Cent. Eur. J. Biol. • 4(1) • 2009 • 41–49
DOI: 10.2478/s11535-008-0064-z

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Central European Journal of **Biology**

Functional discrimination of sea anemone neurotoxins using 3D-plotting

Research Article

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Received 31 August 2008; Accepted 7 December 2008

Abstract: One of the most important goals in structural biology is the identification of functional relationships among the structure of proteins and peptides. The purpose of this study was to (1) generate a model based on theoretical and computational considerations among amino acid sequences within select neurotoxin peptides, and (2) compare the relationship these values have to the various toxins tested. We employed isolated neurotoxins from sea anemones with established specific potential to act on voltage-dependent sodium and potassium channel activity as our model. Values were assigned to each amino acid in the peptide sequence of the neurotoxins tested using the Number of Lareo and Acevedo algorithm (NULA). Once the NULA number was obtained, it was then plotted using three dimensional space coordinates. The results of this study allow us to report, for the first time, that there is a different numerical and functional relationship between the sequences of amino acids from sea anemone neurotoxins, and the resulting numerical relationship for each peptide, or NULA number, has a unique location in three-dimensional space.

Keywords: *NULA • Neurotoxins • 3D plotting • Models • Sequence relationships*

1. Introduction

Identifying structurally similar proteins with different chain topologies can aid studies in homology modeling, protein folding, protein design, and protein evolution [1]. One of the most important intentions, when working with nucleotide or amino acid sequences, is to identify similarities within or among sequences of special interest and ascribe numerical values to those sequence similarities. Determining homology evaluates qualitative and quantitative aspects when assigning similarity. The use of our algorithm in this process helps to generate a unique number from the amino acid sequence of a protein or from the nucleotide sequence of a nucleic acid, which can be plotted in three-dimensional space distinct from any other protein or nucleic acid. The process reported here, termed the Number of Lareo and Acevedo algorithm, has been developed in our group and has been given the acronym NULA [2].

In this paper, NULA it is applied to one of the most interesting protein groups, the sea anemone neurotoxins, in order to understand their physiological and pharmacological effects, and generate reasonable receptor-binding interaction models.

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2. Experimental Procedures

2.1 NULA Algorithm

The numerical method and its graphical three-dimensional representation allow the comparison of sequences and the evaluation of similarities between proteins with related functions. The proposed method does not require an initial sequence alignment. The NULA value relates (1) the number of monomers in a given sequence, (2) the position of each nucleotide or amino acid in the sequence and (3) the frequency that each type of nucleotide or amino acid appears in the same sequence [2]. Briefly, each amino acid in a given protein sequence is numbered from 1 to n starting with the amino terminus. Consecutive prime numbers, starting with 2, are assigned, one to each one of the twenty amino acids, as they appear in the protein sequence. The X-coordinate is the natural logarithm of Gödel's number, *i.e.*, the product of a, where "a" is the prime number that identifies the given amino acid and "I" is the position it occupies in the amino acid sequence. The Y coordinate is $\sum p^n$, where "p" is each prime assigned to each amino acid and "n" is the frequency with which such an amino acid appears in the sequence. The Z coordinate is the total number of amino acids in the sequence. The amino acids L-selenocysteine and L-pyrrolysine were not considered in our model study, as they are not known to occur in anemone neurotoxin proteins.

2.2 Biological model

The sea anemone toxins provide excellent models to study structural and functional relationships [3-5]. Even with the sea anemone toxins being very diverse, the toxins are of theoretical interest due to the molecular targets affected and their high specificity of action on those targets. The neurotoxins from sea anemones affect the nervous and muscular systems and include two main groups: those that act on sodium- voltage dependent channels [6-8], and those which affect potassium-voltage dependent channels [9-15]. The sea anemones neurotoxins have been or will be useful tools for studying the structure and function of specific ion channels [5]. Moreover, the most of the known sodium channel toxins delay channel inactivation by binding to the receptor site 3 and most of the known potassium voltage-dependent channels toxins selectively inhibit Kv1 channels [5]. Several studies have demonstrated that the following peptide toxins are functionally unique among the known sodium or potassium channel toxins: APETx2, which inhibits acid-sensing ion channels in sensory neurons [16]; BDS-I and II, which show selectivity for Kv3.4 channels [9], and APETx1, which inhibits human *ether-a-go-go*-related gene potassium channels [17]. In addition, structurally novel peptide toxins, such as an epidermal growth factor (EGF)-like toxin (gigantoxin I), have also been isolated from some sea anemones although their functions remain to be clarified [5]. Recent study by Zaharenko and coworkers demonstrated the possibility of using sodium channel toxins from sea anemones as tools for dissecting the biophysical properties of inactivation in voltage-gated sodium channels [18]. Cangitoxin (CGTX) is a peptide containing 48 amino acid residues and was formerly purified from *Bunodosoma cangicum*. Based on the observation made by Zaharenko *et al.*, nevertheless, previous works reporting the isolation procedures for such peptide from *B. cangicum* secretions are controversial and may lead to incorrect information. In addition, a simple and rapid procedure, consisting of two chromatographic steps, in order to obtain a CGTX analog directly from sea anemone venom has been reported by this group. This group also reported a substitution of N16D in this peptide sample and the co-elution of an inseparable minor isoform presenting the R14H substitution. Peptides are named as CGTX-II and CGTX-III, and their effects over Nav1.1 channels in patch clamp experiments are demonstrated [18].

