Pure & Appl. Chem., Vol. 61, No. 3, pp. 497–500, 1989.
Printed in Great Britain.

Structure—function relationship of polypeptide toxins: modifying gating mechanism of sodium channel

S.E. Odinokov, A.A. Nabiullin, E.P. Kozlovskaya and G.B. Elyakov

Pacific Institute of Bioorganic Chemistry, Far East Branch of USSR Academy of Sciences, Vladivostok, 690022, U.S.S.R.

Abstract - Sea anemone toxins are systematically classified according to amino acid sequence, topography of hydrophobic regions and known pharmacological and immunochemical activities. On basis of above properties these toxins are divided into two groups. The significance of each property is discussed in view of structure-function relationship. It is shown that pharmacological activity of sea anemone toxins correlates with topography of hydrophobic regions.

INTRODUCTION

Last years progress in studies of immunological properties and chemical modifications of polypeptide toxins as well as in determination of amino acid sequences and, for some of them, spatial structures reveal that their action on exitable membrane is polyfunctional. This action depends on such factors as toxin spatial arrangement features, distribution of charge and its polarizability, peripheral hydrophobic structure, accessibility of individual amino acid residues and their ability to interact with invironment. Being relatively small in size and having a high affinity to receptor, sea anemone toxins fall within distinct class of polypeptides, which is most suitable for investigation of gating mechanism of Na^+-channel. In the present paper primary and secondary structures as well as topography of hydrophobic regions of 14 polypeptide sea anemone toxins are compared with their pharmacological and immunochemical activities.

CLASSIFICATION OF SEA ANEMONE TOXINS

To date amino acid sequences have been determined for 14 toxins isolated from 7 species of sea anemone. Studied by us *Radianthus macrodactylus* species extends known data on chemical structure of sea anemone toxins (Table 1). Our studies make it possible to include during classification more number of properties characterizing structure-function relationship. As shown in Table 1, high similarity is observed for sequences of all toxins investigated. For elucidation of homology several gaps were introduced to maximise alignment and exchange of amino acids possessing similar side chains or twinned exchange of oppositively charged residues, which fail to effect the probable salt bridges, was not taken into account. According to amino acid sequence homology sea anemone toxins may be divided into two groups; namely those isolated from *Radianthus* sp. and *Stoichactis helianthus* (R-group) and those isolated from *Anemonia sulcata* and *Anthopleura* sp. (A-group).

Difference between R- and A-group toxins is manifested by content of some amino acids, total charge of molecule, receptor binding ability (see Table 2) and in immunochemical behaviour (ref. 1-2). All A-toxins are positively charged, but R-toxins have negative or zero charge at pH 7.0. Number of possible salt bridges in toxins of R-group is higher because of content of twinned oppositively charged residues in higher then that in A-toxins. Comparison of immunochemical behaviour and data of competition experiments on rat brain synaptosomes (ref. 1-2) shows that representatives of A- and R-group belong to two different immunological groups and receptor binding ability of A-toxins is higher then that of R-toxins. Note, sea anemone

