On the Convergent Evolution of Animal Toxins

CONSERVATION OF A DIAD OF FUNCTIONAL RESIDUES IN POTASSIUM CHANNEL-BLOCKING TOXINS WITH UNRELATED STRUCTURES

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B goût is a K⁺ channel-blocking toxin from the sea anemone Bunodosoma granulifera. It is a 37-residue protein that adopts a novel fold, as determined by NMR and modeling. An alanine-scanning-based analysis revealed the functional importance of five residues, which include a critical lysine and an aromatic residue separated by 6.6 ± 1.0 Å. The same diad is found in the three known homologous toxins from sea anemones. More strikingly, a similar functional diad is present in all K⁺ channel-blocking toxins from scorpions, although these toxins adopt a distinct scaffold. Moreover, the functional diads of potassium channel-blocking toxins from sea anemone and scorpions superimpose in the three-dimensional structures. Therefore, toxins that have unrelated structures but similar functions possess conserved key functional residues, organized in an identical topology, suggesting a convergent functional evolution for these small proteins.

Functional properties of proteins are frequently associated with a small number of important residues. For example, enzyme activities depend on a few residues that are essential for catalysis. Also, protein-protein recognition processes have been predicted (1) and recently demonstrated (2) to be energetically driven by a small proportion of the residues forming the contacting areas in protein-protein complexes, as identified by x-ray studies (3, 4). Among the proteins whose major functions require protein-protein interactions are animal toxins, which bind to various molecular targets, such as receptors or ion channels, using a small number of binding residues (5–8). As has been shown for enzymes (9), toxins with different architectures are capable of exerting similar functions (10). However, in contrast to enzymes, the molecular basis associated with the conservation of the function in structurally unrelated toxins remains unknown. In this paper, we show that two families of animal toxins with different folding patterns but a comparable capacity to bind to potassium channels include similar functional diads, composed of a critical lysine and an aromatic amino acid separated from each other by 6.6 ± 1.0 Å.

MATERIALS AND METHODS

Synthesis of Toxin and Mutants—The amino acid sequence of BgK1 was proposed a few years ago (11). However, chemical synthesis attempts, based on these data, systematically failed. The proposed amino acid sequence was therefore questioned, re-examined, and ultimately corrected.2 The revised amino acid sequence of BgK from Bunodosoma granulifera is: VCRDWFKETCRHAKSGLCRTSQQYRYANCATKC-

1 The abbreviations used are: BgK, B. granulifera K⁺ channel-blocking toxin; HPLC, high performance liquid chromatography; r.m.s.d., root mean square deviation; ShK, S. helianthus K⁺ channel-blocking toxin; ChTX, charybdotoxin; AgTX, agitoxin; Tricine, N-tris(hydroxymethyl)methylglycine; NOE, nuclear Overhauser effect; DQF-COSY, double quantum filtered correlated spectroscopy.

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A shift increase is expected in cell and a protein concentration of 1.5–2.0 suppressed by low power irradiation at all times except during acquisition in the directly detected dimension. The water signal was method (16) in the indirect dimension and using simultaneous mode were recorded. Quadrature detection was performed using the States spectrum (12), a nuclear Overhauser spectroscopy spectrum (300 ms ambiguities. At each temperature and in each solvent, a DQF-COSY Bruker spectrometer) at 20 and 30°C, in order to resolve assignment ambiguities. The spectra were recorded with 128 \(10^0\) matrices after reduction (1000 at 280 mm, using a molar extinction coefficient of 8100, calculated on the basis of the known amino acid content (11). For analogs where Trp or Tyr were deleted, the peptide concentrations were determined on the basis of quantitative amino acid analyses.

Circular dichroism spectra were recorded using a Jobin-Yvon CD6 dichrograph, driven by an IBM PC operating with a CD6 data acquisition and manipulation program. Spectra in the range 180–250 nm were recorded, either a mixture of 400 \(m\) of water and 40 \(m\) of D,O, or 440 \(m\) of D,O. The DQF-COSY in H\(_2\)O (respectively D\(_2\)O).

**Signal Transformation and Molecular Modeling**—All data were transformed using FELIX software (17). Prior to Fourier transformation, spectra were weighted with a 18° shifted sine bell (90° for the concerned residue is represented by an arrow, directed upward for a positive difference and downward for a negative difference. A shift increase is expected in β-sheet or extended strand, while a helical conformation will yield a decrease of the chemical shift.