For this application of the NULA Algorithm, the amino acid sequences of several neurotoxins that affect sodium or potassium channels isolated from sea anemones were used (see: Figures 1 and 2).

3. Results

Figure 3 depicts the corresponding three-dimensional representation of the data described in Table 1, which are the values of the 3 coordinates described in the methods for the 38 isolated neurotoxins of sea anemone that affect Na^+ channels. The first cluster (numbers 36, 37 and 38) includes the toxins ATX-III, ATX-IV of the Actiniidae family and PA-TX from the Stichodactylidae family. ATX-III, ATX-IV and PA-TX have 27, 25 and 31 amino acids, respectively. The toxin numbered as 1, Ae I (family Actiniidae) with 53 amino acids is distinct in location from the other groups. The largest group contains AETX I toxin (number 2) to Halcurin (number 35), with primary sequencing ranging from 46 and 49 amino acids and include those common to several families, namely Actiniidae, Stichodactylidae, Hormathiidae and Halicuridae.

With respect to the isolated neurotoxins of sea anemone that affect K^+ channels, the values of the 3 coordinates are shown in Table 2. The corresponding three-dimensional representation in Figure 4 results in

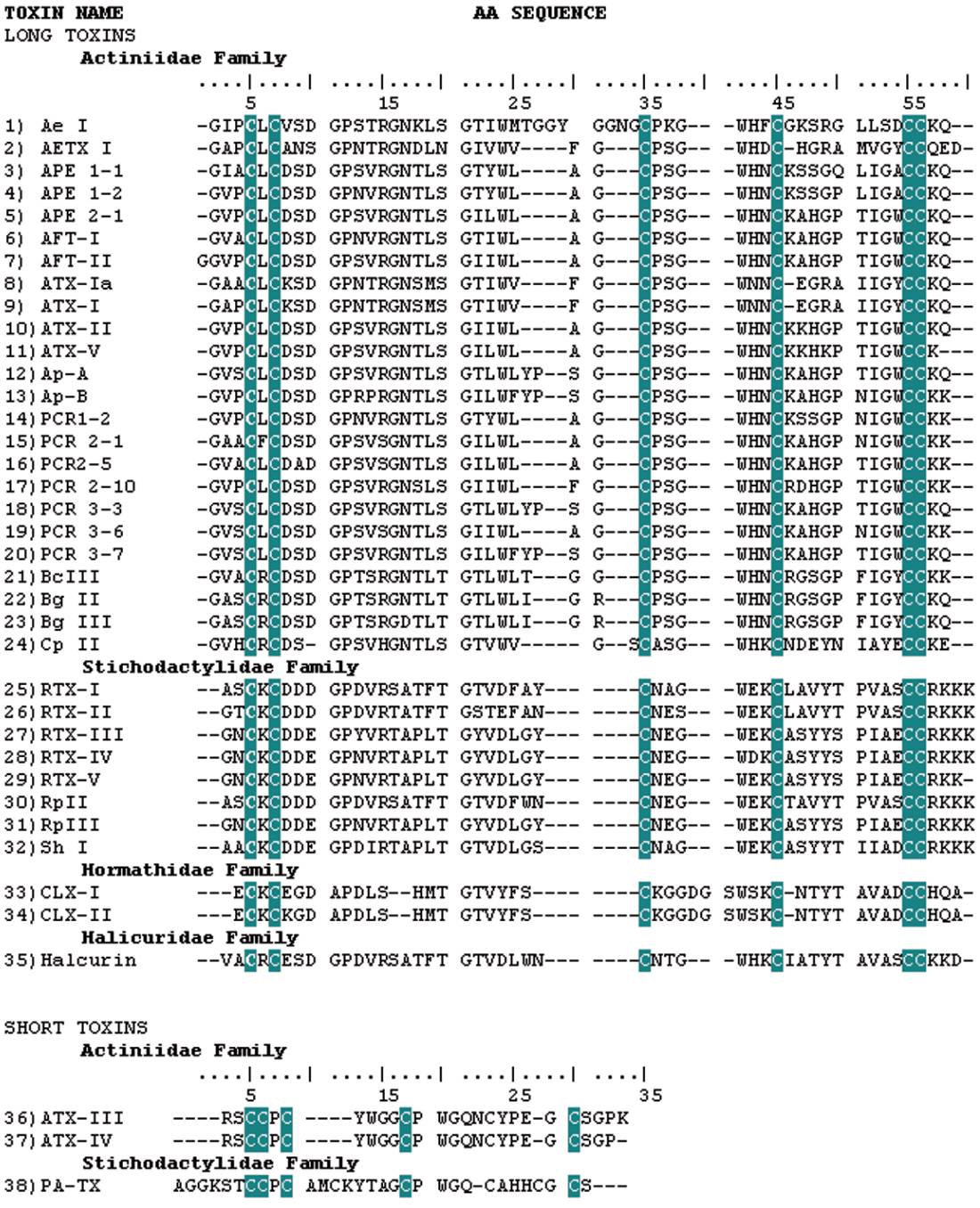

Figure 1. Sea anemone neurotoxins that affect sodium channel.
The sequences were obtained from the following papers: AeI (*Actinia equina*): [27], AETX I (*Anemonia erythraea*): [28], APE 1-1, APE 1-2, APE 2-1 (*Anthopleura elegantissima*): [29], AFT-I, AFT-II (*Anthopleura fuscoviridis*): [30], ATX-Ia, ATX-I, ATX-II, ATX-V ATX-III, ATX-IV (*Anemonia sulcata*): [31-36], Ap-A, Ap-B, PCR1-2, PCR 2-1, PCR2-5, PCR 2-10, PCR 3-3, PCR 3-6, PCR 3-7 (*Anthopleura xanthogrammica*): [37-39], BcIII (*Bunodosoma caissarum*): [40], Bg II, Bg III (*Bunodosoma granulifera*): [41], Cp II (*Condylactis passiflora*): [42], RTX-I, RTX-II, RTX-III, RTX-IV, RTX-V (*Radianthus macrodactylus*/previously *Heteractis macrodactylus*): [43-45], RpII, RpIII (*Radianthus paumotensis*/previously *Heteractis magnifica* or *Heteractis paumotensis*): [46-47], Sh I (*Stichodactylae helianthus*): [48], CLX-I, CLX-II (*Calliactis parasitica*): [19], Halcurin (*Halcurias carlgreni*): [49], PA-TX (*Parasicyonis actinostoloides*/previously *Entacmaea quadricolor*): [50].

