Biochimica et Biophysica Acta, 1026 (1990) 248–250
Elsevier

BBAMEM 74932

Detection of transient capacitance increase associated with channel formation in lipid bilayers

Alexander N. Chanturiya

A.V. Palladin Biochemistry Institute, Kiev (U.S.S.R.)

(Recieved 8 January 1990)
(Revised manuscript received 23 April 1990)

Key words: Black lipid membrane; Latrotoxin; Sea anemone toxin; Ionic channel; Membrane capacitance; Lipid–protein interaction

The insertion of α- and β-latrotoxins and sea anemone *(Radianthus macrodactilus)* toxin into bilayer lipid membranes (BLMs) was investigated using the method of simultaneous conductance/capacitance measurement. All the toxins investigated induced capacitance changes which preceded toxin-induced conductance increases. The processes that may underlie the observed effect are discussed.

Introduction

The mechanism of protein translocation from the aqueous phase to the lipid bilayer is of great importance in the formation of integral membrane structures. The investigation of hydrophilic protein insertion into bilayer lipid membranes (BLMs) could be regarded as a valuable experimental approach to the determination of basic principles of this process.

Capacitance measurements are well suited to characterize the changes in the electromechanical properties of bilayers [1,2]. This method has mostly been applied to nonconducting membranes while routine conductance measurements are widely used to investigate bilayers modified by channel-forming proteins. Simultaneous recording of both bilayer and channel changes may yield much more interesting information. For this reason we made an attempt to use both methods in the same experimental system.

The initial aim of this study was to confirm our previous finding [3] that the channel-forming protein, α-latrotoxin, can be adsorbed onto the membrane without the formation of an ionic channel. In the course of the experiments, we discovered the effect of the toxin on membrane capacitance and went on to determine how general this behaviour is.

We present in this paper some experimental data on the interaction of three channel-forming proteins with phospholipid membranes. These proteins are exotoxins that are spontaneously incorporated into lipid bilayer membranes from aqueous solution [4–6].

Correspondence: A.N. Chanturiya, Department of Physiology, University College London, Gower Street, London, WC1E 6BT, U.K.

Materials and Methods

We used α- and β-latrotoxin (α-LtX and β-LtX, M_r 130000 and 70000, respectively) [4,5] isolated from the crude venom of *Latrodectus mactans tredecimguttatus* spider by FPLC liquid chromatography (Pharmacia) with a MONO-Q column, according to the procedure published in Ref. 6. Purified *Radianthus macrodactilus* toxin (RTX, M_r 20000) [7] was the gift of Dr. E.P. Kozlovskaya and Dr. M.M. Monasturnaya (Pacific Institute of Bio-organic Chemistry, Vladivostok). Egg albumin (M_r 45000) and poly(ethylene glycol) (M_r 6000) from Sigma Chemical Co. were used in the control experiments. The BLMs were formed by the technique of Mueller [8] across a 0.6 mm diameter hole in a teflon cup placed in a glass cell, from a mixture of phosphatidylcholine and cholesterol (Serva) (2 : 1, w/w). Heptane was used as the solvent. The lipid concentration was 20 mg/ml.

The solution bathing the BLM contained the required concentration of corresponding chlorides and 10 mM Tris (pH 7.4). The protein solutions (10 μl) were added to one side of the membrane outside the teflon cup (approx. vol. 9 ml). This volume was permanently stirred in all the experiments except for a few with RTX. Zero potential was at the inner side of the cup. Ag/AgCl electrodes were placed directly in the bathing solution.

Fig. 1 shows the scheme of an experimental system. As can be seen from the diagrams of voltage (A and B) and current (C), two constant values of voltage enable us to measure the value of an active component of the transmembrane current. While there was a linear decrease of membrane potential (A), we registrated the

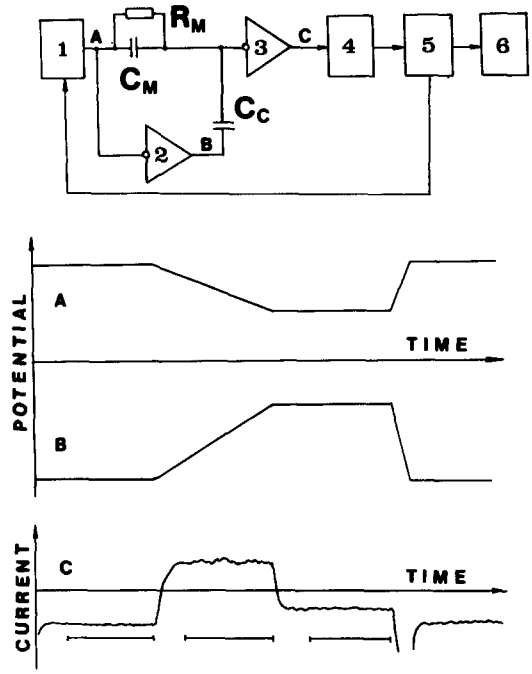

Fig. 1. The scheme of the experimental system and diagrams of the potentials. 1, Generator; 2, invertor; 3, operational amplifier; 4, analog/digital convertor; 5, computer; 6, printer. R_m, C_m, equivalent scheme of BLM; C_c, compensating capacitor; ———, region of the current measurements.

