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Lipid-Mediated Inactivation of Colicin E1 Channels by Calcium Ions

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Abstract—Based on the model of a toroidal protein–lipid pore, the effect of calcium ions on colicin E1 channel was predicted. In electrophysiological experiments Ca^{2+} suppressed the activity of colicin E1 channels in membranes formed of diphytanoylphosphatidylglycerol, whereas no desorption of the protein occurred from the membrane surface. The effect of Ca^{2+} was not observed on membranes formed of diphytanoylphosphatidylcholine. Single-channel measurements revealed that Ca^{2+}-induced reduction of the colicin-induced current across the negatively charged membrane was due to a decrease in the number of open colicin channels and not changes in their properties. In line with the toroidal model, the effect of Ca^{2+} on the colicin E1 channel-forming activity is explained by alteration of the membrane lipid curvature caused by electrostatic interaction of Ca^{2+} with negatively charged lipid head groups.

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Calcium ions play a great and manifold role in vital activities of cells. In particular, they are involved in the regulation of activity of ionic channels [1-3]. Some channel-forming proteins are shown to directly interact with Ca^{2+} [4, 5]. On the other hand, calcium is also reported to regulate the structure and function of ion channels by influencing membrane lipids [6, 7]. But such data are very scarce.

Without overestimation, lipids play a tremendous role in the functioning of all ion channels. A model of the toroidal protein–lipid pore has been recently proposed for channels formed by a number of antibacterial peptides [8-12] and also some proteins [13]. This model suggests a direct involvement of lipids in formation of the channel walls. Based on our data [14], the toroidal model was supposed to fit for explanation of the structure of the channel formed by a water-soluble bacterial toxin colicin E1. The spontaneous membrane curvature plays an essential role in the model of the protein–lipid pore. Lipids with a positive curvature stimulated the formation of colicin E1 channels, whereas lipids with a negative curvature inhibited the channel formation [14].

Calcium is known to decrease the repulsion of charged lipid head groups in the negatively charged membrane, and this increases the negative curvature of the membrane [15-17]. Thus, it was suggested that an increase in the spontaneous negative curvature of the membrane lipids because of calcium binding should inhibit formation of colicin channels. In fact, in the present work the colicin-induced current is shown to decrease under the influence of Ca^{2+} in a negatively charged membrane but not in a neutral membrane.

Abbreviations: BLM) bilayer lipid membrane; P178) colicin E1 channel-forming domain; DPhPC) diphytanoylphosphatidylcholine; DPhPG) diphytanoylphosphatidylglycerol; BrPC) 9,10-dibromopalmitoylphosphatidylcholine.

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MATERIALS AND METHODS

The C-domain (178 amino acids) of colicin E1, P178, was prepared by proteolysis of whole colicin E1

with thermolysin [18]. Gramicidin A was from Sigma (USA). All lipids used were from Avanti Polar Lipids (USA).

The bilayer lipid membrane was prepared from 2% lipid solution in decane or squalene by Muller's technique on a 0.55-mm hole in a Teflon partition separating two compartments of the cell filled with buffer containing 120 mM KCl and 10 mM β-alanine (pH 4.0) in experiments with measurements of the integral current and 1 M KCl and 10 mM CH_3COOK (pH 4.0) in experiments with measurements of single channels. The current was measured using U5-11 (USSR) and BC-525C (Warner Instruments, USA) amplifiers (for integral current and single channels, respectively), digitized with a LabPC 1200 plate (National Instruments, USA), and analyzed with a PC using WinWCP Strathclyde Electrophysiology Software program developed by J. Dempster (University of Strathclyde, UK). Ag–AgCl electrodes were dipped directly into the cell. P178 was added into the surrounding bathing from the *cis*-side of the membrane, and gramicidin A and $CaCl_2$ were added from both sides of the membrane.