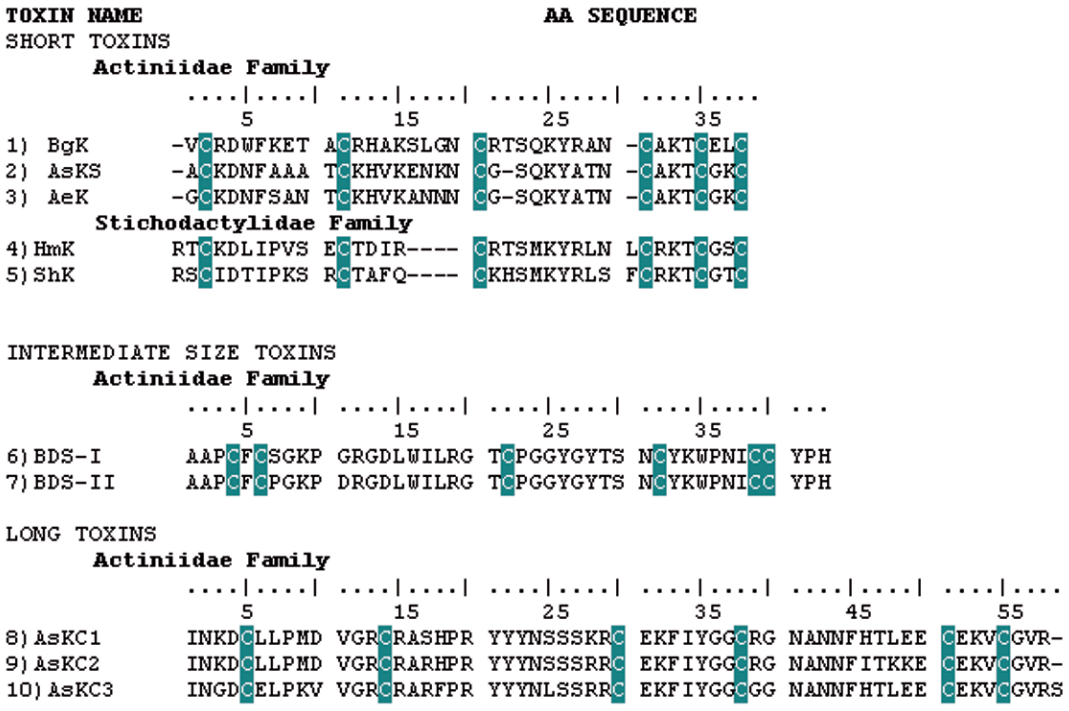

Figure 2. Sea anemone neurotoxins that affect potassium channel.
The sequences were obtained from the following papers: BgK (*Bunodosoma granulifera*): [12], AsKS, BDS-I, BDS-II, AsKC1, AsKC2, AsKC3 (*Anemonia sulcata*): [20,23,24], AeK (*Actinia equina*): [22], HmK (*Heteractis magnifica*): [21], ShK (*Stichodactyla helianthus*): [13].

3 differentiated clusters. One of them corresponds to the numbered toxins 1 to 5, which are BgK, AsKS, AeK, HmK and ShK, with primary sequencing ranging from 35 to 37 amino acids. In this group there are toxins isolated from specimens of family Actiniidae (BgK, AsKS, AeK) and Stichodactylidae (HmK and ShK). Another cluster corresponds to the toxins BDS-I and BDS-II (Actiniidae family) numbered as 6 and 7, with 43 amino acids in each one. The last cluster includes the AsKC1, AsKC2 and AsKC3 toxins, of Actiniidae family having 58 and 59 amino acids, respectively.

Interestingly, when both groups of neurotoxins are graphed together, using green to indicate the neurotoxins that affect Na^+ channels and red to indicate those that affect K^+ channels, Figure 5 demonstrates that the particular groupings for each type remain distinct, demonstrating the selectivity of our algorithm in distinguishing common features to the toxins mechanism of action.

4. Discussion

The novel NULA algorithm assigns a unique number to a given protein, based on its primary amino acid sequence and demonstrates that a new level of complexity can be distilled from amino acid features, not found using simple surveys of the amino acid sequences or from common homology alignment algorithms. From our data, it may be argued that function depends on particular three dimensional features that are reflected in the NULA number and not by chance alone. If this was the case, proteins with similar activities should have NULA numbers that would be group in the same cluster in a 3D-plotting. Further, when plotted as peptides, overlapping functions could be ascertained among very dissimilar proteins.

