Volume 270, number 1,2, 127-131 FEBS 08859 September 1990

Cloning and structure of cDNA encoding α-latrotoxin from black widow spider venom

N.I. Kiyatkin, I.E. Dulubova, I.A. Chekhovskaya and E.V. Grishin

Shemyakin Institute of Bioorganic Chemistry, USSR Academy of Sciences, Moscow, USSR

Received 12 July 1990

cDNA encoding the putative α-latrotoxin precursor was isolated from spider venom glands cDNA library and sequenced. The cDNA contained the 4203 base-pair open reading frame corresponding to the 156 855-Da protein composed of 1401 amino acids. Computer analysis of the deduced primary structure revealed the presence of various internal imperfect repeats mainly in its central and C-terminal regions.

α-Latrotoxin precursor; cDNA nucleotide sequence; Deduced primary structure; Amino acid sequence homology

1. INTRODUCTION

A high molecular weight neurotoxic protein (α-latrotoxin) isolated from venom glands of black widow spider *Latrodectus mactans tredecimguttatus* acts selectively on presynaptic nerve endings of vertebrates and stimulates secretion on neuromediators. The α-latrotoxin molecular mass is about 130 kDa, and its isoelectric point is 5.5. It is not a glycoprotein and shows no enzymic activity [1].

Specialized membrane receptors of α-latrotoxin are identified in brain preparations of some animals and in the PC12 cell line by radioligand analysis. Apparently, interaction of the neurotoxin with a presynaptic receptor leads to increase of the Ca^{2+} concentration inside the cell, and stimulates hydrolysis of phosphoinositides [2]. At the same time α-latrotoxin enhances cation conductivity of the bilayer lipid membrane due to the incorporation of the toxin molecule into the lipid layer with the formation of the cation-selective ion channel [3]. Results which evidence fusogenic properties of the toxin were also obtained [4].

Information on the α-latrotoxin structure is essential for thorough elucidation of the mechanism of its action. The paper presents the total amino acid sequence of the α-latrotoxin deduced from cDNA sequencing and describes a few unusual features of its structure.

2. MATERIALS AND METHODS

Total RNA was isolated from the frozen spider venom glands by the Feramisco method [5]. The mRNA fraction was obtained after two cycle chromatography on oligo(dT)-cellulose [6].

Correspondence address: N.I. Kiyatkin, Shemyakin Institute of Bioorganic Chemistry, USSR Academy of Sciences, Moscow 117871, USSR

Double-stranded cDNA synthesized according to the Gubler method was inserted into the *Sma*I-site of plasmid vector pSP65 and cloned in *E. coli* MHI [7]. The cDNA library was screened with γ-^{32}P-labeled synthetic oligodeoxynucleotide probe 5′-TC$^{C}_{T}$TT$^{T}_{C}$TCNGT$^{A}_{G}$TC$^{A}_{G}$AA$^{A}_{G}$TA-3′, its synthesis was based on the structure of one of the toxin tryptic peptides [8].

Hybridization was performed at 30°C for 16 h in buffer containing 5×SSPE, 5×Denhardt's solution, 0.25 mg/ml of yeast RNA and 0.1% sodium pyrophosphate [9]. The filters were washed 3 times for 10 min at 28°C in 0.33 M Tris-HCl, 0.5 M NaCl, 0.5% SDS, then washed for 20 min at 30°C in the same solution.

Nucleotide sequences of cDNA inserts from positively hybridized clones were determined by the Maxam-Gilbert technique in solid-phase modification [10]. A continuous nucleotide chain was deduced from sequences of overlapping fragments as described in [11].

3. RESULTS AND DISCUSSION

The cDNA library of 80 000 recombinant clones was obtained on the basis of poly(A)$^+$ RNA from venom glands of the spider *Latrodectus mactans tredecimguttatus* in plasmid vector pSP65.