The P178 binding with the membrane was measured using liposomes containing a brominated lipid, which is known to quench the fluorescence of tryptophan [19]. Liposomes containing 9,10-dibromopalmitoylphosphatidylcholine (BrPC) were prepared from 2% solution of BrPC/DPhPG (70/30 mole %) in chloroform. On evaporation of chloroform under a nitrogen current and hydration of the lipid, the mixture was shaken and after a freezing–thawing cycle pressed across a polycarbonate membrane with 0.1-μm pores (Nucleopore, USA) using a mini-extruder (Avanti Polar Lipids). Buffer for preparing the liposomes contained 10 mM β-alanine and 0.12 M KCl (pH 4.0). The same buffer was used in experiments on quenching the tryptophan fluorescence with brominated liposomes.

RESULTS

Colicin-induced current across the bilayer lipid membrane (BLM). Figure 1 presents recordings of the electric current induced by the channel-forming domain of colicin E1 (P178) across a BLM. In the membrane formed of the negatively charged lipid diphytanoylphosphatidylglycerol (DPhPG) (Fig. 1a) the addition of $CaCl_2$ at a concentration from 35 μM (curve *1*) to 5 mM (curve *4*) decreased the colicin-induced current, whereas in the membrane prepared of the neutral lipid diphytanoylphosphatidylcholine (DPhPC) (Fig. 1b) the addition of $CaCl_2$ had no effect on the current. In both cases, before the addition of $CaCl_2$ the colicin-induced current was voltage-dependent (Fig. 1b).

For comparison, we have studied the effect of Ca^{2+} on the gramicidin A-induced current across a BLM. Figure 2 shows changes in the current on addition of 1 mM $CaCl_2$ to the membrane containing only a neutral lipid DPhPC (curve *1*) or only a charged lipid DPhPG (curve *2*). The gramicidin-induced current was reduced only in the case of the membrane formed of DPhPG. These findings are in agreement with data of the work [7] on the calcium-induced decrease in the gramicidin A-induced conductivity of the membrane containing 100% dioleoylphosphatidylserine.

Colicin binding with BLM. The presence of Ca^{2+} in solution was shown to decrease the binding constant of colicin with membranes containing negatively charged lipids [20]. In our studies on the effect of Ca^{2+} on the colicin-induced current, we added $CaCl_2$ after the sorption

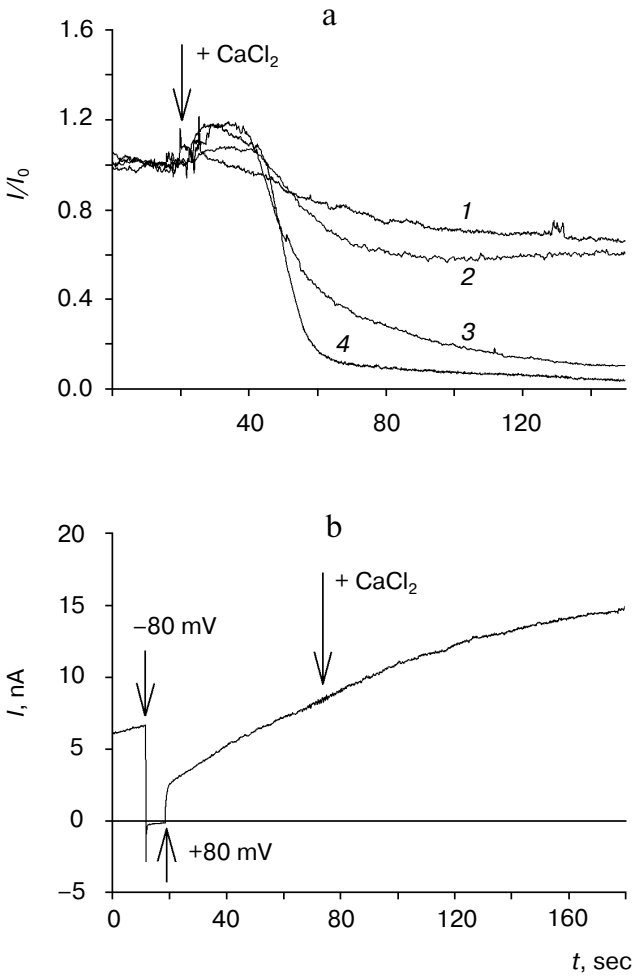

Fig. 1. Effect of calcium chloride on the colicin E1-induced current across a BLM. The buffer solution contained 10 mM β-alanine and 0.12 M KCl. a) The membrane was prepared from DPhPG solution in squalene. Concentration of $CaCl_2$: 33 μM (*1*), 82 μM (*2*), 330 μM (*3*), 5 mM (*4*). The applied voltage was 80 mV. The initial current was 2 nA. b) The membrane was prepared from DPhPC solution in squalene. Concentration of $CaCl_2$ was 5 mM. Values of the applied voltage are indicated at the corresponding arrows.