In order to test this possibility, we studied 48 sea anemone neurotoxins for which amino acid sequences had been published. These neurotoxins were chosen because they have relatively short (25-59 amino acids) sequences and well-characterized activities. When NULA numbers were assigned to the 48 neurotoxins and plotted, it was apparent that they were grouped into distinct regions of sequences according to their known activity. Thus, those peptides that affected voltage-dependent Na^+ channels fell into three well-defined clusters, while those affecting K^+ channels fell into two well-defined and distinct clusters. Interestingly, some of these clusters intermingled in sequences but did not overlap. Even though there is clear discrimination among the cluster for each type of neurotoxin, no

Number	Toxin	X	Y	Z
1	Ae I	1840.405070	65.499323	53
2	AETX I	1355.828874	56.092274	47
3	APE 1-1	1423.695378	56.497454	47
4	APE 1-2	1425.964202	58.097542	47
5	APE 2-1	1418.646681	57.294019	47
6	AFT-I	1420.560358	54.312150	47
7	AFT-II	1475.419321	58.278328	48
8	ATX-Ia	1432.248049	52.783089	46
9	ATX-I	1436.183311	54.115528	46
10	ATX-II	1513.624068	59.687481	47
11	ATX-V	1389.474596	57.131743	46
12	Ap-A	1611.528516	61.036288	49
13	Ap-B	1561.776194	62.974564	49
14	PCR1-2	1483.001469	59.559493	47
15	PCR2-1	1397.596476	53.823443	47
16	PCR2-5	1397.596476	53.823443	47
17	PCR2-10	1443.584809	60.125717	47
18	PCR3-3	1611.528516	61.036288	49
19	PCR3-6	1392.073769	58.751428	47
20	PCR3-7	1589.662383	61.948046	49
21	BclII	1606.701005	55.999066	48
22	Bg II	1559.896035	57.397670	48
23	Bg III	1545.988327	56.483856	48
24	Cp II	1354.445736	56.033702	47
25	RTX-I	1429.358111	49.759854	48
26	RTX-II	1448.146580	49.434135	48
27	RTX-III	1444.680497	56.405529	48
28	RTX-IV	1441.566874	56.154990	48
29	RTX-V	1371.673969	54.546464	47
30	RpII	1486.916013	50.112250	48
31	RpIII	1441.566874	56.154990	48
32	Sh I	1369.359002	47.662549	48
33	CLX-I	1272.229189	47.002470	46
34	CLX-II	1274.812338	47.619770	46
35	Halcurin	1365.549081	47.353093	47
36	ATX-III	495.838104	35.079913	27
37	ATX-IV	412.229275	31.791440	25
38	PA-TX	482.082619	29.740022	31

Table 1. Coordinates to the sea anemone neurotoxins that bind to voltage-dependent Na^+ channels.

