# Reconstitution in Bilayer Lipid Membranes of the Crab *Potamon Transcaspicum* Spider Venom Sensitive Glutamate Receptors

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**Abstract.** Membrane proteins have been isolated from neuromuscular synapses of the crab *Potamon transcaspicum* using a specific blocker of glutamatergic synapses, the neurotoxin of the spider *Argiope lobata*. These membrane components have been shown to induce glutamate-sensitive conductance in bilayer lipid membranes (BLM) in the presence of sodium ions. As an agent blocking desensitization of glutamatergic synapses, concanavalin A was shown to enhance the conductance and to abolish desensitization. Diethylester of glutamic acid as a blocker of glutamatergic synapses inhibited glutamate-induced conductance. No similar change in conductance was seen when BLM was modified by a membrane proteins - neurotoxin complex. Conductance current fluctuations induced by these receptor protein components were monitored by the single-channel registration method.

**Key words:** Neurotoxin — Glutamate receptors — Membrane conductance — Current fluctuations

## Introduction

The toxin of the *Nephila clavata* spider venom has been shown to block the postsynaptic membranes of neuromuscular junctions in lobster (Kawai et al. 1982). Recent electrophysiological studies of effects of venoms of more than 20 different families of spiders carried out by the authors of the present paper have shown that venoms of the *Araneidae* family contain toxins blocking the postsynaptic membranes of insect neuromuscular junctions. In particular, the venom of *Argiope lobata* spider was found to block irreversibly the miniature amplitude potentials and responses to glutamate application in locust neuromuscular synapse (Tashmukhamedov et al. 1983a). Neurotoxin fractions of this venom were used as a ligand for affinity chromatography. This procedure enabled the isolation of a glycoprotein capable of inducing glutamate-sensitive conductance on BLM from the synapses of the cockroach *Periplaneta americana* (Tashmukhamedov et al. 1983b). The present paper contains experimental results obtained with the

isolation and reconstitution of glutamate receptors from the neuromuscular synapses of the crab *Potamon transcaspicum*.

## Materials and Methods

The homogenate of crab muscles was precipitated at 2500 g in a solution containing (in mmol/l): 134 NaCl; 6 $NaH_2PO_4$; 6 KCl; 4 $KHCO_3$; pH 7.4. The resulting supernatant was used as a crude synaptosomal fraction. These membrane fragments were sonicated on a UZDN-1 apparatus at 35 kHz, $t = 0$ °C and pH 9.6. The activated polyamide was used as a sorbent-carrier, the neurotoxin fraction from *Argiope lobata* isolated by gel chromatography on G-100 and subsequently on Bio-gel P-4 was used as a ligand. One g of the polyamide powder treated by glutaraldehyde was incubated for 24 hours with 11 mg of the toxic fraction in a borate buffer at 20 °C, pH 9. The bound protein yield was 73 %. The remaining free carrier aldehyde groups were blocked by ethanolamine. The sonicated membrane fractions were incubated with this affinity sorbent in the presence of 0.02 % $NaNO_2$ during 48 hours at 2 °C. The unbound proteins were carefully washed out. A pH gradient of 9.2—5.6 was used for the elution of specific proteins bound to the neurotoxin. Effects on BLM of proteins obtained this way were studied. BLM were formed according to Mueller and Rudin (1963). Measurements were made at 25 °C. The electronic equipment used for conventional BLM conductance determination has been described by Liberman (1970). The electric resistance of BLM was measured using silver-silver chloride electrodes and a high-input-impedance ($10^{13}$ $\Omega$) electrometer Radelkis. Ox Brain white matter phospholipids in decan as a membrane-forming solution were spread under water across a circular hole in a teflon septum. The circular hole area was 0.017 $cm^2$.

To record single channel current fluctuations the area of the circular hole in the teflon septum was 0.0007 $cm^2$. In this case a U-1-7 amplifier with an input impedance of $10^{10}$ $\Omega$ was used. The records were made by means of a potentiometer KSP-4I.