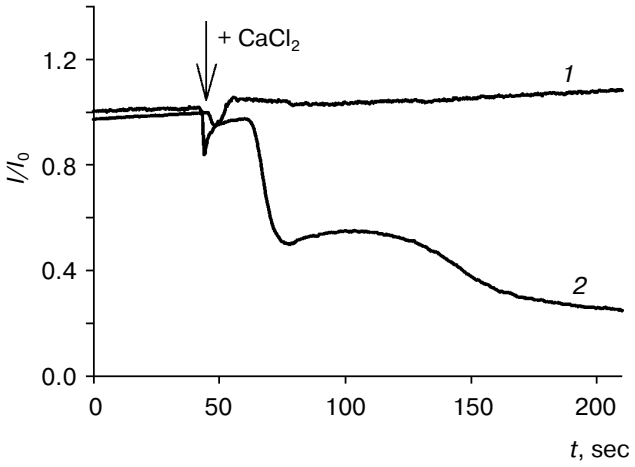

Fig. 2. Effect of calcium chloride on the gramicidin A-induced current across a BLM. The membrane was prepared from DPhPC (*1*) and DPhPG (*2*) solutions in decane. The applied voltage was 30 mV; the initial current was 15 nA. Concentration of $CaCl_2$ was 1 mM. Other conditions were the same as in Fig. 1.

of colicin and its incorporation into the bilayer. Therefore, it was important to make sure that Ca^{2+} did not cause desorption of the protein from the membrane. We determined the binding of P178 with the membrane by measuring the quenching of fluorescence of colicin tryptophans using liposomes containing brominated phospholipids (Fig. 3). Addition of the brominated liposomes to the buffer solution containing P178 significantly decreased the tryptophan fluorescence (curves *1* and *2*), which suggested the protein binding with liposomes. Our findings confirmed the data of Zakharov et al. [20]: the addition of calcium to liposomes before addition of P178 both decreased the protein sorption and slowed down its kinetics (data not shown).

The addition of Ca^{2+} to the protein sorbed on liposomes had virtually no effect on the tryptophan fluorescence of P178 (Fig. 3, curve *3*). Thus, the protein was not desorbed from the membrane in the presence of calcium. Note that a similar effect caused by the increase in pH of the medium was described in work [20]. The sorption of colicin on the membrane was impeded at high values of the medium pH; however, if the protein had already been presorbed at lower pH values, a shift to alkaline values caused virtually no effect on its sorption.

Single channels of colicin E1. The integral current across a lipid bilayer depends on the number of single channels, their lifetime, and amplitude. Therefore, it was important to find out how calcium influences single channels of colicin E1.

Single P178 channels were measured on membranes formed from lipid solutions in decane. As earlier shown [21], under these conditions colicin formed channels with two conductance states: a small channel with the amplitude of about 65 pS and a large channel (which appears only in the presence of the small channel) with the amplitude of about 600 pS. Figure 4a gives typical recordings from such channels. The figure presents colicin channels in the membrane formed from 100% neutral lipid DPhPC. With increase in the fraction of the negatively charged lipid in the membrane, the conductivity of both types of channels decreased. In the completely negatively charged membrane conductivities of the small and large channels decreased tenfold and about twofold, respectively, resulting in 6 and 280 pS (Fig. 4, b and c).

In our experiments, calcium did not change the conductivity and lifetime of the channels in the membrane of pure DPhPC (Fig. 4d). In the negatively charged membrane Ca^{2+} had no effect on the conductivity of small colicin channels (Fig. 4e) and slightly increased the conductivity of large channels (to 310 pS, Fig. 4f), which seems to be associated with an increase in the membrane-adjacent concentration of chloride ions because of a decrease in the negative charge of the membrane on Ca^{2+} binding.