Number	Toxin	X	Y	Z
1	BgK	820.234211	39.551243	37
2	AsKS	752.904945	34.301024	36
3	AeK	725.616977	36.120996	36
4	HmK	830.803940	40.242231	35
5	ShK	807.476549	39.071917	35
6	BDS-I	1285.924560	52.403152	43
7	BDS-II	1280.579409	51.850795	43
8	AsKC1	2172.096715	74.663289	58
9	AsKC2	2193.914397	75.336328	58
10	AsKC3	2227.691702	72.899240	59

Table 2. Coordinates to the sea anemone neurotoxins that bind to voltage-dependent K^+ channels

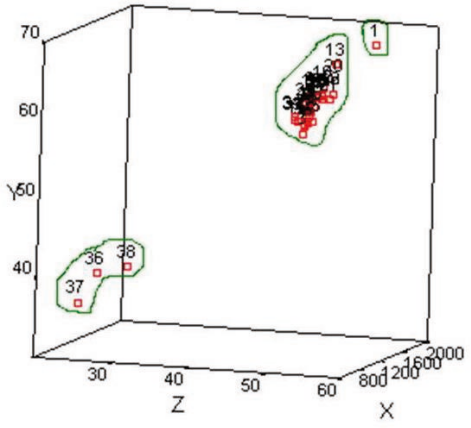

Figure 3. Three-dimensional representation of neurotoxins of sea anemone that bind to voltage-dependent Na^+ channels.

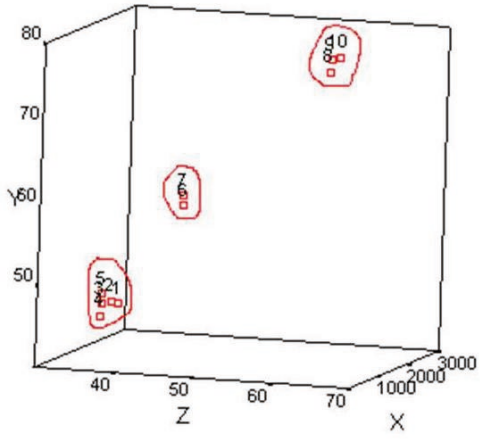

Figure 4. Three-dimensional representation of neurotoxins of sea anemone that bind to voltage-dependent K^+ channels.

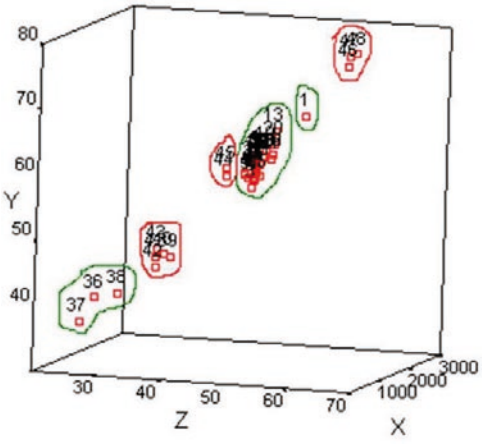

Figure 5. Three-dimensional representation of neurotoxins of sea anemone that bind to voltage-dependent Na^+ and K^+ channels.

modern bioinformatics tool exists today to explain such discrimination in function. The fact that the obtained clusters include toxins isolated from species of different families, shows that there is not a relationship to the degree of similarity among the sequences and the families and there is no taxonomic dependency among them.

The results of this study show that the clusters obtained for each type of neurotoxin are independent of the homology between each group. In the case of the neurotoxins that affect Na^+ channels, for example, CLX-I and CLX-II toxins of Hormathiidae family [19] do not present or display a high level of homology with those of the Actiniidae and Stichodactylidae families, but belong to the same group of Halcurin toxin of Halicuridae family; even with these differences in homology, all of the four toxins are grouped in the same cluster (numbered 33 and 34 for CLX-I and CLX-II and 35 in the case of Halcurin), as demonstrated in Figure 3.

In relation to the K^+ channels neurotoxins, the comparison of the sequences between families for short toxins shows similarity between two toxins of Stichodactylidae family, ShK and HmK, and between toxins of Actiniidae family, as AsKS, BgK and AeK. Although differences exist in the sequences when comparing families, the 6 cysteines are absolutely conserved [12,13,20-22]. However, no matter the similarities or differences in the sequences of both families, the 5 toxins are grouped in the same cluster as seen in Figure 4.

