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Latrotoxin-like properties of a protein from brain

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In bovine brain cortex cytoplasm we have identified a soluble protein (L-protein) of M_r ~90 kDa interacting with polyclonal antibodies to α-latrotoxin. The L-protein forms potential-dependent and cation-selective ion channels in BLM, which are blocked by Cd^{2+}. The fusogenic activity of the L-protein was demonstrated on liposomes. We have arrived at the conclusion that the action mechanisms of the L-protein and α-latrotoxin are similar.

Latrotoxin; L-protein; Bilayer lipid membrane; Liposome fusion; Channel; Bovine brain

1. INTRODUCTION

Different neuronal cell membrane receptors were investigated using natural toxins specifically interacting with neuron components. It might be supposed that if highly specific binding sites do exist on the neuronal membrane, endogenous analogs of neurotoxins could be found in neuronal tissue. Indeed, the soluble endogenous peptide analogs of apamine and tetrodotoxin were found in mammalian brain [1,2].

The molecular mechanisms of neurotransmitter secretion are still obscure. α-Latrotoxin is a natural toxin from *Latrodectus mactanus tredecimguttatus* venom, which influences the secretion process by binding with the presynaptic membrane [3]. In bovine brain cortex we identified a soluble protein (L-protein) of M_r ~90 kDa interacting with polyclonal antibodies against α-latrotoxin [4]. The channel forming and fusogenic activity of α-latrotoxin were recently demonstrated in artificial membranes and liposomes [5,6]. This study is the first attempt to compare the properties and behavior of L-protein and α-latrotoxin in model membrane systems.

2. MATERIALS AND METHODS

2.1. *Purification of L-protein*

Bovine brain cortex cytoplasm was obtained as described in [4]. We applied anionic chromatography on DEAE-Toyopearl 650M (Toyo Soda, Japan) as the first step of purification. A protein fraction containing L-protein was eluted with 0.4 M NaCl, 10 mM Tris-HCl, pH 7.4 [4]. Then the fraction was chromatographed on a hydrophobic Butyl-Toyopearl 650M column (Toyo Soda, Japan) equilibrated with 10 mM Tris-HCl, pH 7.4, 400 mM NaCl. Proteins were eluted from the hydrophobic column with a NaCl concentration gradient (from 400 to 0.0 mM). The L-protein-containing fraction was concentrated on an Amicon XM-50 filter (Amicon, USA) and chromatographied on a Bio-Gel A 0.5m column (Bio-Rad, USA) in 10 mM Tris-HCl, pH 7.4.

The L-protein was identified with rabbit monospecific polyclonal antibodies against α-latrotoxin as described in [4].

2.2. *BLM experiments*

BLM were formed by the technique of Mueller [7] in a hole (d 0.6 mm) of a Teflon cup placed in a glass cell. The membrane was formed from a PC/cholesterol mixture (2:1, w/w) in heptane with a total lipid concentration of 20 mg/ml. Electrolyte solutions bathing the membrane contained 10 mM Tris-HCl, pH 7.4 as well as the necessary amounts of chloride salts of different metal cations. We used two Ag/AgCl electrodes for conducting the current through the system under voltage-clamp conditions. The protein was added just to the nearest membrane area from the *cis*-side. The potential at the *trans*-side of the membrane was taken as zero. All experiments were carried out at room temperature, 20–22°C.

2.3. *Liposome preparation*

Large unilamellar vesicles consisting of a mixture of PC/PE/cardiolipin in a molar ratio 2:3:5, respectively were prepared by the reverse phase evaporation method [8].

2.4. *Fusion assay*

The Tb-dipicolinic acid fusion assay was used for fusion monitoring [9]. The measurements were carried out on a Hitachi 650-10S fluorescence spectrofotometer in a medium of 100 mM NaCl, 5 mM HEPES, 0.1 mM EDTA, pH 7.4, and Tb- plus DPA-vesicles (each of 50 μM lipid).

2.5. *Gel electrophoresis*

The protein fraction were analyzed in 7% polyacrilamide gel electrophoresis according Laemmly [10].

Abbreviations: BLM, bilayer lipid membrane; Tris, tris(hydroxymethyl)aminomethane; PC, phosphatidylcholine; PE, phosphatidylethanolamine, SDS, sodium dodecyl sulfate; DPA, dipicolinic acid.

