Vol. 201, No. 1, 1994 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 30, 1994 Pages 186-193

SECONDARY STRUCTURE OF NOXIUSTOXIN AND CHARYBDOTOXIN FROM HYDROPATHY POWER SPECTRA

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Received March 25, 1994

The analysis of the hydropathy profile power spectra provides a basis for studies of pattern matching between the primary and secondary structure of peptides. The structural motif obtained with Noxiustoxin (NTX), the first K^+ channel blocking peptide described, is composed of a N-terminal ß-strand, a central α-helix and a final ß-strand zone, probably forming a ß-sheet. These results were compared with those of Charybdotoxin (ChTX), a potent inhibitor of the high conductance Ca^{2+}-activated K^+ channel, which presents about 48% similarity with NTX in the amino acid sequence. Our prediction for ChTX secondary structure, which is known by 2D-NMR spectroscopy, yielded a Chou-Fasman quality index Q = 90 %. The comparison between the two toxins has guided the interpretation of the data obtained.

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0006-291X/94 $5.00

In recent years several short peptidic toxins (37 to 39 residues, 3 disulfide bridges) have been purified from scorpion venoms according to their blocking activity on potassioum selective channels [1]. These neurotoxins are very important probes not only for identifying and studying the biophysical properties of channels, but are invaluable tools for isolating and characterizing K^+ channel proteins [2]. The primary structures of these peptides are similar and possibly have a three-dimensional structure with a conserved motif, but the molecular mechanism of action in terms of affinity and specifivity varies considerably. Thus, in order to increase our knowledge on the structure-activity relationships of toxins versus K^+ channels we present here a study on the secondary structure of NTX, a 39 amino acid residues peptide, purified from the venom of the mexican scorpion *Centruroides noxius* [3], which blocks the voltage-dependent K^+ channel of squid axon [4]. The method used for the prediction allows to obtain plots of the two-dimensional power spectrum of the hydropathy profile, revealing ordered structures [5]. The adequacy of this prediction has been confirmed by the analysis of ChTX, a protein isolated from the venom of the scorpion *Leiurus quinquestriatus*, which was shown to block Ca^{2+}-activated K^+ channel from rat skeletal muscle [6], and whose secondary structure was elucidated by Bontems et. al. using NMR spectroscopy [7].

Due to the fact that both NTX and ChTX display an important similarity in their primary structure and both recognize Ca^{2+}-activated K^+ channels of skeletal muscle transverse tubules [8], we surmise that our proposed secondary structure of NTX is correct.

METHODS

NTX and ChTX are relatively small peptides strongly compacted by three disulfide bridges. Kyte and Doolittle [9] have proposed a general and reliable method for prediction of possible amphipathic α-helices and ß-strands, that can be applied to such peptides. These authors have treated the hydrophobicity concept in a quantitative manner. Each individual amino acid residue of a paricular sequence has an intrisic property manifesting the free-energy involved in desplacing that amino acid residue from the lipophilic to the aqueous phase. Several hydropathy tables were generated for each residue and and can be used to calculate the hydropathy profile of a given sequence. Positive hydropathy values means hydrophobicity.

In this work we used a method based on two-dimensional plots obtained by the Fourier transform of the hydropathy profile (amphipathic plots) within a certain sequence length, called window, to identify possible amphipathic ordered structures [5]. The Gilbrat hydropathy scale, based on the formalism of information theory, has been used [10,11].

The transform is performed on a fixed window that, residue by residue, will span all the sequence. The length of the window has to be near the length of the ordered structure under investigation. In our prediction windows of 7 and 11 residues were used, in order to conform the best as possible ordered structures.

Regular structures show periodicity in the hydrophobic profile, so that the Fourier transform of amino acid hydrophobicities, as a function of sequence, will have peaks at the frequencies given by 1/P, where P is the period in residues [5]. In this way amphipathic ordered conformations, due to

the alternance of hydrophobic with hydrophilic residues, are said to have an "ideal" hydropathy profile oscillation of period P = 2 residues for ß-strands and period P = 3.6 residues for α-helices, and the frequency peaks at 1/2 and 1/3.6 (residues^{-1}) respectively, while frequency 1/4 means the tendency to a loop conformation of the chain [5].