sum of the active and reactive components of the current. The true value of the reactive current was calculated by subtracting the active component from the full current. By using a compensating capacitor, placed between the input of the operational amplifier and the invertor of the BLM's applied potential, it was possible to compensate for almost all the reactive component of the current. This enables us to use a high speed of change of potential and obtain high resolution of measurements because large values of he reactive transmembrane current do not overload the amplifier.

Each value of transmembrane current was calculated as the average of 50 elementary measurements. During the experiment, values of the capacitance and conductance were being stored in computer memory. The time resolution of the system was approx. 300 ms. Mean values of the determined number of measurements were calculated to obtain each point for the hardcopy.

After BLM formation, membrane capacitance achieved a relatively stable level within 5–15 min. All experiments were conducted when the drift of this parameter was lower than 1%/min. It was noted that the capacitance was extremely sensitive to mechanical vibration. For this reason we selected only these experiments in which the capacitance changes appeared at least 10 s after the addition of the test solution. Between 10 and 15 experiments selected by this criteria were carried out with each protein.

Results

In most cases the addition of α- or β-LTX induced only a small alteration of membrane capacitance (usually an increase). In some experiments, however, we observed capacitance changes of variable amplitude but very similar in time course (Figs. 2A and 2B). It can be seen that there was first an increase and then a decrease of membrane capacitance after addition of the toxin, prior to the conductance increase (i.e., before the channel formation). The capacitance changes appeared 30–40 s after addition of the toxin and had a duration of 60–100 s. The amplitude of capacitance increases varied from 5 to 30 pF. (Probably there were some experiments with lower changes in amplitude, but to observe a change of shape in the time course was possible only when the effect was 4–5-times more than the noise. The number of successful experiments, with the above given limits of variability, was approx. 30%.)

One of the proteins investigated, RTX, had a different effect. The addition of this protein in the range of 50–100 nM (final concentrations) never induced effects similar to those described above. To positive membrane potentials, the increase of capacitance was observed only after RTX channel formation. To negative ones, a decrease of capacitance was observed (data not shown);

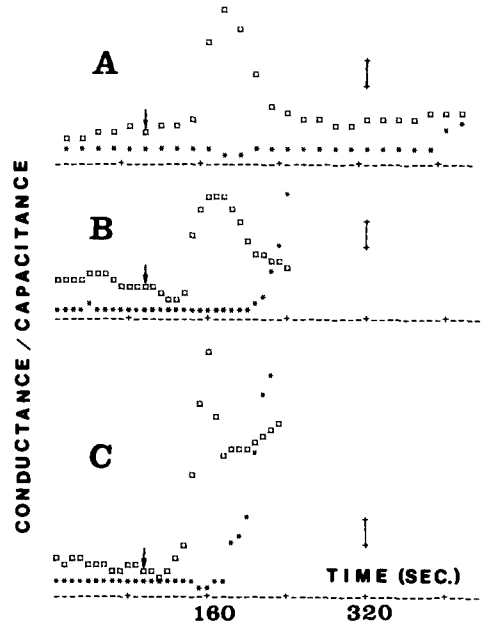

Fig. 2. Changes of BLM conductance (*) and capacitance (□) induced by different proteins. (A) α-LTX (final concentration 2 nM), (B) β-LTX (40 nM), (C) RTX (10 μl of 50 μM RTX solution was added near the BLM). Solution: A and B, 10 mM $CaCl_2$; C, 100 mM KCl. Membrane potential: A, −50 mV ± 40 mV; B and C, +50 mV ± 40 mV. Speed of potential change 2 V/s. Horizontal axes indicates zero level of BLM's conductance and the base level of capacitance (A, 520 pF; B, 740 pF; C, 830 pF). Vertical scale markers correspond: A, 6 pF, 200 pS; B, 5 pF, 100 pS; C, 20 pF, 100 pS. The arrows indicates the moment of protein addition.

we suppose it was an artefact caused by the fast closing of RTX channels at potentials of more than + 40 mV [9].

The routine procedure of toxin addition did not permit us to substantially increase the RTX concentration. For this reason we conducted the experiments adding RTX close to the BLM, without stirring. Under such conditions, the protein concentration close to the membrane was hard to determine, but evidently it was much higher than in the previous case. In two experiments from 16, transient capacitance increase was observed after RTX addition (Fig. 2C).

In control experiments the addition of poly(ethylene glycol) (final concentration 0.15 μM, 12 experiments), or egg albumin (final concentration 0.1 μM, 10 experiments), never induced the capacitance changes.