Table 1. Homology alignment for sea anemone toxins

Toxin[a]	Sequence	Group
Rm-III	G-NCKCDDEGPYVRTAPLTGYVDL--GYCNEGWEKC-ASYYSPIAECCRKKK	R
Rm-IV	G-NCKCDDEGPNVRTAPLTGYVDL--GYCNEGWEKC-ASYYSPIAECCRKKK	
Rm-V	G-NCKCDDEGPNVRTAPLTGYVDL--GYCNEGWDKC-ASYYSPIAECCRKK	
Rm-II	G-TCKCDDDGPDVRTATFTGSIEF--ANCNESWEKCLAVY-TVPASCCRKKK	
Rp-II	ASCKCDDDGPDVRSATFTGTVDF--WNCNEGWEKCTAVY-TPVASCCRKKK	
Sh-I	AACKCDDEGPDIRTAPLTGTVDL--GSCNAGWEKC ASYYTIIADCCRK	
As-I	GA(P/A)PCLCKSDGPNTRGNSMSGTIWV--FGCPSGWNNCEGRA--IIGYCC KQ	A
As-II	G(V/I)PCLCDSDGPSVRGNTLSGIIWL--AGCPSGWHNCKKHGPT-IGWCC KQ	
As-V	GVPCLCDSDGPSVRGNTLSGILWL--AGCPSGWHNCKKHK(P/G)T-IGWCC-K	
Af-I	GVACLCDSDGPNVRGNTLSGTIWL--AGCPSGWHNCKAHG(P/T)-IGWCC-KQ	
Af-II	GGVPCLCDSDGPSVRGNTLSGIIWL--AGCPSGWHNCKAHGPT-IGWCC-KQ	
Ae-I	GVPCLCDSDGPSVRGNTLSGILWL--**AGCPSGWHNCKAHGPT-IGWCC-KQ**	
Ax-I	GVSCLCDSDGPSVRGNTLSGTLWLYPSGCPSGWHNCKAHGPT-IGWCC-KQ	
Ax-II	GVPCLCDSDGPRPRGNTLSGILWFYPSGCPSGWHNCKAHGPN-IGWCC-KK	

[a]Sea anemones: Radianthus macrodactylus (Rm), R. paumotensis (Rp), Stoichactis helianthus (Sh), Anemonia sulcata (As), Anthopleura fuscoviridis (Af), A. elegantissima (Ae) and A. xanthogrammica (Ax).

Table 2. Some physico-chemical and pharmacological properties of sea anemone toxins

Toxin	Total charge at pH 7	Possible salt bridges	Total hydrophobicity[a]		LD_{50}[b] on mice μg/kg	K_b[c] rat brain synaptosome
			Middle region	C-terminal region		
Rm-III	0	5	21.7	8.7	25	
Rm-IV	0	5	21.7	8.7	40	30
Rm-V	-1	5	21.7	8.7	350	
Rm-II	0	5	22.9	64.3	1 650	
Rp-II	0	5	13.9	44.1	4 200	100
Sh-I	-2	4	27.4	49.3	20 000	
As-I	+2	2	65.0	41.1	3 800	7
As-II	+2	2	94.8	13.3	100	0.15
As-V	+3	2	89.9	9.5	19	0.05
Af-I	+1	2	61.1	13.3	98	
Af-II	+1	2	94.8	13.3	450	
Ae-I	+1	2	89.9	13.3		
Ax-I	+1	2	27.2	13.3	66	0.12
Ax-II	+3	2	54.3	4.5	8	0.03

[a] Determined as area of "positive peaks" on hydropathic profiles, calculated by Kyte and Doolittle method (ref. 11).
[b] Lethal dozes on mice after intraperitoneal injection (our results and data from ref. 1-2).
[c] Constant of toxin binding to rat brain synaptosomes found in competition experiments involving either [^{125}I]As-II or [^{125}I]AaH-II, a toxin from scorpion Androctonus australis Hector (ref. 1-2).

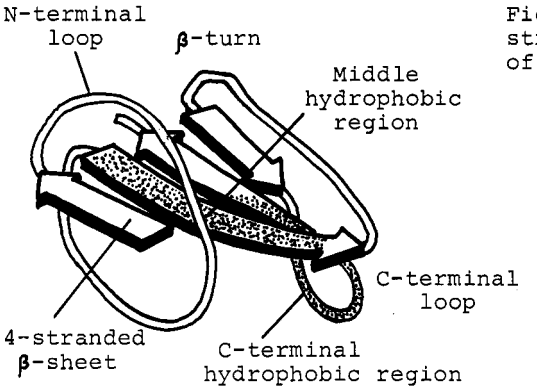

Fig. 1. Elements of secondary structure and hydrophobic regions of sea anemone toxins.

toxins are divided into two groups according to structural and immunochemical properties, but not the toxicity. By other words, analysis of these properties is not quite enough to explain differences in their pharmacological effect values. Below we shall discuss if comparison of toxin spatial organization can disolve this problem.