From the Fourier transform, the power spectrum is then calculated by computer, within the same window of a given size. The plot of the two-dimensional power spectrum, function of the sequence residues versus the frequency shows the peaks revealing the conformation of the polipeptide investigated, shown in different levels of grey-color. It is worth noting that different brightness in the same ordered structure is due to either numerical bord effects or to different amplitudes of the hydrophobic signal. The information is only carried by the position in the axis and not by the brightness.

These plots constituite a 'fingerprint' of the sequence. They carry information about the identification of amphipathic ordered structures and may be used also for recognition of structural similarities between different proteins.

RESULTS and DISCUSSION

The structure of ChTX, a 37 amino acid residue peptide was studied by Bontems et al. [7] by means of proton Nuclear Magnetic Resonace (NMR) spectra data analysis. They proposed for ChTX a α+ß model, that seems to be a structural motif common to other scorpion toxins.

In this model ChTX has an initial ß-strand (5-9), a central α-helix (10-20) and two final consecutive ß-strands

(26-30,31-35) that give rise to a ß-sheet. This particular folding is stabilized by three disulfide bridges. The Cys-7 and Cys-28 links the intial ß-strands with the first strand of the final ß-sheet, Cys-13 with Cys 33 and Cys 17 with Cys-35 links the helix to the sheet.

Our prediction of the secondary structure based on the hydropathy power spectrum for ChTX (see Fig. 1b) also reveals an N-terminal ß-strand (3-8) a central α-helix (10-20) and a final ß-strand zone (28-36) and it allows the same package, due to disulfide.bridges, with a final ß-sheet, similar to the model proposed by Bontems et al. [7].

The results for ChTX compared with NMR solved structure yields a Chou-Fasman quality index [12] <Q> = 90 % .

The amino acid sequence of NTX is 48.6% identical to that of ChTX. The Cys residues are conserved, suggesting similar three disulfide bridge arrangements, which should strongly influence the folding of NTX.

Our prediction of the NTX secondary structure is very similar to that of ChTX, yielding an N-terminal ß-strand (3-8), a central α-helix (10,20), and a final ß-strand zone (29-37) (Fig. 1a) separed by the residue Gly-32. This Gly-32 may play a central role as a ß-turner, and it is feasable that, like in ChTX, the ß-strands could give rise to a ß-sheet. The Cys-7 and Cys-29 links the first and the second ß-strands, Cys-13 with Cys-34 and Cys-17 with Cys-36 links the central helix with the third ß-strand, in analogy with the Bontems et al. model for ChTX [Fig. 2].

From this figure it is evident a high similarity between the predicted secondary structure of NTX and ChTX, that strongly supports a strict correlation between the structure and the activity of the two toxins versus K^+ channels

