STRUCTURE OF ANTIGENIC DETERMINANTS OF NEUROTOXIN II FROM COBRA VENOM

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A knowledge of the structure of antigenic determinants will permit the synthesis of "artificial antigens" based on them, with the aim of obtaining highly effective antisera [1]. In addition, an investigation of the interaction of the antigenic determinant sections with elements of the immune system will permit a deeper understanding of the mechanism of antibody formation [2].

Convenient antibodies for solving these problems are the neurotoxins of the venom of the cobra Naja naja oxiana, the structures of which have been well studied [3]. It appeared of interest to establish the structures of the antigenic determinants of neurotoxin II (NT-II) with the object of their chemical synthesis.

Neurotoxin II was isolated by the method of [4] with some modifications and with additional purification on the ion-exchange sorbent Bio-Rex 70 in a concentration gradient of ammonium acetate of from 0.2 to 0.5 M. The purity of the NT-11 isolated was evaluated by electrophoresis in PAAG in the presence of Na DDS [5] and by analysis of its amino acid composition, which corresponded to that given in [3].

In view of the fact that the sections including Lys^{15}, Lys^{25}, Arg^{32}, Arg^{38}, and Arg^{55} are concentrated on the external surface of the NT-II molecule [6], to identify the determinant sections it was desirable to analyze tryptic peptides of NT-II. The reduction with dithioerythritol, carboxymethylation, and tryptic hydrolysis of NT-II were performed as in [7]. The tryptic hydrolysate was separated on a column (0.4 × 125 cm) of Sephadex G-25 Sf equilibrated with 0.1 M ammonium acetate buffer, pH 4.5. Detection was effected with a Uvicord S flow-through spectrometer (LKB) and a Beckmann spectrometer at wavelengths of 280 and 206 nm, respectively.

Monospecific antiserum to NT-II was obtained by immunizing rabbits with a conjugate prepared from NT-II and a suspension of oxidized cellulose. The antibodies were isolated on the immunosorbent NT-II–Tsellopor (Tsellopor consists of porous cellulose beads oxidized with sodium metaperiodate to dialdehydocellulose). To isolate the peptides containing the antigenic determinants of NT-II, to increase the capacity of the immunosorbent, and to prevent the nonspecific absorption of peptides an affinity sorbent was synthesized with orientedly immobilized $F_{ab'}$ fragments of antibodies to NT-II [8]. The peptides isolated by means of this sorbent were identified by analysis of the N-terminal amino acid residues, by determination of amino acid compositions, and, where necessary, by a determination of primary structures.

The amino acid sequences of the antigenic determinants of NT-II were determined on the basis of the known primary structure of NT-II [1] and the results of an analysis of the information on the N-terminal sequences and amino acid analyses of the peptides isolated. The antigenic determinant sections of NT-II from the venom of the cobra Naja naja oxiana responsible for binding with the active sites of antibodies to the native NT-II are localized in the following fragments of the polypeptide chain of the neurotoxin: Leu^{1}–Lys^{15}, Tre^{16}–Lys^{25}, Gly^{33}–Arg^{38}, Pro^{47}–Arg^{55}.

LITERATURE CITED

1. R. V. Petrov, R. M. Khaitov, and R. I. Ataullakhanov, Immunogenetics and Artificial Antigens [in Russian], Meditsina, Moscow (1983), p. 5.
2. Sh. K. Kasymov, B. Z. Dalimov, F. Yu. Garib, and A. S. Sadykov, 3rd Soviet–Swiss Symposium on Biological Membranes: Structure and Functions, Tashkent (1983), p. 153.

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3. N. V. Grishin, A. P. Sukhikh, N. L. Slobodyan, Yu. A. Ovchinnikov, and V. M. Sorokin, FEBS Lett., 45, No. 1, 118 (1974).
4. Ya. Kh. Turakulov, D. N. Sakhibov, V. M. Sorokhin, and L. Ya. Yukel'son, Biokhimiya, 34, No. 6, 1119 (1969).
5. U. K. Laemmli, Nature (London), 227, 680 (1970).
6. V. T. Ivanov, V. I. Tsetlin, I. I. Mikhaleva, O. M. Volpina, A. R. Nuriddinov, Yu. N. Utkin, A. S. Arseniev (Arsen'ev), V. S. Pashkov, E. Karlson, and A. M. Surin, Peptides 1978, Wroclaw (1979), p. 41.
7. Yu. N. Utkin, V. S. Pashkov, K. A. Pluzhnikov, A. V. Kuryatov, A. S. Arsen'ev, V. I. Tsetlin, V. F. Bystrov, and V. T. Ivanov, Bioorg. Khim., 9, No. 4, 437 (1983).
8. V. T. Skvortsov and A. E. Gurvich, Byull. Éksp. Biol. Med., No. 2, 179 (1984).

ACTIVATION OF ANGREN KAOLIN – AN ADSORBENT FOR SEPARATION OF AMINO ACIDS BY THIN-LAYER CHROMATOGRAPHY

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After heat treatment (at 800-850°C, 4 h) and acid activation (20% HCl, 4 h), Angren gray kaolin is not inferior in its chromatographic activity to adsorbents widely employed in TLC.

The results of the separation of a number of natural compounds in layers of silica gel–gypsum and of activated kaolin–starch show that the latter possesses a higher separating capacity and sensitivity.

To determine the activity of activated Angren kaolin we have determined the R_f values of standard azo dyes: azobenzene and Sudan I. For comparison, we give the R_f values of azobenzene and Sudan I on alumina (Brockmann activity II) and a fixed kaolin–starch layer (the R_f values on alumina are taken from the literature):

Adsorbent	Azobenzene	Sudan I
Alumina	0.59	0.01
Kaolin–starch	0.60	0.12

We have studied the chromatographic separation of amino acids in a thin layer of kaolin–starch. The methods of preparing the plates with a fixed layer corresponded to those given in the literature [1-3]. The most suitable solvent system for such separation is butan-1-ol–acetic acid–water (4:1:1). The revealing agent was a 1% solution of ninhydrin in acetone.

Below we give the R_f values of a number of amino acids in a layer of silica gel–gypsum and in a layer of kaolin–starch (the R_f values in the silica gel–gypsum layer have been taken from the literature [1, 2]; * denotes a hydrochloride):

Amino acid	Silica gel–gypsum*	Kaolin–starch
Alanine	0.27	0.54
Aspartic acid	0.21	0.32
Leucine	0.47	0.87
Valine	0.35	0.70
Threonine	0.25	0.45
Serine	0.22	0.17
Tryptophan	0.56	0.69

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