Twenty-five tryptic peptides and the N-terminal amino acid sequence of α-latrotoxin were earlier characterized [8] and peptide 51-5 (Tyr-Phe-Asp-Thr-Glu-Lys-Glu) was used to derive the 20-base oligonucleotide hybridization probe (see Materials and Methods). The initial screening of the cDNA library by hybridization with this probe revealed two positive clones (pT-1 and pT-2) containing inserts of 1200 and 800 bp, respectively. According to analysis of the nucleotide cDNA sequence of the isolated clones, they contained overlapping fragments of the α-latrotoxin structural gene. Additional screening was carried out since neither of the two clones contained the entire protein-coding sequence. cDNA from clone pT-1 was used as a probe to screen the cDNA library and the last screening was performed with the C-terminal fragment

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/90/$3.50

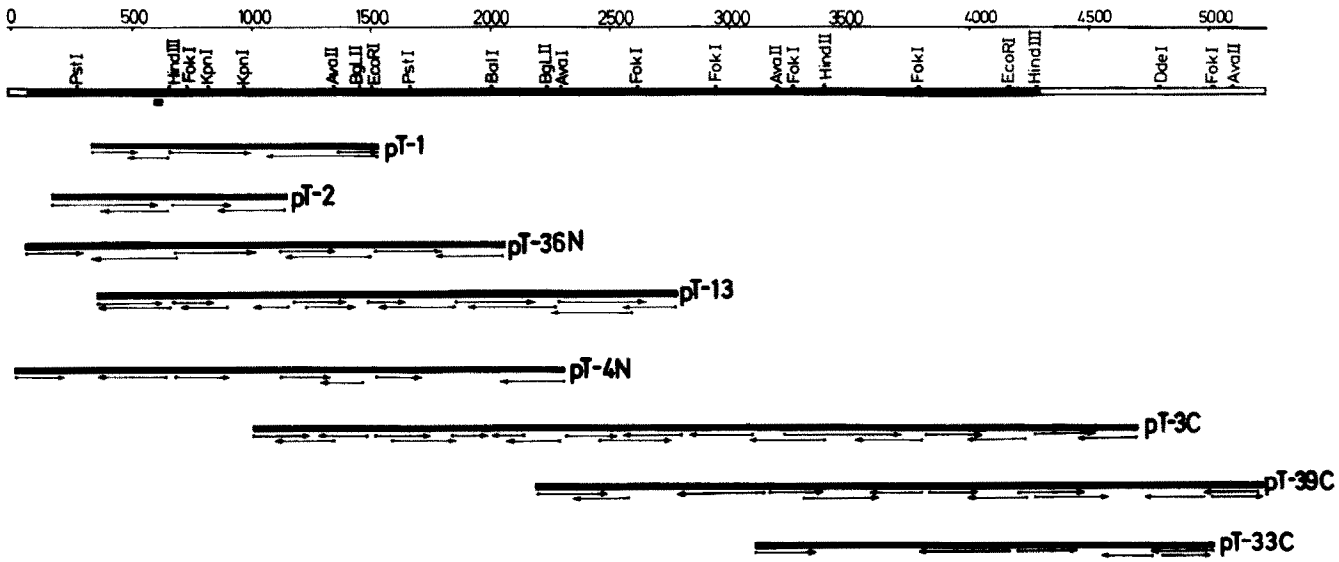

Fig. 1. Location of cDNA fragments of isolated clones on partial restriction map of α-latrotoxin cDNA. The protein coding region is filled. Horizontal arrows show the direction of sequencing. Filled rectangle indicates position of oligonucleotide probe used in cDNA cloning.

*Ava*II (3079)/*Eco*RI (4158) from clone pT-3C (Fig. 1). Structural analysis of overlapping cDNA clones revealed a continuous nucleotide sequence 5408 bp long. The given sequence contains an open reading frame of 4203 bp encoding a polypeptide of 1401 amino acids (calculated M_r 156 855) starting with the first Met residue (see below) and ending at the termination TAG codon. (Fig. 2). The cDNA includes a full-size structural gene of mature α-latrotoxin. This is confirmed by structural analysis, which showed that all the tryptic peptides of the toxin, including the N-terminal fragment, with the structure previously deciphered by the protein chemistry methods were found in the deduced amino acid sequence.

Comparison of this sequence with the N-terminal structure of the purified α-latrotoxin (Glu-Gly-Glu-Asp-Leu-Thr-Leu-Glu-Glu-Lys-Ala-Glu), showed that the mature toxin started with Glu-1. Upstream of this position there are 3 methionine residues (−3), (−13), (−20), each being a possible candidate for initiating an amino acid residue. Since the open reading frame extends to the 5′-end, a possibility of additional coding sequence cannot be completely ruled out. However, one can suggest that the Met (−20) residue should correspond to the site of translation initiation. A nucleotide sequence around this Met shows obvious homology to the translation initiation site consensus (AAXATGA) for a number of studied toxins; in addition, the length of a putative signal peptide for α-latrotoxin in this case is 20 amino acid residues that accords well with the length of signal peptides found in other toxins [12–14].