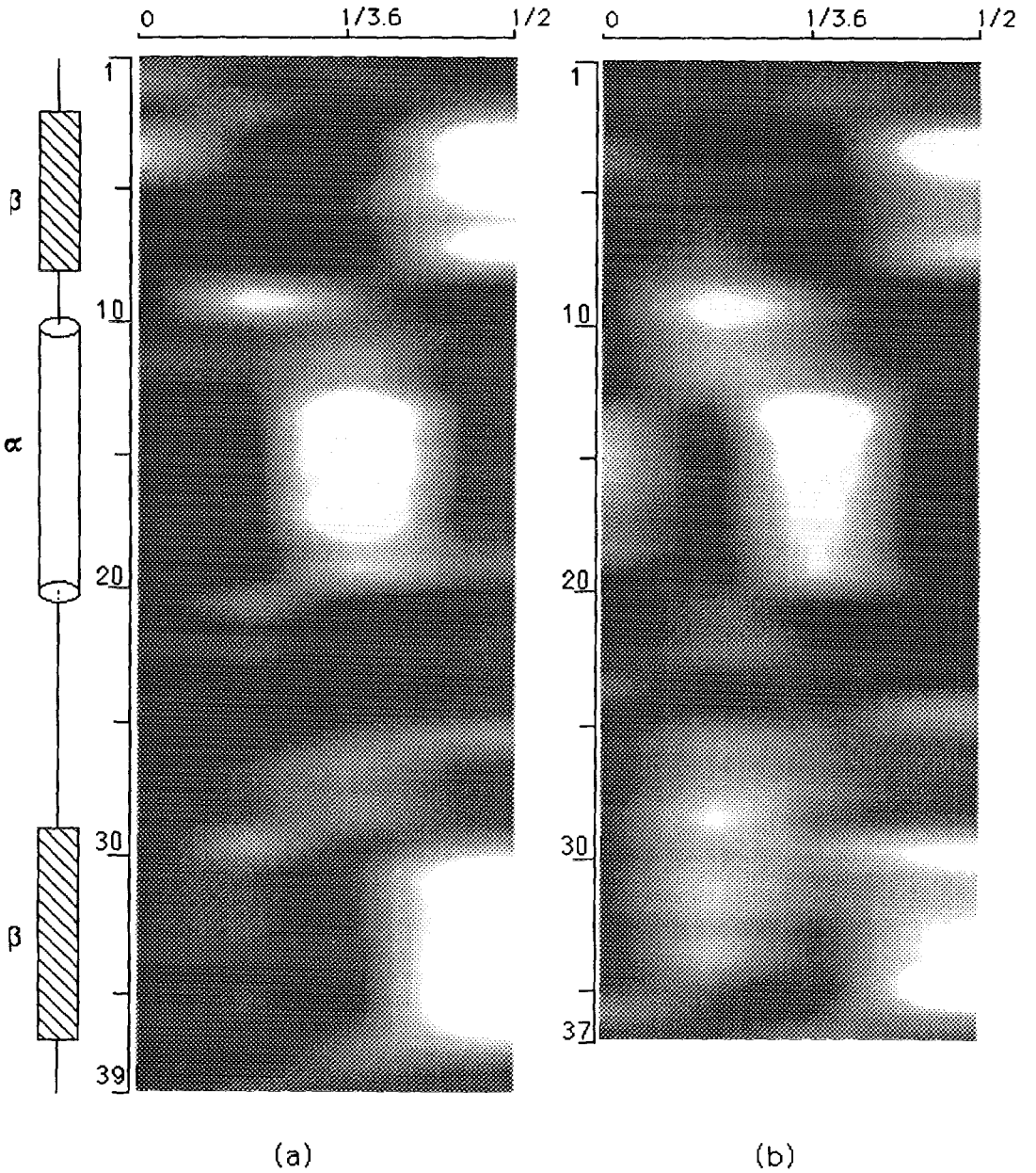

Fig. 1.
Power spectra plots. Window 7 has been used. In x-axis the frequencies, in Y-axis the residues. a) NTX b) ChTX. The polypetides conformation is shown by means of grey levels. The spot under frequency 1/3.6 indicates the presence of an α-helix conformation while the spots under frequency 1/2 are relative to ß-conformations. See text for details. On the left: a cartoon representation of the prediction for NTX.

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          1        10        20        30        39
ChTX      EFTNVSCTTSKECWSVCQRL-HNTSRGKCMNKKCRCYS
NTX       TIINVKCTSPKQCSKPCKELYGSSAGAKCMNGKCKCYNN
            ßßßßßß aaaaaaaaaaa        ßßßßßßßßß
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Fig. 2.
Aligned ChTX and NTX and their predicted secondary structures. The symbols α and ß refer to the α-helix and ß-strand conformations, respectively. In bold underlined characters, the Cys residues.

Indeed, as shown by Valdivia et al. [8], both NTX and ChTX affect the Ca^{2+}-activated K^+ channels of skeletal muscle transverse tubules, reducing the open probability of the channel. However, the blocking effect was different; ChTX induced long-term blockade, while NTX drove the channel into a brief blocked state.

The presence of prolines in the α-helix region (Pro-10 and Pro-16), (see Fig. 2), should also be analysed with care. Proline residues are known to be disruptive of α-helix formation. Howewer, several examples of experimentally prooved prolines in α-helix have been reported, for example in Myoglobin [13]. Pro-10 is at the beginning our proposed α-helix of NTX, while Pro-16 is nearly Cys-17 that could overcome through the disulfide bridge formation any constraint to the proposed model.

In order to increase our knowledge of the toxin structures involved in the recognition of the channel receptor site, it is essential to solve the three-dimensional structure of NTX.

At the moment, from our data, we can suggest that the spatial folding of NTX have to be close to that of the solved ChTX.

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