For BDS-I and BDS-II isoforms, classified together with toxins of intermediate size, and grouped in the same cluster, the sequences differ in positions 7 and 11, (Ser and Gly for BDS-I and Pro and Asp for BDS-II). However, regarding isoform condition, unlike the RTX-III and RTX-IV isoforms discussed for Na^+ channels, the toxins BDS-I and BDS-II, in addition to binding to K^+ channels, also show antiviral and antihypertensive effects [23,24]. This suggests the occurrence of particular functional motifs within the same sequence that correlate with different activities. On the other hand, it is of great interest to report topological similarity -demonstrated by binding and displacement tests- among toxins that affect K^+ channels of different Phyla, such as dendrotoxin and ShK (toxins isolated from snakes and sea anemones, respectively), that allows them to bind to the same sites in the K^+ channels [11].

Consequently, the use of this novel algorithm can predict complete neurotoxins that have not been deciphered yet. Once the amino acid sequence of a new neurotoxin becomes available, it will be assigned a NULA number that can be plotted in three dimensions and its localization in the coordinates could tell us about its structural topology, particularly in the binding site of the receptor. Frequently the comparison of new sequences with others previously studied can lead to the inference of functions and relations, and to outline the methodology to follow in future investigations. The sequences codify biological information within an intricate group of norms that up to this day have not been completely deciphered. The NULA tool will assist in elucidating close relations from the patterns that help to determine the set of rules that are not known.

The algorithm of Lareo and Acevedo has many applications for studying molecular systems and to bring studies of phylogeny relationships to a molecular level, which will allow for biological correlations. This model could be utilized to expand the understanding of Archaea bacterial proteins or post-translational modifications of known residues, and couple that information to proteomics research. Even though biologically meaningful 3D models have been generated from the simultaneous alignment of several protein structures, which have allowed a better understanding of active sites [25], or to discriminate between different ligand-binding sites [26], an additional advantage of the NULA algorithm is the evident reduction in the time of calculation since it does not need an alignment of sequences. Additional uses are expected to be found for NULA, which could be included as a novel bioinformatics tool for screening proteins or peptides, and for overlapping functions and the assignment of new functions to orphan peptides.

Acknowledgements

We thank Dr. William Kem for his worthy information about the sequences of the sea anemone toxins, and the valuable comments of Dr. Leonardo Lareo, Mrs. Sandra L. Siedlak and Mr. Hector Palacios for their critical review. This work was supported by a grant from COLCIENCIAS.

References

[1] Dundas J., Binkowski T., DasGupta B., Liang J., Topology independent protein structural alignment, BMC Bioinformatics, 2007, 8, 388

[2] Lareo R., Acevedo O., Sequence mapping in a three-dimensional space by a numeric method and some of its applications, Acta Biotheor., 1999, 47, 123-128

[3] Kem W., Pennington M., Dunn B., Sea anemone polypeptide toxins affecting sodium channels: initial structure-activity investigations, In: Hall S., Strichartz F. (Eds.), Marine Toxins: Origin, Structure and Molecular Pharmacology, ACS Symp. Ser., 1990, 279-289

[4] Watters M., Tropical marine neurotoxins: venoms to drugs, Semin. Neurol., 2005, 25, 278-289

[5] Honma T., Shiomi K., Peptide toxins in sea anemones: structural and functional aspects, Mar. Biotechnol. (NY), 2006, 8, 1-10

[6] Norton R., Polypeptide modulators of sodium channel function as a basis for the development of novel cardiac stimulants, In: Veerapandian P. (Ed.), Structure Based Drug Design, Marcel Dekker, Inc., New York, 1997, 295-319

[7] Kem W., Pennington M., Krafte D., Hill R., Sea anemone toxins affecting sodium channels: are the similarities greater than the differences? In: Lazarovici P., Spira M., Zlotkin E. (Eds.), Biochemical aspects of Marine Pharmacology, Alaken Press, 1996, 98-120

[8] Norton R., Structure and structure-function relationships of the sea anemone proteins that interact with the sodium channel, Toxicon, 1991, 29, 1051-1084

[9] Yeung S., Thompson D., Wang Z., Fedida D., Robertson B., Modulation of Kv3 subfamily potassium currents by the sea anemone toxin BDS: significance for CNS and biophysical studies, J. Neurosci., 2005, 25, 8735-8745

[10] Kem W., Pennington M., Norton R., Sea anemone toxins as templates for the design of immunosuppressant drugs, Perspectives Drug Discov. Design, 1999, 15/16, 111-129

[11] Dauplais M., Lecog A., Song J., Cotton J., Jamin N., Gilquin B., et al., On the convergent evolution of animal toxins. Conservation of a diad of functional residues in potassium channel-blocking toxins with unrelated structures, J. Biol. Chem., 1997, 272, 4302-4309