It should be noted that the molecular mass of the protein deduced from the cDNA (M_r 156 855 Da) considerably differs from that earlier determined for α-latrotoxin by means of SDS gel electrophoresis (130 kDa) [1].

So, α-latrotoxin either has abnormal electrophoretic mobility or it can be coded as a precursor, and processing takes place in the C-terminal region of the polypeptide chain during its maturation. The latter seems more reasonable because all studied peptides of the tryptic hydrolysate are equally distributed in the toxin fragment with coordinates 1-1170. Molecular mass of this fragment (M_r 131 kDa) is in good agreement with the apparent molecular mass of the isolated toxin. Moreover the C-terminal fragment of over 200 amino acid residues with coordinates 1171–1381 was absent in the products of toxin tryptic hydrolysate despite a lot of cleavable regions. Though these data confirm possible processing of the α-latrotoxin C-terminal region, only direct determination of the C-terminal amino acid sequence might provide the final conclusion.

Computer analysis of the deduced amino acid sequence revealed the presence of various internal imperfect repeats mainly in its central and C-terminal region (Fig. 3). According to this analysis it is possible to divide the molecule of the processed toxin into two structural regions: N-terminal fragment including some 500 amino acid residues and practically free of internal repeats; and the C-terminal one (about 500–1200 amino acid residues) of high intrinsic homology (Fig. 3). Functional significance of such a structure organization of α-latrotoxin is still unknown. One can assume that these structural domains overlap with functional domains responsible for binding to presynaptic receptor or for the ion channel arrangement [15]. Of much interest is an imperfect repeat of 7 amino acids occurring in the structure of α-latrotoxin for 19 times with an interval of 26–28 amino acid residues (Fig. 2), except for the interval between repeats 13 and 14. It is noteworthy that analogous repeats, though few in number, are found in the structure of *Drosophila* Notch-protein [16]