In all experiments, the water solution contained 50 mmol/l NaCl and 5 mmol/l tris-HCl, pH 7.5, on both sides of BLM. The glycoproteins were applied to both sides of BLM, the protein content was 10 mg/l. The protein concentration was determined according to Lowry et al. (1951), and contents of common sugars were estimated according to Dubois et al. (1956). Ox brain white matter phospholipids were obtained by the method of Folch et al. (1957).

This paper reports on experiments with conventional conductance of black lipid membrane in time and on single channel current fluctuations in the presence of glutamate receptor protein preparations obtained in a six-step procedure.

## Results and Discussion

In our experiments the protein fraction specifically bound to the neurotoxin has been observed to induce BLM conductance in the presence of sodium ions (50 mmol/l). The conductance of cations increased and was dependent on the concentration of the receptor protein. Ten to over 100-fold variations in the induced conductance were observed. This may be probably explained by the active state of certain receptor channels in the absence of mediator (Popo et al. 1977). An increase in the glutamate-induced conductance may have been seen at protein concentrations not affecting membrane resistance. It is worth noting that the kinetics of the glutamate-induced conductance change was characterized by a number of variations in our experiments. The most typical one was a spontaneous

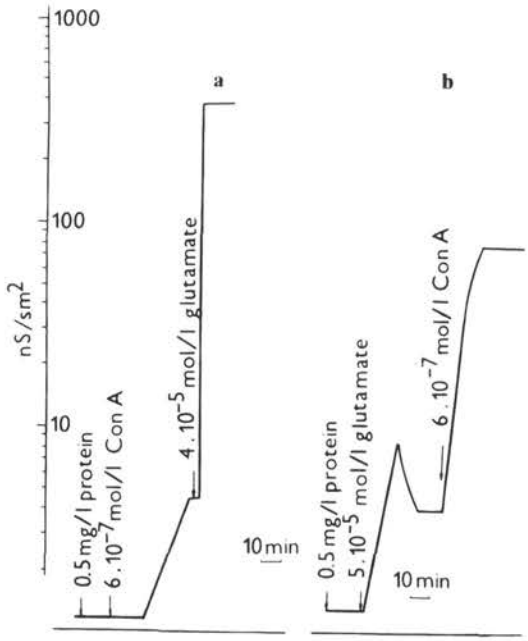


**Fig. 1.** Effect of glutamate and concanavalin A on BLM modified by receptor proteins. The medium contained 50 mmol/l NaCl; 10 mmol/l tris, pH 7.5.

decrease of the conductance following its maximum value. This may be a result of the well known physiological desensitization. Similar results were obtained with carbamoylcholine-induced BLM conductance in the presence of acetylcholine receptors (Schindler and Quast 1980).

Our further experiments dealt with concanavalin A as an agent able to abolish glutamate receptor desensitization (Usherwood 1979). Figure 1*a* shows that concanavalin A induced a certain increase in conductance in absence of the mediator. The addition of glutamate resulted in a considerable increase in conductance without any subsequent decrease. A similar increase in conductance could be observed with concanavalin A added following receptor desensitization (Fig. 1*b*). The blocking effect of concanavalin A on glutamate receptor desensitization is due to the interaction between this lectin and the carbohydrate portion of the receptor (Usherwood 1979). The interaction of concanavalin A with carbohydrate components of membrane fractions may bring about a functionally active receptor conformation. It is important to note that no increase in conductance was observed when the *Argiope lobata* neurotoxin + receptor glycoprotein complex is used as BLM modifier even in the presence of concanavalin A.

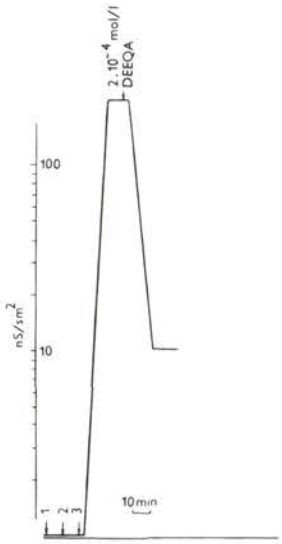


**Fig. 2.** Effect of glutamate diethyl ester on the glutamate-induced conductance in modified BLM. For the medium see the legend to Fig. 1. 1 — 0.5 mg/l protein; 2 — 0.5 mg/l protein; 3 — $2 \times 10^{-5}$ mol/l glutamate.

