# ISOLATION OF α-LATROTOXIN FROM VENOM OF THE SPIDER Latrodectus tredecimguttatus BY AFFINITY CHROMATOGRAPHY

B. Z. Dalimov, Sh. K. Kasymov,
and Sh. I. Salikhov

UDC 577.1.112

An affinity method is decribed for isolating α-latrotoxin, a neurotoxin with a presynaptic action from the venom of the spider Latrodectus tredecimguttatus, by two different antibody immunosorbents enabling the neurotoxin to be obtained in the homogeneous state according to the result of immunoelectrophoresis and electrophoresis in polyacrylamide gel after one chromatographic isolation. The first immunosorbent consisted of monospecific antibodies to α-latrotoxin bound to the $F_c$ fragment of protein of protein A from Staphylococcus aureus which, in its turn, was covalently conjugated with a cellulose sorbent – Tsellopor; and the second consisted of the active $F_{ab'}$ fragments of the same antibodies covalently conjugated with Tsellopor. It was shown that the capacity of the $F_{ab'}$-Tsellopor immunosorbent was 1.5-2.0 times higher than the capacity of the antibody-protein A-Tsellopor immunosorbent.

The isolation of α-latrotoxin, a neutroxin with a presynaptic action, from the venom of the karakurt spider Latrodectus tredecimguttatus with the aid of the gel, ion-exchange, and high-performance liquid variants of chromatography is associated with a number of technical difficulties which sharply lower the yield of the substance and require expensive equipment [1, 2].

It was desirable to develop an affinity method for the single-stage isolation of the neurotoxin directly from the whole venom with the aid of immunosorbents consisting of monospecific antibodies to α-latrotoxin bound to an affinity sorbent – protein A-Tsellopor (Tsellopor consists of porous cellulose beads oxidized with sodium metaperiodate to dialdehydecellulose) and active $F_{ab'}$ fragments of antibodies to the neurotoxin immobilized in oriented fashion on the sorbent. It is known that the immobolization of antibodies on any sorbents whatever is made difficult by the fact that immunoglobulins bind to the sorbent in unordered fashion both by the $F_c$ fragment and by the $F_{ab'}$ fragments, because of which the capacity of the immunosorbent falls sharply. To avoid this, the antibodies were immobilized on Tsellopor with the aid of protein A from Staphylococcus aureus (Pharmacia). In this case, the immunoglobulins are bound to protein A, which is covalently attached to the support, only through the $F_c$ fragment, and the active sites of the molecule remain free for binding with the neurotoxin. The neurotoxin was isolated with the aid of the immunosorbent by a procedure described previously [3] with some modification. Unfortunately, the capacity of this immunosorbent was not sufficiently high: when the neurotoxin was eluted from the column, a certain part of the ligands was washed out, which led to a rapid fall in the capacity of the sorbent. We therefore attempted to synthesize an immunosorbent with an oriented immobilization of the $F_{ab'}$ fragments of the antibodies.

$F(ab')_2$ fragments of the antibodies were isolated [4] and reduced with cysteine. The ordinary $F_{ab'}$ fragments so obtained were separated on a column of Bio-Gel P-6 (1 × 15 cm) equilibrated with 0.1 M Tris-HCl buffer, pH 8.2, containing 0.0001 M sodium ethylenediaminetetraacetate and were immobilized on the sorbent. The capacity of the immunosorbent obtained in this way was 1.5-2.0 times higher than that of the antibody–protein A–Tsellopor immunosorbent; i.e., calculated to 1 g of $F_{ab'}$-Tsellopor immunosorbent an average of 180 mg of neurotoxin was isolated, in contrast to the antibody-protein A-Tsellopor immunosorbent, 1 g of which isolated an average of 100 mg of neurotoxin.

A. S. Sadykov Institute of Bioorganic Chemistry, Uzbek Academy of Sciences, Tashkent.
Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 679-682, September-October, 1988. Original article submitted February 3, 1988.

0009-3130/88/2405-0577$12.50  

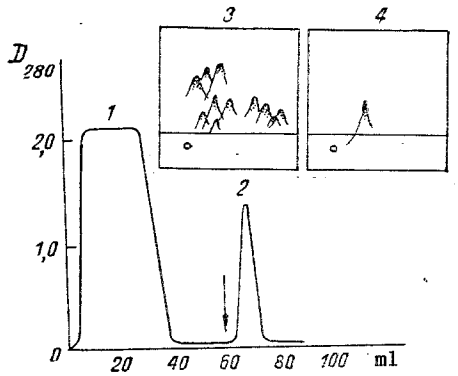


Fig. 1. Results of the affinity chromatography of α-latrotoxin: 1) protein components of the venom not bound to the immunosorbent; 2) α-latrotoxin. The inserts to the figure show the results of cross-immunoelectrophoresis before (3) and after (4) affinity chromatography. The arrow shows the beginning of elution with 0.01 M HCl in 0.15 M sodium chloride solution, pH 2.2.

The results of the affinity chromatography of α-latrotoxin are shown in Fig. 1. The bulk of the protein substances, having no affinity for the active centers of the antibodies, passed through the column without being bound to the immunosorbent. When the column was washed with 0.01 M HCl in 0.015 M sodium chloride solution, pH 2.2, the neurotoxin was eluted in the form of a narrow peak analysis of which in cross-immunoelectrophoresis revealed a single precipitation peak in the anodic region.