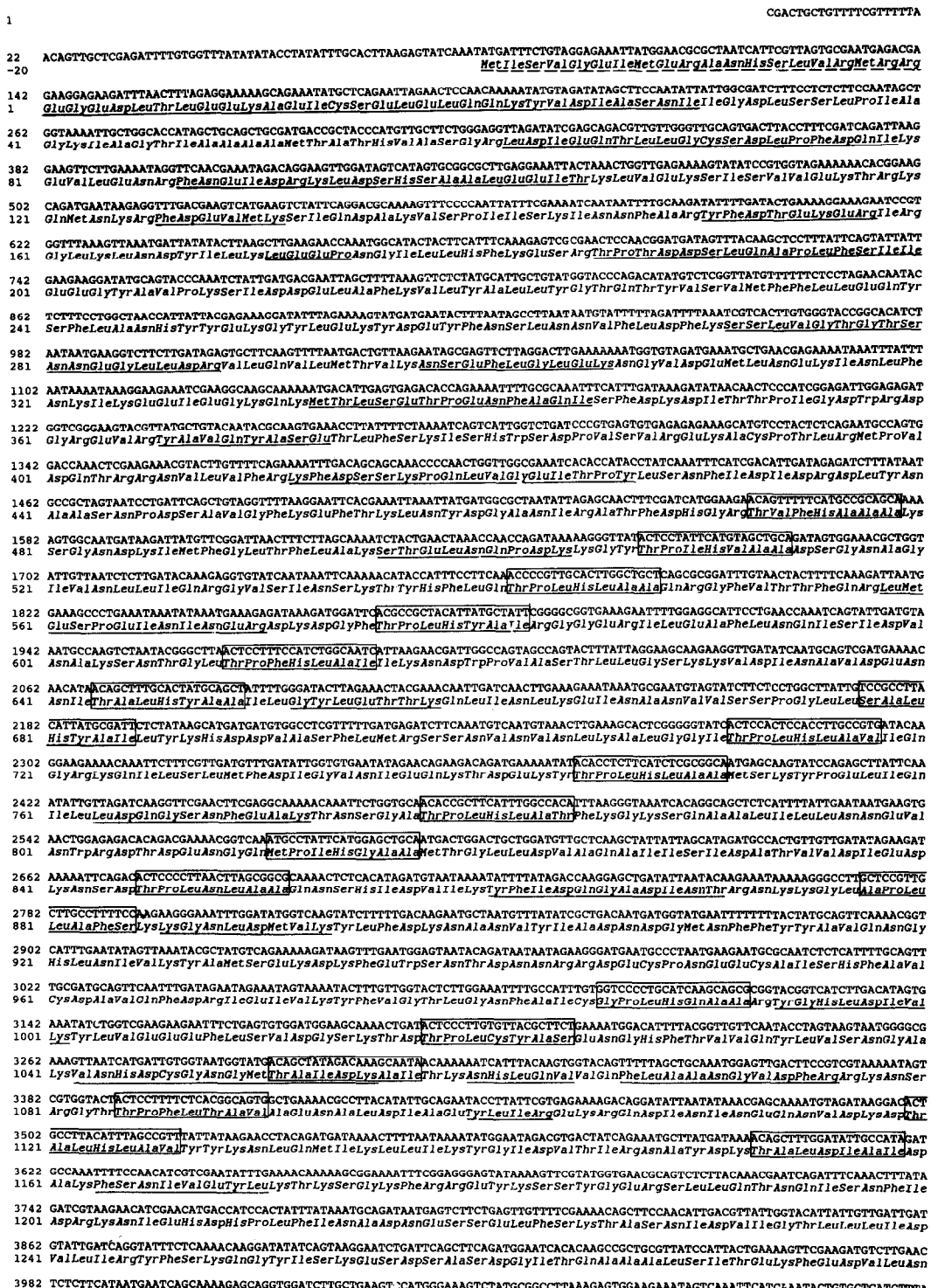

Fig. 2. Nucleotide sequence of cDNA coding chain and amino acid sequence of α-latrotoxin precursor from black widow spider. Amino acid sequences determined by peptide analysis are underlined. Putative signal peptide is indicated by dotted line. 19 tandemly arranged imperfect repeats are boxed.