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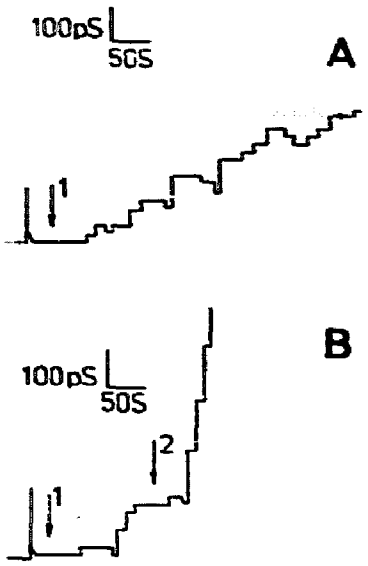


Fig. 1. Channel forming activity of L-protein. Current fluctuations of the membrane at −100 mV were applied. L-protein was added to the *cis*-side of BLM at a final concentration 0.1 μg/ml. The bathing solution contained from both sides: (A) 10 mM $CaCl_2$; (B) 100 mM KCl; 1, L-protein addition; 2, ~5 mM $CaCl_2$ addition from the *cis*-side.

3. RESULTS AND DISCUSSION

The protein sample enriched with a component of M_r ~90 kDa was obtained after purification on anion exchange DEAE-Toyopearl 650M, Butyl-Toyopearl 650M and Bio-Gel A 0.5m columns. Addition of the protein to the BLM *cis*-side entailed a stepwise increase

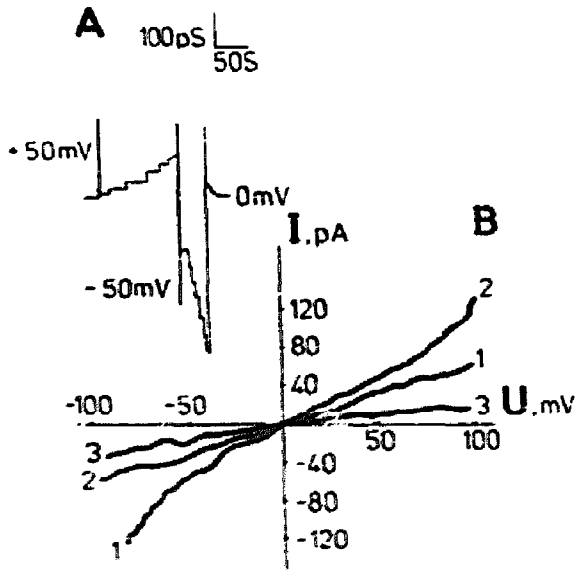


Fig. 2. Current–voltage curves of BLM treated by L-protein. (A) Potential-dependent insertion of L-protein into BLM under different potentials (+50 mV to −50 mV) in symmetric solutions with 5 mM $CaCl_2$. (B) L-protein was added: 1, from the *cis*-side; 2, from the *trans*-side. The solution contained 50 mM KCl from both sides; 3, 1 mM Cd^{2+} was added from *cis*-side.

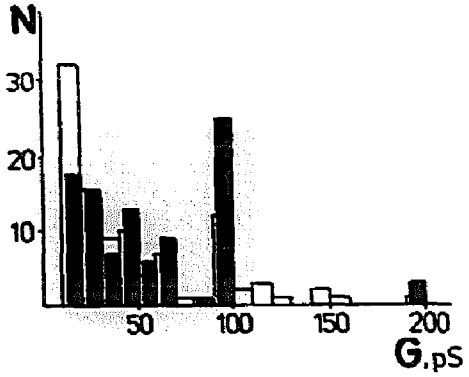


Fig. 3. Amplitude histograms of single channel conductance in the membrane treated with L-protein. Current fluctuations on the membrane: white boxes – at +100 mV; black boxes – at −100 mV were applied. A 10 mM $CaCl_2$ solution was present at both sides of BLM.

in the membrane current (Fig. 1A). The insertion of the L-protein occurred slower in the presence of monovalent cations than in a $CaCl_2$ solution. Addition of 5 mM $CaCl_2$ much activated the process (Fig. 1B). The same results were obtained for α-latrotoxin [5].

The membrane polarization much influences the process of the L-protein insertion into BLM: a negative potential activates this process more than a positive one (Fig. 2A). It is interesting that α-latrotoxin incorporation into BLM under the same conditions was activated by the positive potential [5]. The opposite sign of the potential stimulating a channel-forming effect of the L-protein and α-latrotoxin accords with the sideness of the natural application of these proteins: while the toxin penetrates into the membrane from the extracellular space, the L-protein is localized inside the cell.