It should be noted that addition of Ca^{2+} into the surrounding bathing did not change the number of open channels in the membranes of DPhPC but significantly decreased their number in the membranes of DPhPG (data not shown).

DISCUSSION

The role of Ca^{2+} in formation of ion channels of colicin was not elucidated for a long time, because in many studies on colicin, Ca^{2+} was added to the buffer solution bathing the membrane to increase its stability, and the

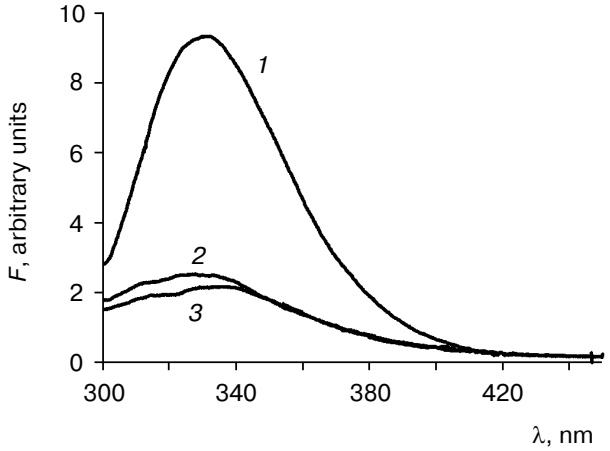

Fig. 3. Effect of calcium chloride on colicin binding with a BLM. The fluorescence spectra of P178 (10 μg/ml): *1*) without additions; *2*) after addition of liposomes containing BrPC (320 μg/ml); *3*) after the subsequent addition of 5 mM $CaCl_2$. The exciting wavelength was 282 nm. The buffer solution contained 10 mM β-alanine, 10 mM MES, 10 mM Tris-HCl, 0.12 M KCl (pH 4.0).