**RESULTS**

**Structure Determination**—BgK was isolated from \textit{B. granulifera} (11), and its amino acid sequence (see Fig. 1) has been recently corrected.\(^6\) BgK, like a number of scorpion toxins such as charybdotoxin, (i) binds to potassium channels, (ii) contains 37 residues, and (iii) possesses 6 half-cystines. However, our attempts to align the amino acid sequences of BgK and scorpion toxins have failed, suggesting that BgK adopts a structure that differs from the well-known α/dβ fold of scorpion toxins (22). We therefore decided to elucidate its structure by NMR and molecular modeling.

**Structural Assignment**—Three unique amino acids having specific spin systems were used as starting points for sequential assignments. These are Trp\(^{5}\), His\(^{13}\), and Gly\(^{18}\). The unique tyrosine (Tyr\(^{20}\)) was also used to assign the C-terminal part of the amino acid sequence of BgK. As no proline was present in the protein, these four starting points as well as the existence of all but one sequential NH-NH connectivities allowed us to achieve the complete assignment of the amino acid sequence of BgK (Table I).

**Experimental Restraints, r.m.s.d., and Energetical Parameters**—A total of 767 distance restraints deriving from NOEs (316 intra-residual correlations, 172 sequential, 155 short range (\(|i-j| \leq 4\), 124 long range (\(|i-j| > 4\) and 12 dihedral restraints (7 from ϕ angles, 5 from χ1 angles) deriving from coupling constants was used. This large set of constraints (21 restraints by residue) yielded a well defined structure, as reflected by the mean r.m.s.d. value of its backbone, which was as low as 0.8 ± 0.2 Å between two structures. The calculated structures were consistent with both experimental data and the standard covalent geometry. The structures had no distance violations larger than 0.32 Å, and only three distance violations were larger than 0.3 Å. All dihedral restraint violations were lower than 5°. The covalent geometry was respected, as revealed by the low (r.m.s.d.) values of the bond length (0.003 Å) and the valence angles (1.52°).
Two Conserved Critical Residues in K\(^+\) Channel Blocking Toxins

Measurements were made at 293 K and pH 3.7. Data were obtained in H\(_2\)O/D\(_2\)O, 10:1 (v/v), and in D\(_2\)O. Chemical shifts are in ppm relative to TSP-d_4. Protein concentration was 4.9 mM.