SECONDARY STRUCTURE OF SEA ANEMONE TOXINS

Recently, elements of secondary structure of three toxins (Ax-I, Rp-II and As-I) have been established by two-dimentional proton NMR-spectroscopy (ref. 3-5). We have compared the CD-spectra of wider series of representatives of A- and R-toxins (not shown). The shape and amplitudes of CD-spectra of both toxin groups are similar. The main elements of spatial structure of sea anemone toxins drawn according to NMR-spectroscopy data are schematically are represented in fig. 1. As shown, antiparallel 4-stranded β-sheet seems to be twisted due to "rigid" arrangement of three disulfide links. This is particularly confirmed by evidence that indol rings of TRP-23 and Trp-33 in A-toxins are closered to each other (ref. 3). Finally, comparison of NMR- and CD-spectroscopy data as well as high homology of amino acid sequences within an every group raise serious reason to suggest that polypeptide chains of all studied sea anemone toxins have to fold in the similar manner. Secondary structure of these toxins has following common characteristic features: a) absence of α-helix; b) antiparallel 4-stranded β-sheet core connecting regions 2-6 ↔ 18-24 ↔ 41-48 ↔ 30-36 of polypeptide chain; c) reverse β-turn in position 26-33 and d) formation of two loops in positions 6-17 (N-terminal loop) and 34-42 (C-terminal loop).

Such a strong stress in spatial structure folding of sea anemone toxins due to three disulfide links and a number of salt bridges imposes limitation in progress of secondary prediction. In fig. 2 results of some calculation of toxin secondary structure by methods of Chou and Fasman (C & F, ref. 8), of Dufton and Hider (D & H, ref. 9) and of Deleage and Roux (D & R, ref. 10) are shown. The comparison of results of these predictions with NMR-spectroscopy data (NMR in fig. 2) reveals that used methods describe in outline correctly main elements of secondary structure; the greatest accuracy of prediction demonstrates method of Dufton and Hider. For R-toxin (Rp-II) success rate is lower especially at the N- and C-terminal regions, where there is a number of easily ionizable side chains being able to form salt bridges.

Together with experimental data results of prediction method confirm additionally a close similarity of secondary structure of R- and A-toxins. Absence of clear differences in overall spatial structure of sea anemone toxin series indicates that significant variability of their toxicity is probably caused by surface properties of toxin molecules.

HYDROPATHIC PROFILES OF SEA ANEMONE TOXIN SEQUENCES

Calculation of hydropathic profiles by method of Kyte and Doolittle (ref. 11) demonstrates that all investigated toxins are characterized by two hydrophobic regions. The first one lies in the middle part of amino acid sequence and the second occupies position near C-terminal loop (see fig. 1). Hydropathic profiles of the middle region of A- and R-toxins are different, however its total hydrophobicity varies slightly in the toxins of every group (Table 2).