[12] Aneiros A., Garcia I., Martinez J.R., Harvey A.L., Anderson A.J., Marshall D.L., et al., A potassium channel toxin from the secretion of the sea anemone Bunodosoma granulifera. Isolation, amino acid sequence and biological activity, Biochim. Biophys. Acta, 1993, 1157, 86-92

[13] Castañeda O., Sotolongo V., Amor A.M., Stöcklin R., Anderson A.J., Harvey A.L., et al., Characterization of a potassium channel toxin from the Caribbean Sea anemone Stichodactyla helianthus, Toxicon, 1995, 33, 603-613

[14] Pennington M., Byrne M., Zaydenberg I., Khaytin I., de Chastona J., Krafte D., et al., Chemical synthesis and characterization of Shk toxin: a potent potassium channel inhibitor from a sea anemone, Int. J. Pept. Prot. Res., 1995, 46, 354-358

[15] Pennington M., Mahnir V., Krafte D., Zadenberg I., Byrnes M., Khaytin I., et al., Identification of three separate binding sites on Shk toxin, a potent inhibitor of voltage-dependent potassium channels in human T-lymphocytes and rat brain, Biochem. Biophys. Res. Commun., 1996, 219, 696-701

[16] Diochot S., Baron A., Rash L.D., Deval E., Escoubas P., Salinas M., et al., A new anemone peptide, APETx2, inhibits ASIC3, a major acid-sensitive channel in sensory neurons, EMBO J., 2004, 23, 1516-1525

[17] Zhang M., Liu X., Diochot S., Lazdunski M., Tseng G., APETx1 from sea anemone Anthopleura elegantíssima is a gating modifier peptide toxin of the human ether-a-go-go- related potassium channel., Mol. Pharmacol., 2007, 2, 259-268

[18] Zaharenko A.J., Ferreira W.A., de Oliveira J.S., Richardson M., Pimenta, D.C., Konno K., et al., Proteomics of the neurotoxic fraction from sea anemone Bunodosoma cangicum venom: novel peptides belonging to new classes of toxins, Comp. Biochem. Physiol. Part D: Genomics and Proteomics, 2008, 3, 219-225

[19] Spagnuolo A., Zanetti L., Cariello L., Piccoli R., Isolation and characterization of two genes encoding calitoxins, neurotoxic peptides from Calliactis parasitica (Cnidaria), Gene, 1994, 138, 187-191

[20] Schweitz H., Bruhn T., Guillemare E., Moinier D., Lancelin J.M., Beress L., et al., Kalicludines and kaliseptine. Two different classes of sea anemone toxins for voltage sensitive K^+ channels, J. Biol. Chem., 1995, 270, 25121-25126

[21] Gendeh G., Young L., de Medeiros C., Jeyaseelan K., Harvey A., Chung M., A new potassium channel toxin from the sea anemone Heteractis magnífica: isolation, cDNA cloning, and functional expression, Biochemistry, 1997, 36, 11461-11471

[22] Minagawa S., Ishida M., Nagashima Y., Shiomi K., Primary structure of a potassium channel toxin from the sea anemone Actinia equina, FEBS Lett., 1998, 427, 149-151

[23] Diochot S., Schweitz H., Beress L., Lazdunski M., Sea anemone peptides with a specific blocking activity against the fast inactivating potassium channel Kv3.4, J. Biol. Chem., 1998, 273, 6744-6749

[24] Doppelfeld I., Henschen-Edman A., Graf E., Zwick J., Beress L., Etschenberg E., Patent: 3324689, -DE 17-JAN-1985

[25] Nebel J., Generation of 3D templates of active sites of proteins with rigid prosthetic groups, Bioinformatics, 2006, 22, 1183-1189

[26] Najmanovich R., Kurbatova N., Thornton J., Detection of 3D atomic similarities and their use in the discrimination of small molecule protein-binding sites, Bioinformatics, 2008, 24, i105-i111

[27] Lin X., Ishida M., Nagashima Y., Shiomi K., A polypeptide toxin in the sea anemone Actinia equina homologous with other sea anemone sodium cannel toxins: Isolation and amino acid sequence, Toxicon, 1996, 34, 57-65

[28] Shiomi K., Qian W., Lin X., Shimakura K., Nagashima Y., Ishida M., Novel polypeptide toxins with crab lethality from the sea anemone Anemonia erythraea, Biochim. Biophys. Acta, 1997, 1335, 191-198

[29] Bruhn T., Schaller C., Schulze C., Sanchez-Rodriguez J., Dannmeier C., Ravens U., et al., Isolation and characterisation of five neurotoxic and cardiotoxic polypeptides from the sea anemone Anthopleura elegantissima, Toxicon, 2001, 39, 693-702