The L-protein-formed channels were incorporated into BLM in an orientated manner. Thus, if the L-protein was added to the *trans*-side, we obtained an inverted type of the current–voltage curve similar to the

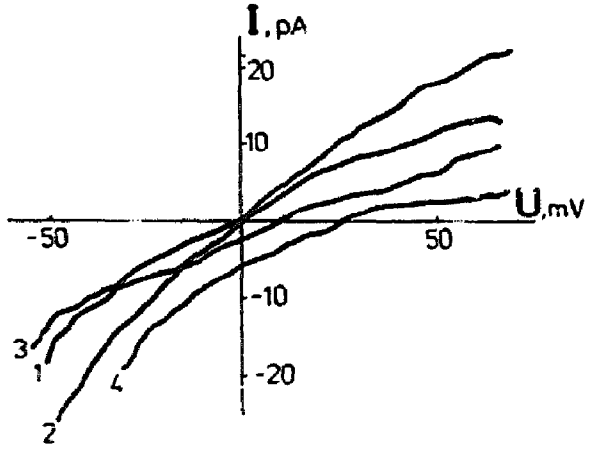


Fig. 4. Current–voltage curves of BLM modified with L-protein under different metal ions applications. From the *cis*-side: (1) 5 mM $CaCl_2$; (2) 5 mM $BaCl_2$; (3) 5 mM $MgCl_2$; (4) 5 mM KCl.

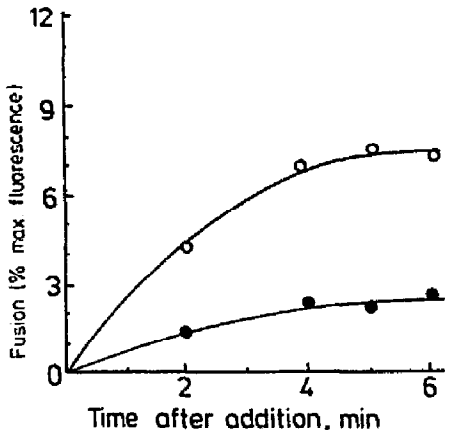


Fig. 5. Time courses of liposome fusion induced by L-protein and α-latrotoxin. The maximum fluorescence (at 100% of fusion) was set at the solubilization of Tb-containing vesicles by 0.5% sodium cholate in the presence of DPA without EDTA. (●) L-protein, 50 μg/ml; (○) α-latrotoxin, 10 μg/ml.

α-latrotoxin one (Fig. 2B, curve 1,2). Cd^{2+} blocked the L-protein channels (Fig. 2A).

The conductance of the major population of channels formed by the L-protein was within the range of 20–200 pS. Amplitude histograms of the channels corresponded to utilitary conductance of 20 and 100 pS under positive and negative potentials, respectively (Fig. 3). In comparison, histograms of the channels formed by α-latrotoxin had the opposite frequency.

α-Latrotoxin and the L-protein channels differ in cation/anion selectivity. Thus, a shift of the reversal potential is 45±2 mV for the L-protein and 55±3 mV for α-latrotoxin. The permeability of the investigated channels for cations decreases in the order $Ba^{2+} > Ca^{2+} > Mg^{2+} > K^{+}$ (Fig. 4). The results correlate with the cation sequence for the channels produced by α- latrotoxin.

As shown, α-latrotoxin possessed a fusogenic activity [6]. It was interesting to find out if the L-protein also possessed the furogenic activity. Two populations of vesicles containing Tb or DPA were mixed and the L-protein was added to the vesicle suspension. The rate of vesicles fusion is shown in Fig. 5. The fusion result resembles that of α-latrotoxin-induced fusion under the same conditions [6].

4. CONCLUSION

In bovine brain cortex cytoplasm we discovered a protein of M_r~90 kDa with α-latrotoxin-like properties: (i) L-protein interacts with polyclonal antibodies to the toxin; (ii) the two proteins form potential-dependent cation-selective channels in BLM, which are blocked by Cd^{2+}. A reverse form of current–voltage curves for different channels could be explained by unidirected incorporation of the toxin molecules (from outside) into the membrane; (iii) the fusogenic activity also has the common characteristics of both proteins. When comparing the results of the present work with those of work on α-latrotoxin we can conclude that the actions of α-latrotoxin and the endogenous L-protein on nerve membranes can have a common mechanism. The functional role of the endogenous latrotoxin-like protein is still unknown but its properties could indicate a possible participation in the secretory process.

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