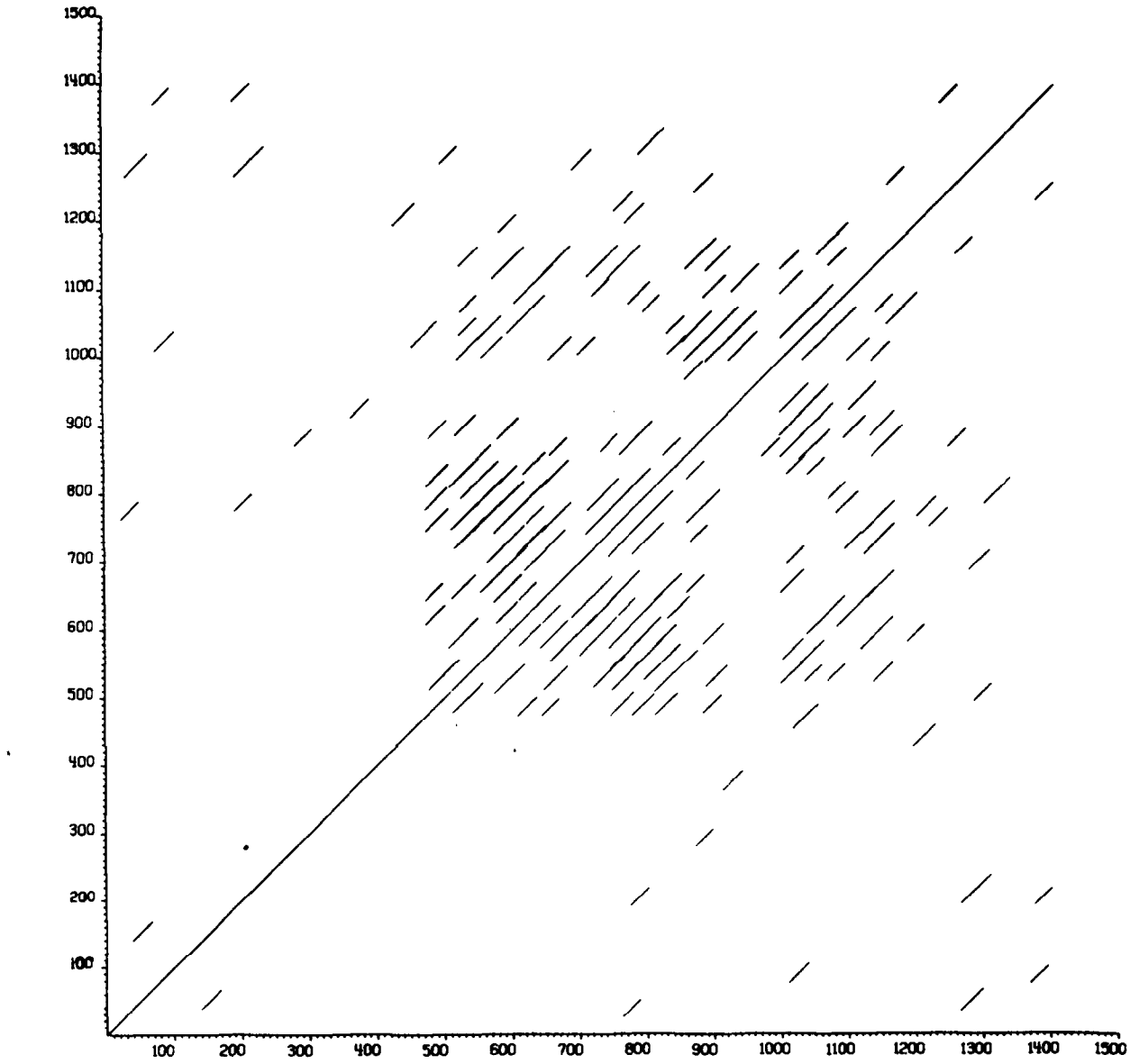

Fig. 3. Computer dot plot analysis of α-latrotoxin to identify amino acid sequences within the toxin. Homologous sequences are indicated by lines parallel to the central diagonal, their lengths corresponding to those of homologous regions of amino acid sequences. Residue numbers are indicated on the axis.

as well as in a virus envelope protein [17], the repeats being located with the same interval. Their functional role is still obscure. Thorough results deal with complete analysis of latrotoxin intrinsic homology and prediction of the toxin secondary structure will be published elsewhere. Further research into the expression of the cloned gene for α-latrotoxin and its mutants will illuminate the molecular mechanism of the interaction of black widow spider neurotoxin and cell membrane.

REFERENCES

[1] Grasso, A. (1988) in: Neurotoxins in neurochemistry (Dolly, J.O. ed), pp. 67-78, Ellis Horwood.
[2] Scheer, H. and Meldolesi, J. (1985) EMBO J. 4, 323-327.
[3] Finkelstein, A., Rubin, L.L. and Tzeng, M.C. (1976) Science 193, 1009-1011.
[4] Sokolov, Yu.V., Chanturia, A.N. and Lishko, V.K. (1987) Biochim. Biophys. Acta 900, 295-299.
[5] Feramisko, J.R., Smart, J., Burridge, K., Helfman, D.M. and Thomas, G.P. (1982) J. Biol. Chem. 257, 11024-11031.
[6] Aviv, H. and Leder, P. (1972) Proc. Natl. Acad. Sci. USA 69, 1408-1413.
[7] Gubler, U. (1988) Nucl. Acids Res. 16, 2726.
[8] Volkova, T.M., Galkina, T.G., Kudelin, A.B., Nazimov, I.V. and Grishin, E.V. (1990) Bioorg. Khim. (in press).
[9] Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) in: Molecular Cloning: a Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
[10] Chuvpilo, S.A. and Kravchenko, V.V. (1985) FEBS Lett. 179, 34-36.

[11] Kostetsky, P.V. and Dobrova, I.E. (1988) Bioorg. Khim. 14, 515–521.
[12] Ando, K., Natori, S. (1988) Biochemistry 27, 1715–1721.
[13] Ducancel, F., Guignery-Frelat, G., Bouchier, Ch., Menez, A. and Boulain, J.-C. (1988) Nucl. Acids Res. 16, 9048.
[14] Tamiya, T., Lamonroux, J., Julien, B., Grima et al. (1985) Biochimie 67, 185–189.
[15] Meldolesi, J., Scheer, H., Madeddu, L. and Wanke, E. (1986) Trends Pharmacol. Sci. 7, 1–5.
[16] Wharton, K.A., Johansen, K.M., Xu, T. and Artavanis-Tsakonas, S. (1985) Cell 43, 567–581.
[17] Tomley, F., Binns, M., Campbell, J. and Boursnell, M. (1988) J. Gen. Virol. 69, 1025–1040.