Discussion

The similarity of the capacitance changes induced by different toxins indicates that the mechanism may be common for different membrane active proteins.

The question emerges, how may the protein adsorption induce such changes? The increase of capacitance could be the result of changes in different BLM parameters: its area, thickness or dielectric constant. The transient character of the capacitance increase indicates that changes of the bilayer are also transient. For this reason it is not worthwhile considering the static changes of lipid order around protein molecules [10,11] or the contribution of the protein dielectric constant to the dielectric constant of the membrane.

Recently, we have investigated the insertion of α-LTX into BLM using a different method [3]. It was shown that application of positive voltage pulses to the BLM, clamped at a potential below 40 mV, stimulated the rapid insertion of the α-LTX channel. The results obtained indicate that the first step of this process is the formation of a nonconductive lipid-protein complex, in which some part of the α-LTX molecule is immersed in the membrane. Obviously, immersion of a large molecule into a bilayer will result in displacement of lipid. It is not impossible that appearance of redundant lipids in one monolayer should produce local deformations of the bilayer. It is also conceivable that such a state of the membrane is not stable, and that lateral movement of lipid molecules in the monolayer restores an energetically favourable state in the membrane. The dissipation of the deformed microareas in the membrane could be accomplished through the induction of a membrane oscillation or wave. The possibility of the existence of different types of wave is discussed. Any type of wave is capable of increasing the membrane area and decreasing its average thickness [12,13].

This hypothesis qualitatively explains the effect we have observed. Moreover, it explains why a small protein (RTX) is effective only at a higher concentration (in comparison with large proteins (α-LTX)) in inducing the capacitance changes: per mol small proteins displace less lipids. Nonetheless, there is a quantitative disagreement between this data and the data obtained previously [3].

The energy changes ΔE in the processes presented in Fig. 2 can be calculated from the equation $\Delta E = \Delta C U^2/2$. For $U = 0.05$ V, and $\Delta C = 10^{-10}$ F, $\Delta E = 10^{-14}$ cal. Supposing that the change of free energy for water-lipid transition of the protein is $\Delta G = 10$ kcal/mol, the amount of the protein needed to produce such a value of ΔE should be -10^{-18} mol, or 10^6 molecules. In the above-mentioned study [3], the number of non-channel molecules of α-LTX on BLM was estimated to be up to 100 and the rate constant of association was three orders lower than that claculated for the natural α-LTX receptor-containing membrane. If the number of bound molecules is much more than a hundred, the rate constant of LTX association should increase too. But it is difficult to understand how non-specific association could have an effectiveness comparable with the specific toxin–receptor interaction.

The reason for the bad reproducibility of the effect is also unclear. It is not consistent with the suggestion that large number of molecules are involved in this process. To account for this effect in detail, further experiments, perhaps on solvent-free membranes, should be performed.

References

1 Chizmadziew, Yu.A., Abidor, I.G. and Glazunow, I.Yu. (1985) Biol. Membr. (in Russian) 2, 906–918.
2 Rojas, E. and Pollard, H.B. (1987) FEBS Lett. 217, 25–31.
3 Chanturiya, A.N. and Lishko, V.K. (1988) Dokl. Acad. Sci. UkSSR (in Russian) 4, 76–79.
4 Mironov, S.L., Sokolov, Yu.V., Chanturiya, A.N. and Lishko, V.K. (1986) Biochim. Biophys. Acta 862, 185–198.
5 Salikhov, Sh.I., Tashmukhamedov, M.S. and Adulbekov, M.T. (1982) Dokl. Acad. Sci. USSR (in Russian) 262, 485–488.
6 Chanturiya, A.N., Sokolov, Yu.V., Grebinozko, E.I., Nikolaenko, A.N., Ivanov, A.P. and Lishko, V.K. (1985) Dokl. Acad. Sci. UkSSR (in Russian) 9, 82–85.
7 Kozlovskaya, E.P., Monasturnaya, M.M. and Elyakov, G.B. (1984) Abstr. 3rd USSR-Switzerland Symposium, Biological Membranes Structure and Function, Tashkent, p. 189.
8 Mueller, P., Rudin, D.O., Tien, T. and Wescott, W.C. (1962) Nature 194, 979–980.
9 Rudnev, V.S., Likhatskaya, G.N., Kozlovskaya, E.P., Monasturnaya, M.M. and Elyakov, G.B. (1984) Biol. Membr. (in Russian) 1, 1019–1024.
10 Scott, H.L. (1986) Biochemistry 25, 6122–6126.
11 Ivkov, V.I. and Berestovskij, G.N. (1982) in Lipid Bilayer of Biological Membranes (in Russian), p. 133, Nauka, Moscow.
12 Iakovenko, L.V., Butulin, A.A. and Tverdislov, V.A. (1987) Biofizika (in Russian) 32, 273–279.
13 Miller, I.R. (1984) Biophys. J. 45, 643–648.