[30] Sunahara S., Muramoto K., Tenma K., Kamiya H., Amino acid sequence of two sea anemone toxins from Anthopleura fuscoviridis, Toxicon, 1987, 25, 211-219

[31] Wunderer G., Eulitz M., Amino-acid sequence of toxin I from Anemonia sulcata, Eur. J. Biochem., 1978, 89, 11-17

[32] Widmer H., Wagner G., Schweitz H., Lazdunski M., Wüthrich K., The secondary structure of the toxin ATX Ia from Anemonia sulcata in aqueous solution determined on the basis of complete sequence-specific 1H-NMR assignments, Eur. J. Biochem., 1988, 171, 177-192

[33] Wunderer G., Fritz H., Wachter E., Machleidt W., Amino-acid sequence of a coelenterate toxin: toxin II from Anemonia sulcata, Eur. J. Biochem., 1976, 68, 193-198

[34] Scheffler J., Tsugita A., Linden G., Schweitz H., Lazdunski M., The amino acid sequence of toxin V from Anemonia sulcata, Biochem. Biophys. Res. Commun., 1982, 107, 272-278

[35] Beress L., Wunderer G., Wachter E., Amino acid sequence of toxin III from Anemonia sulcata, Hoppe-Seyler's Z. Physiol. Chem., 1977, 358, 985-988

[36] Warashina A., Jiang Z., Ogura T., Potential-dependent action of Anemonia sulcata toxins III and IV on sodium channels in crayfish giant axons., Pflugers Arch., 1988, 411, 88-93

[37] Tanaka M., Hainu M., Yasunobu K., Norton T., Amino acid sequence of the Anthopleura xanthogrammica heart stimulant, anthopleurin A, Biochemistry, 1977, 16, 204-208

[38] Reimer N., Yasunobu C., Yasunobu K., Norton T., Amino acid sequence of the Anthopleura xanthogrammica heart stimulant, anthopleurin-B, J. Biol. Chem., 1985, 260, 15, 8690-8693

[39] Kelso G., Blumenthal K., Identification and characterization of novel sodium channel toxins from the sea anemone Anthopleura xanthogrammica, Toxicon, 1998, 36, 41-51

[40] Malpezzi E., de Freitas J., Muramoto K., Kamiya H., Characterization of peptides in sea anemone venom collected by a novel procedure, Toxicon, 1993, 7, 853-864

[41] Loret E., del Valle R., Mansuelle P., Sampieri F., Rochat H., Positively charged amino acid residues located similarly in sea anemone and scorpion toxins, J. Biol. Chem., 1994, 269, 16785-16788

[42] Shiomi K., Lin X., Nagashima Y., Ishida M., Isolation and amino acid sequence of polypeptide toxins in the Caribbean sea anemone Condylactis passiflora, Fish Sci., 1995, 61, 1016-1021

[43] Zykova T., Vinokurov L., Kozlovskaya E., Elyakov G., Amino-acid sequence of neurotoxin III from the sea anemone Radianthus macrodactylus, Bioorg. Khim., 1985, 11, 302-310

[44] Zykova T., Kozlovskaia E., Eliakov G., Amino acid sequence of neurotoxins IV and V from the sea anemone Radianthus macrodactylus, Bioorg. Khim., 1988, 14, 1489-1494

[45] Zykova T., Kozlovskaia E., Amino acid sequence of a neurotoxin from the anemone Radianthus macrodactylus, Bioorg. Khim., 1989, 15, 1301-1306

[46] Wemmer D., Kumar N., Metrione R., Lazdunski M., Drobny G., Kallenbach N., NMR analysis and sequence of toxin II from the sea anemone Radianthus paumotensis, Biochemistry, 1986, 25, 6842-6849

[47] Metrione R., Schweitz H., Walsh K., The amino acid sequence of toxin RpIII from the sea anemone Radianthus paumotensis, FEBS Lett., 1987, 218, 59-62

[48] Kem W., Parten B., Pennington M., Price D., Dunn B., Isolation, characterization, and amino acid sequence of a polypeptide neurotoxin occurring in the sea anemone Stichodactyla helianthus, Biochemistry, 1989, 28, 3483-3489

[49] Ishida M., Yokoyama A., Shimakura K., Nagashima Y., Shiomi K., Halcurin, a polypeptide toxin from the sea anemone Halcurias sp., with a structural resemblance to type 1 and 2 toxins, Toxicon, 1997, 35, 537-544

[50] Nishida S., Fujita S., Warashina A., Satake M., Tamiya N., Amino acid sequence of a sea anemone toxin from Parasicyonis actinostoloides, Eur. J. Biochem., 1985, 150, 171-173