Glutamate-induced conductance of BLM may be effectively blocked by diethylester of glutamic acid (DEEGA) (Fig. 2), the latter appearing to be an inhibitor of glutaminergic synapses.

Thus, the postsynaptic action of the isolated glycoprotein was suggested by the effect of glutamate on glycoprotein-modified BLM as well as by the influence of concanavalin A, DEEGA and the neurotoxin on these effects.

A single-channel registration method allowed us to study conductance fluctuations induced by glycoprotein treatment of BLM. Fig. 3 shows traces of single-channel current fluctuations at 25 °C and a holding potential of ±100 mV in the presence of 50 mmol/l NaCl and 0.1 mmol/l glutamate. It should be stressed that the observed parameters of single-channel current fluctuations were not reproducible. The same applied for experiments with BLM conductance as a function of time in the presence of an acetylcholine receptor and different activators (Shamoo and Eldefrawi 1975). When the acetylcholine receptor was left for two days at room temperature with a resulting loss of its capacity to bind acetylcholine, no ionophoric properties could be detected with $Ca^{2+}$ or $Na^{+}$ and with or without carbamylcholine (Shamoo and Eldefrawi 1975). As the receptor protein ages there is a possible corresponding change in the parameters of single channel current fluctuations. Fig. 3 is representative of traces of single-channel current fluctuations observed most frequently in our experiments. As in the case of

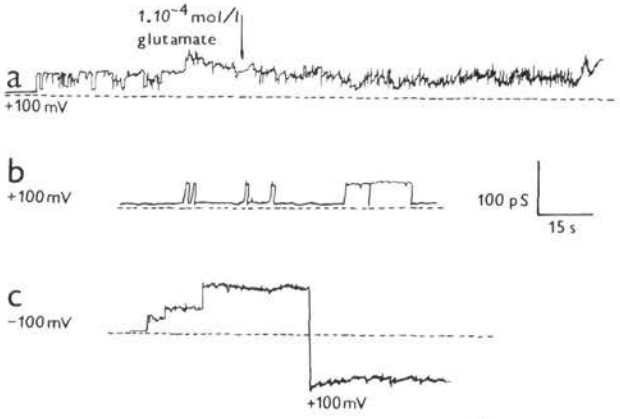


**Fig. 3.** Current fluctuations of receptor-protein-modified BLM. Glutamate ($1 \times 10^{-4}$ mol/l) was added to the medium (For the composition of the medium, see the legend to Fig. 1).

the acetylcholine receptor reconstituted into planar lipid bilayers by Boheim et al. (1981), both the single-opening and multiple-opening events (trace a) as well as persistent-opening events (trace b) could be observed in our model system. More frequently the conductance fluctuations levels were in the 25—150 pS range. Earlier a single-channel conductance value of 130 pS was recorded by the patch-clamp method on locust synapses (Usherwood 1979). Glutamate channel conductance obtained by the voltage-clamp method on crayfish muscle fibers was $23.5 \pm 7$ pS (Stettmeier et al. 1983). Similarly as in the case of the reconstituted acetylcholine receptor system (Boheim et al. 1981), the average life-time of single-opening events in our reconstituted system was longer than that reported for intact postsynaptic membrane (Cull—Candy et al. 1981), and may reach several seconds. In comparing quantitatively the reconstituted glutamate receptor system with the intact membrane current patterns, differences in species used and in experimental conditions should be taken into account. These differences include ions concentrations, lipid composition of cell and artificial membranes, the presence of solvent in BLM, etc.

We succeeded in reconstituting the glutamate receptor protein in BLM; however, improvements are still required to approach the in vivo properties of the receptor function.

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