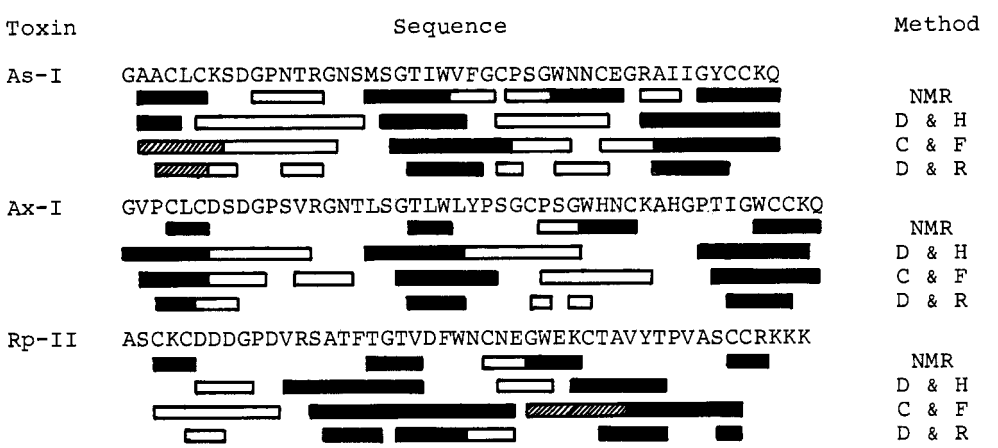

Fig. 2. Comparison of NMR-spectroscopy data and results of prediction methods on secondary structure of three sea anemone toxins: ▨ α-helix; ■ β-sheet and □ β-turns (for comments see the text).

As seen from Table 2 total hydrophobicity of this region of A-toxins is, as a rule, higher then that of R-toxins. This correlates rather well with higher binding constants (K_b) of A-toxins to rat brain synaptosomes. As distinct from the middle region, C-terminal hydrophobic region varies appreciably within both groups and its total hydrophobicity correlates with polypeptide toxicity expressed in terms of LD_{50} values (Table 2). As it is obvious from fig. 1, this highly variable hydrophobic region occupies zone of C-terminal region, where active exchange of amino acid residues is observed in both toxin series.

Analysis of the results of toxin chemical modifications and of immunological studies gives further conclusions about important roles of middle region in receptor binding ability and of C-terminal hydrophobic region in toxicity of the polypeptides. Thus, modification of Arg-13 and Trp-30 residues lieing not far from middle region of toxin Rm-III (ref. 12) induces diminution of LD_{50} values in 5 and 3 times, but binding constants K_b decrease in 15 and 20 times, respectively. On our opinion chemical modification of Asp-7 and/or Asp-9 residues affects on the C-terminal region and, hence, on the toxicity of A-toxins (as discribed in ref. 13). Indeed, in accordance with immunochemical studies (ref. 14) being not participated in interaction with receptor these residues may alter lysine residue state near C-terminal loop owing to disruption of salt bridge after modification.

REFERENCES

1. H. Schweitz, J.-N. Bidard, C. Frelin, D. Pauron, H.P.M. Vijverberg, D.M. Mahasneh, M.Lazdunski, F. Vilbois and A. Tsugita, Biochemistry, 24, 3554-3561 (1985).
2. H. schweitz, J.P. Vincent, J. Barhanin, C. Frelin, G. linden, M. Hugues, M. lazdunski, Biochemistry, 20, 5245-5252 (1981).
3. P.R. Gooley and R.S. Norton, Biochemistry, 25, 2349-2356 (1986).
4. D.E. Vermer, N.V. Kumar, R.M. Metrione, M. Lazdunski, G. Drobny and N.R. Kallenbach, Biochemistry, 25, 6842-6849 (1986)
5. H. Widmer, G. Wagner, H. Schweitz, M. Lazdunski and K. Wüthrich, Eur. J. Biochem.,171, 177-192 (1988).
6. A.A. Nabiullin, S.E. Odinokov, E.I. Vozhova, E.P. Kozlovskaya and G.B. Elyakov, Bioorgan. Khim., 8, 1644-1648 (1982).
7. P.R. Gooley, J.W. Blunt, and R.S.Norton, FEBS Lett., 174, 151-159 (1984).
8. P.Y. Chou and G.D.Fasman, Biochemistry, 13, 222-245 (1974).
9. M.J. Dufton and R.C. Hider, J. Mol. Biol., 115, 177-193 (1977).
10. G. Deleage and B. Roux, Protein Engineering, 1, 289-294 (1987).
11. J. Kyte and R.F. Doolittle, J. Mol. Biol., 157, 105-132 (1982).
12. V.M. Mahnyr, E.P. Kozlovskaya and G.B. Elyakov, in preparation.
13. T.R. Norton, Fed. Proc., 40, 21-25 (1981).
14. M. El Ayeb, E.M. Bahraoui, C. Granier, L. Beress and H. Rochat, Biochemistry, 25, 6755-6761 (1986).