Short communication

Current Response of Bilayer Lipid Membrane to Killer Factor from *Saccharomyces cerevisiae* T158C

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Killer yeasts secrete proteins (killer toxins) lethal to sensitive strains of yeasts. The process of the killer toxin action involves binding to a cell wall receptor (Al-Aidroos and Bussey 1978; Bussey et al. 1979). The proton-aminoacid symport as well as proton pumping are inhibited immediately after the binding of toxin molecules (Peña et al. 1980, 1981). It was shown in our previous paper (Vondrejs et al. 1982) that the observed effects are very likely due to an interaction of the toxin with the cytoplasmic membrane since fluorescence of a membrane probe (N-phenyl-l--naphthylamine) increased after the killer toxin had been added to sensitive yeast cells. Results summarized in the present report demonstrate that the killer factor from *S. cerevisiae* T158C forms aquous pores in artificial bilayer lipid membranes (BLM).

BLMs were formed by the method of Mueller et al. (1962) over a hole of 0.5 mm in diameter. Egg-lecithin (Kharkov Plant of Chemical Preparations U.S.S.R.) and cholesterol in n-heptane (20 mg/ml) were used in a mixture 4:1 (w/w). The conductance of BLM was measured using the method described by Haydon et al. (1972). One of the calomel electrodes was connected to the inverting input of a high input impedance, low-bias current operating amplifier (WSH 223, Tesla, see Dostál 1981) in the virtual-ground mode. This enabled recording of signal-channel current transitions with an amplitude $< 10^{-13}$ A using a stripchart recorder TZ4100 (Laboratorní přístroje, Prague). All our experiments were performed at room temperature ($T = 20$ °C). Compartments on both sides of the membrane were filled with 3 ml of electrolyte each (1 mol/l KCl) in monodistilled water, unbuffered, pH about 5). It took about 30 min to obtain steady-state conductance at a voltage of 100 mV reached. Fluctuations of the membrane current occurring after the addition of 10 μl of the killer toxin solution are illustrated in Fig. 1 *A*. The killer toxin solution was prepared according to

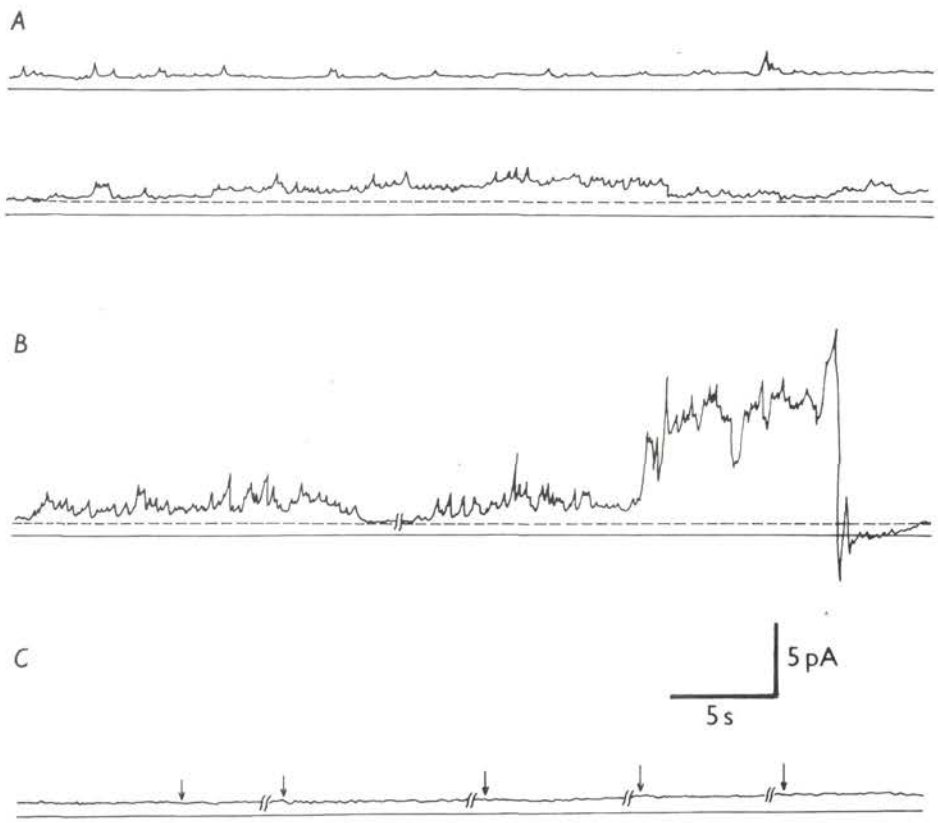

Fig. 1 *A:* Current fluctuations of BLM prepared from egg-lecithin and cholesterol (4:1 w/w) in n-heptane (20 mg/ml) after the addition of 10 μl of killer toxin solution. Temperature $T = 20°C$, voltage $U = 100$ mV, electrolyte 1 mol/l KCl. *B:* An example of a multilevel conductance of BLM under the same condition as in *A*. *C:* Control: Current response of BLM to killer-free medium. Repeated additions of 10 μl of killer-free solution(arrows). A 60 min record.

Vondrejs et al. (1982) as a cell free medium from a culture of the superkiller strain *S. cerevisiae* T158C. The discrete quantised nature and the amplitude of these current fluctuations were typical for the formation and disappearance of ion-permeable channels. The conductance of a single channel calculated from the current fluctuations was $\gamma = 7.36 \pm 0.51$ pS. The life time of the channel conductivity state was $\tau = 5.1 \pm 2.0$ s. In some cases a multichannel conductance was observed (Fig. 1*B*). The increase rate of the toxin-induced integral conductance depended on the toxin concentration. When 30 μl of the killer toxin solution was added to both compartments a larger increase was observed. No fluctuations were observed following repeated addition of toxin-free cultivation medium to both sides of the

membrane (Fig. 1*C*). The results presented above are similar to those obtained for the killer toxin from *Pichia kluyveri* (Kagan 1983).

We propose that the channels introduced in BLM by the killer factor molecules also account for the killer effect in vivo. Moreover, it seems possible that the channel formation in vivo need not be strain specific.

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