Terminal amino acid analysis showed that the N-terminal amino acid was isoleucine.

## EXPERIMENTAL

The α-latrotoxin was isolated by a method described previously [1] with slight modifications consisting in the preliminary gel chromatograph of the whole venom on a column (1.5 × 100 cm) of Ultragel AcA 34 equilibrated with a 0.15 M solution of sodium chloride buffered with phosphates, pH 7.2.

Cross-immunoelectrophoresis was carried out by Osterman's method [5] on a "Multiphor" instrument (LKB), using antibodies against the whole venom of the karakurt spider. Electrophoresis was conducted in 1% agarose gel (Sigma) in a buffer solution containing 0.07 M Tris and 0.02 M veronal, pH 8.5, at 20 V/cm for 1.5 h, and immunoelectrophoresis in the same gel containing 0.038% of antibodies to the whole venom at 10 V/cm for 18 h. The precipitation peaks obtained were stained with Coomassie R-250.

Electrophoresis in PAAG in the presence of NaSDS was carried out by a known method [6].

The neurotoxin homogeneous according to the results of immunoelectrophoresis and NaSDS electrophoresis was used to obtain antiserum. Rabbits were immunized by a scheme which we have described previously [7].

The affinity sorbent Tsellopor was obtained by precipitating an emulsion of a cuprammonium solution of cellulose with chloroform–benzene (1:5) in a 6% solution of sulfuric acid in acetone. The beads obtained were fractionated by free sedimentation in a cylinder and the fraction of particles with dimensions of 70-200 μm, determined under a microscope, were separated. The sorbent was oxidized with a 0.02 M solution of sodium metaperiodate at room temperature for 18 h and was washed with water [8].

The immobilization of the α-latrotoxin and protein A on the Tsellopor was carried out in 0.1 M carbonate-bicarbonate buffer solution, pH 9.0. The remaining aldehyde groups of the sorbent were reduced with a 0.2 M solution of sodium tetrahydroborate. The amount of protein added to the support was determined from the binding of the Bromophenol Blue [9].

All processes for the concentration and dialysis of the protein preparations were carried out in a Schleicher und Schüll ultrafiltration chamber with a Thomapor-50 membrane filter (FRG).

The specificity of the antibodies isolated was tested in Ouchterlony's double radial immunodiffusion reaction [10], where a single precipitation band was obtained, which showed the monospecificity of the antibodies obtained and the homogeneity of the neurotoxin.

In the production of the antibody-protein A-Tsellopor immunosorbent, the bond between the antibody molecules and protein A was stabilized with glutaraldehyde.

The immobilization of the $F_{ab'}$ fragments of the antibodies on the Tsellopor was carried out on the sorbent that had first been activated with epichlorohydrin and iodoacetamide.

The N-terminal amino acid of the isolated neurotoxin was determined by the method of Gray et al. [11].

## SUMMARY

The immobilization of antibodies and active $F_{ab'}$ fragments of antibodies to α-latrotoxin from the venom of the spider Latrodectus tredecimguttatus on a cellulosic sorbent has given immunosorbents – antibodies-protein A-Tsellopor and $F_{ab'}$-Tsellopor – for the one-stage isolation of α-latrotoxin directly from the whole venom.

It was shown that the capacity of the $F_{ab'}$-Tsellopor immunosorbent was 1.5-2.0 times higher than the capacity of the antibody–protein A-Tsellopor immunosorbent.

The neurotoxin isolated by means of these solvents has been characterized immunoelectrophoretically and its N-terminal amino acid has been determined as isoleucine.

## LITERATURE CITED

1. A. S. Sadykov, A. A. Akhunov, and Sh. I. Salikov, The Venom of the Karakurt Spider [in Russian], FAN, Tashkent (1985), p. 72.
2. Yu. A. Ushkarev and E. V. Grishin, Bioorg. Khim., 12, No. 1, 71 (1986).
3. G. Gyka, V. Ghetie, and J. Sjöquist, J. Immunol. Methods., 57, 227 (1983).
4. M. W. Fanger and D. G. Smith, Biochemistry, 127, 757 (1972).
5. L. A. Osterman, The Investigation of Biological Macromolecules by Electrofocusing, Immmunoelectrophoresis, and Radioisotopic Methods [in Russian], Nauka, Moscow (1983), p. 144.
6. U. K. Laemmli, Nature, 227, 680 (1970).
7. Sh. K. Kasymov, B. Z. Dalimov, Sh. I. Salikhov, and U. K. Inogamov, Symposium on Structure, Biosynthesis and Functions of the Molecular Elements of the Immune System. Pushchino (1987), p. 101.
8. A. E. Gurvich and A. V. Lekhttsind, Byull. Eksp. Biol. Med., No. 12, 752 (1981).
9. A. E. Gurvich, Biokhimiya, No. 5, 550 (1955).
10. H. Friemel and J. Brock, Grundlagen der Immunologie, 3rd edn., VEB Akademie Verlag, Berlin (1975) [Russian translation, Mir, Moscow (1979), p. 31].
11. W. R. Gray, S. P. Colowick and N. O. Caplan, Meth. Emzymol., 11, 139 (1967).