH = 4.0. *a*) Original current record after long and short depolarizations. The pulse protocol is shown below the current trace. The breaks were for 2–5 minutes, the durations of the depolarization pulses 1, 2 and 3 were 0.07; 0.7; and 25 ms, respectively. *b*) Logarithm of current responses (relative values:  $(I_o - I_i)/(I_o - I_s)$ ) plotted against the length of the depolarization pulses (left trace, circles). The solid line represents the least squares fit with a time constant  $\tau_3 = 25$  ms. Squares represent the current resulting from the subtraction of the exponential relaxation current with time constant  $\tau_3$ . Another regression line can be calculated from these points, giving a second time constant  $\tau_2 = 0.8$  ms. Triangles represent the residual current after additional subtraction of the exponential relaxation current with time constant  $\tau_2$ . A third regression line can be calculated from these points, giving a time constant  $\tau_1 = 0.015$  ms.



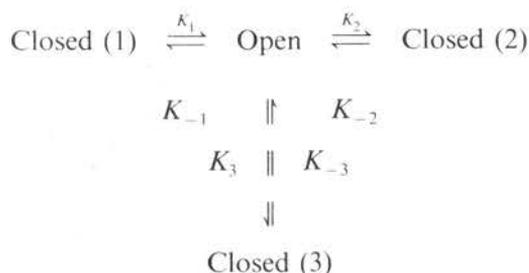
**Fig. 2.** The dependence of the current amplitude on the pulse order in the series. *a*) An original record of the current time course after a series of 0.45 ms pulses. The pulse protocol is shown above the current trace. The breaks were varied from 5 to 60 minutes without changes of results. For other conditions see legend to Fig. 1*a*. *b*) Maximal values (relative units:  $(I_i - I_s)/(I_o - I_s)$ ) of current responses from Fig. 2*a*, plotted against the pulse order ( $N$ ) in the series. The solid line was drawn according to the mathematical model with the parameters  $K$  described in the text.

one short-lived step (0.03–100 ms) to 0 mV the current shows a large value ( $I_i$ ), which then rapidly decreases ( $I_s$ ). The decrease is the greater the longer the time spent at 0 mV. This agrees with the results reported by Menestrina (1986). The dependence of the value of  $I_i$  on the voltage step duration is nonmonotonic

(Fig. 1). This relationship can be fitted sufficiently well with the sum of three purely exponential components with different time constants (0.1–0.3; 10–15; and 20–100 ms). These time constants are similar to those of ion channel activation in excitable biomembranes. In some experiments, the amplitudes showed slight variations. These results suggest the existence of three or more distinct states of lower conductance of ST channel (closed states).

More interesting were observations after a small change of the pulse protocol (Fig. 2). In this experiment a series of identical depolarizing pulses was applied. The maximal value of the instantaneous current in response to a concrete pulse was dependent on the order of the pulse in the series, while being independent of the time interval between the neighbouring pulses. Up to pulse 6–9, the value of  $I_i$  decreased, remaining effectively equal in response to further pulses. If e.g. after pulse 10 the trans-membrane potential remained invariable and equal to 90 mV, during more than one hour the current amplitude in response to the pulse 11 differed from the expected value by only 2%.

Our results suggest that a multi-ST channel membrane can “count” as many as 6–9 identical pulses. This counting mechanism represents some short-time memory of cells. When a long (200–1000 ms) membrane depolarization to 0 mV with a subsequent return to 90 mV was used to restore the initial state, the “counting mechanism” was ready again “to remember” new pulses. A simple scheme can be used to describe our results. The model is based on a working hypothesis assuming one open state and three different closed states of the channel:



where  $K_i$  and  $K_{-i}$  are the forward and backward transition rate constants.

When the membrane potential is small or zero the ST channels tend to occur only in open state. Upon increasing membrane potential, ST channels go over to distinct states of lower conductance. For all the channels changing to closed state, the number of closed channels into state  $i$  ( $C_i$ ) can be written as

$$C_i^0 = K_i C / \sum_i K_i \quad (1)$$

where  $C = \sum_i C_i^o$  is the total number of channels.

The current in response to the first short depolarization pulse (duration  $t$ ) can be expressed as

$$I^1 = j \sum O_i^1 \quad (2)$$

where  $O_i^1 = C_i^o [1 - \exp(-K_{-i} t)]$  is the number of channels, which had gone over from closed state  $i$  to open state;  $j$  is the single channel current.

The current in response to  $n$ -th short depolarization pulse (of identical duration) can be expressed as

$$I^n = j \sum_i O_i^n \quad (3)$$

where  $O_i^n = C_i^n [1 - \exp(-K_{-i} t)]$

$$C_i^n = C_i^{n-1} [\exp(-K_{-i} t)] + K_i \sum_i O_i^{n-1} / \sum_i K_i$$

An analysis of the above equations revealed that the rate of this voltage-dependent activation/inactivation processes is determined by the constant  $K_1$  ( $s^{-1}$ ). Let  $K_{-1} \gg K_1$  at 90 mV,  $K_1 \ll K_{-1}$  at 0 mV; since, in addition, the rate constants of ST channels transitions from distinct closed to open state are very different,  $K_{-1} > K_{-2} \gg K_{-3}$ . Then, a series of short depolarization pulses ( $t \approx 1/K_{-1}$ , s), shall redistribute the closed states of ST channels, with a tendency to closed (3) state. It is clear that the current amplitude in response to each consecutive pulse shall be smaller than the preceding one until the transition rates from distinct closed states to open states become equal.

A computer analysis of the kinetic equations system showed that the following values of the kinetic constants are sufficient to fit the experimental observations (Figs. 1 and 2):

$$K_1 = 0.044 s^{-1}, \quad K_2 = 0.073 s^{-1}, \quad K_3 = 0.48 s^{-1} \text{ (at 90 mV);}$$

$$K_{-1} = 6700 s^{-1}, \quad K_{-2} = 1250 s^{-1}, \quad K_{-3} = 40 s^{-1} \text{ (at 0 mV).}$$

The observed property of an ion channels pool may provide the molecular basis for receipt, storage and transmission of information in the nervous system. Also, it may be useful for future biotechnical devices.

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