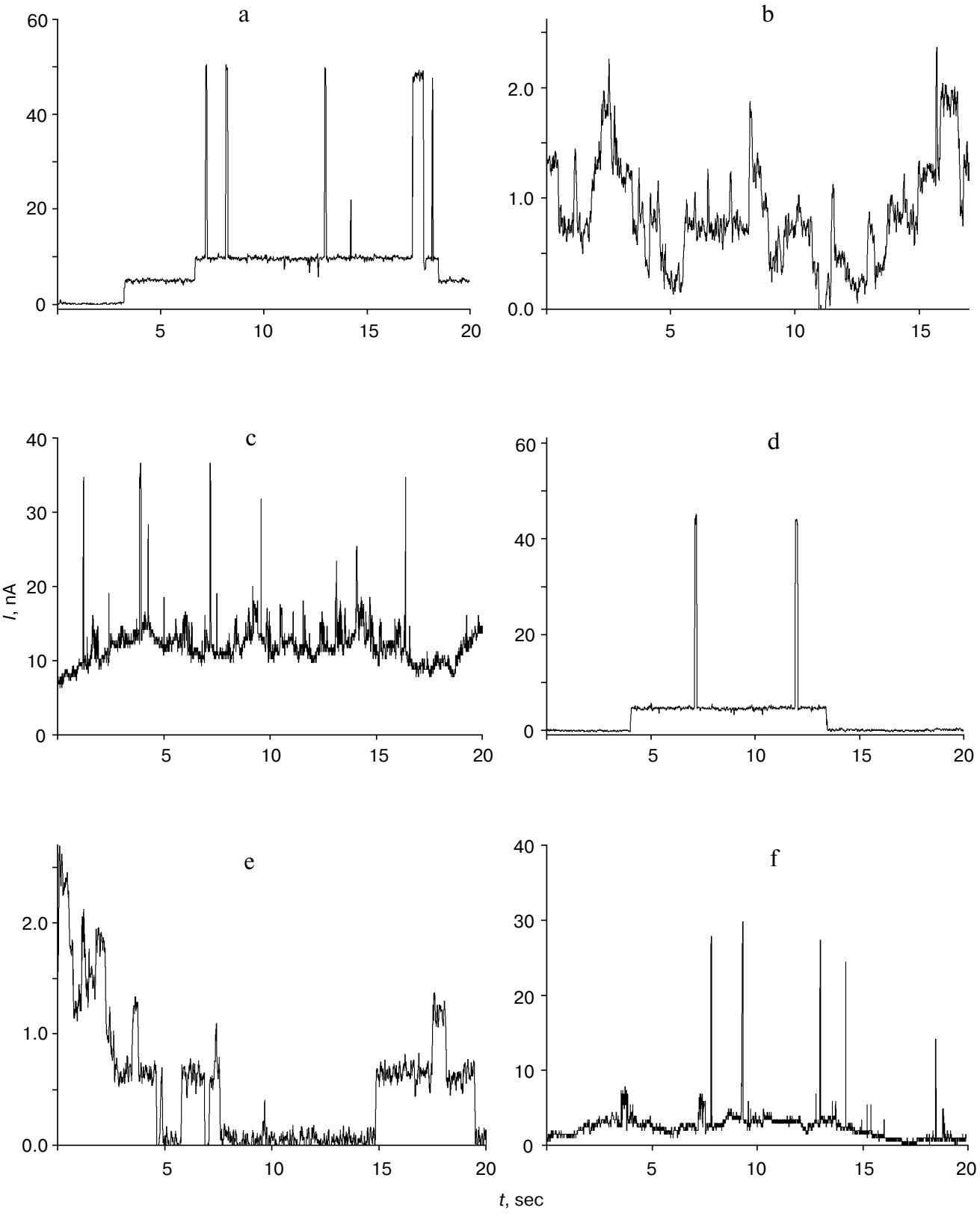

Fig. 4. Single channels of colicin E1. Typical recordings are presented from colicin channels in the membrane formed of DPhPC/decane (a, d) and DPhPG/decane ((b, e) only small colicin E1 channels are shown; (c, f) large colicin E1 channels are shown on the background of small ones). Typical recordings of colicin channels (in the presence of 5 mM $CaCl_2$) in the membrane formed of DPhPC/decane (d) and DPhPG/decane (e, f). The applied voltage was 80 mV.

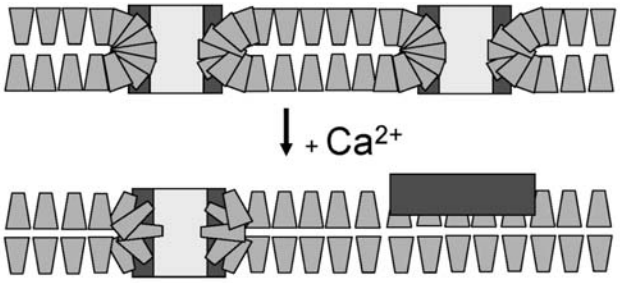

Fig. 5. Scheme of action of Ca^{2+} on formation of a colicin E1 ion channel. The binding of Ca^{2+} with the membrane changes the spontaneous curvature of lipids (the ratio between the cross-sectional areas of lipid heads and tails schematically presented as trapeziums) and, as a consequence, makes difficult the formation of a protein–lipid pore with a high surface curvature.

effect of Ca^{2+} on the channels themselves was not taken into account and studied.

We have shown that addition of Ca^{2+} into the solution bathing the BLM of DPhPG lowers the colicin E1-induced current across the membrane. Such a suppression of the conductivity was not caused by changes in the amplitude and lifetime of single channels and not associated with desorption of P178 from the membrane surface. Thus, this decrease in the conductivity was due to a decrease in the number of protein molecules forming the channels. However, Ca^{2+} did not influence the colicin-induced conductivity of BLM formed of neutral lipid. This result suggests that the effect of Ca^{2+} on the colicin channels is mediated by the lipids.

The channel activity of colicin E1 is very sensitive to the spontaneous curvature of the BLM: the formation of channels increases with an increase in the positive curvature of the membrane and decreases in the opposite case. Consequently, the effect of Ca^{2+} on colicin channels is caused by the cation binding with negatively charged lipid head groups that decreases the repulsion between them and changes the spontaneous lipid curvature. Figure 5 schematically shows the reduction of the spontaneous curvature of negatively charged lipids on Ca^{2+} binding that decreases the number of open channels in the membrane.

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