### TABLE I

Proton chemical shifts of BgK

| Residue | NH   | Hα  | Hβ  | Hγ  | Others   |
|---------|------|-----|-----|-----|----------|
| Val\(^1\) | 7.84 | 4.35 | 2.18 | 0.99 |          |
| Cys\(^2\) | 9.00 | 4.92 | 3.07 | 2.82 |          |
| Arg\(^3\) | 8.46 | 4.52 | 1.58 | 1.31 | 0.53  |
| Asp\(^4\) | 8.73 | 4.84 | 3.28 | 2.68 | 0.67  |
| Trp\(^5\) | 8.68 | 4.94 | 3.47 | 3.34 | 1.33  |
| Phe\(^6\) | 7.84 | 5.23 | 3.44 | 3.34 | 1.33  |
| Lys\(^7\) | 8.56 | 4.27 | 2.10 | 1.87 | 1.65  |
| Gly\(^8\) | 8.90 | 4.07 | 2.24 | 2.13 | 2.43  |
| Thr\(^9\) | 8.42 | 3.91 | 1.46 | 1.30 |        |
| Ala\(^{10}\) | 6.92 | 4.38 | 1.67 |       |        |
| Cys\(^{11}\) | 8.59 | 4.83 | 3.05 | 2.90 | 1.64  |
| Arg\(^{12}\) | 9.22 | 3.89 | 1.98 |       |        |
| His\(^{13}\) | 8.02 | 4.47 | 3.41 | 3.46 | 1.49  |
| Ala\(^{14}\) | 8.14 | 3.75 | 0.90 |       | 1.43  |
| Lys\(^{15}\) | 8.56 | 4.16 | 2.04 | 1.66 | 1.49  |
| Ser\(^{16}\) | 8.00 | 4.29 | 4.02 |       |        |
| Leu\(^{17}\) | 7.15 | 4.36 | 1.52 | 1.20 |        |
| Gly\(^{18}\) | 7.71 | 4.41 | 4.01 |       | 1.20  |
| Asn\(^{19}\) | 8.78 | 4.74 | 2.69 | 2.25 | 2.49  |
| Cys\(^{20}\) | 8.69 | 4.35 | 3.12 | 3.92 | 1.27  |
| Arg\(^{21}\) | 7.59 | 4.35 | 2.01 | 1.95 | 1.27  |
| Thr\(^{22}\) | 7.38 | 4.43 | 4.41 | 1.27 | 1.93  |
| Ser\(^{23}\) | 7.66 | 4.96 | 4.41 | 1.42 | 1.93  |
| Gln\(^{24}\) | 9.31 | 4.00 | 2.12 | 2.49 | 1.93  |
| Lys\(^{25}\) | 8.22 | 3.89 | 1.85 | 1.42 | 1.93  |
| Tyr\(^{26}\) | 7.58 | 4.04 | 2.69 | 3.92 | 1.93  |
| Arg\(^{27}\) | 8.42 | 3.77 | 1.76 | 1.69 | 1.93  |
| Ala\(^{28}\) | 7.61 | 4.20 | 1.37 | 1.93  |
| Asn\(^{29}\) | 7.13 | 4.84 | 1.45 | 1.29 | 1.93  |
| Cys\(^{30}\) | 8.05 | 5.54 | 3.44 | 3.24 | 1.93  |
| Ala\(^{31}\) | 9.38 | 3.83 | 1.46 | 1.93  |
| Lys\(^{32}\) | 7.51 | 4.10 | 1.69 | 1.24 | 1.93  |
| Thr\(^{33}\) | 10.61 | 3.88 | 4.00 | 1.06 | 1.93  |
| Cys\(^{34}\) | 8.95 | 4.67 | 3.20 | 2.88 | 1.93  |
| Gln\(^{35}\) | 8.07 | 4.15 | 2.36 | 2.29 | 1.93  |
| Leu\(^{36}\) | 8.86 | 4.52 | 1.74 | 1.85 | 1.93  |
| Cys\(^{37}\) | 7.76 | 4.40 | 3.39 | 2.99 | 1.93  |

### Backbone Structure Description

The existence of two helices, running from residues 9 to 16 and residues 24 to 31, was clearly indicated by the presence of numerous short-range NOEs between h\(_i\) and hn\(_{i+3}\) protons and between h\(_{i}\) and hβ\(_{i+3}\) protons (Fig. 1). No other regular secondary structure emerged from these data. Fig. 2a shows the 15 best structures of BgK, which result from transformation of NMR data and molecular modeling. The two helical stretches are well defined with r.m.s.d. values of 0.4 Å and 0.5 Å for the position 9–16 and 24–31 stretches, respectively. Though to a lesser extent, the other parts of the toxin structures were also relatively well defined. Panels b and c of Fig. 2 show, respectively, the overall fold of BgK and the spatial organization of its secondary elements. The N- and C-terminal regions are maintained in spatial proximity by the disulfide 2–37, whereas the third disulfide 20–34 brings the loops 16–24 and 31–37 inside the center of the molecule, providing the toxin with a globular shape. The 11–30 disulfide, which links the two helices, adopts a unique conformation, which is almost left-handed (23). It is centrally located in the structure, consistently with a tight organization of the local hydrophobic core. The other disulfide bonds adopt either two conformations (disulfide 20–34) or no prevailing conformation (disulfide 2–33).

### Mutational Analysis

To identify functional residues of BgK, we submitted the toxin to an alanine-scanning experiment, producing all single point variants by chemical synthesis, as described under “Materials and Methods.” Competition experiments were performed between the variants and radio-labeled α-dendrotoxin on membrane from rat brain synaptosomes. Twenty-five variants have been synthesized and investigated regarding their effects on dendrotoxin binding. Fig. 3 shows five inhibition curves obtained with native BgK and four variants, which display a substantially lower competition activity, as compared with native BgK. Except for the variant T33A, all inhibition curves are parallel to that observed with the native toxin. Similar parallel inhibition curves were obtained for other variants, except K25A, for which no inhibition was observed even in the presence of 3×10⁻³ M protein. In the presence of the variant F6A, a 10% increase in binding of labeled dendrotoxin was observed. Although we are not able to explain this observation, as yet, one should recall that different subtypes of potassium channels are present in brain and are composed of heterooligomeric mixtures of different protein subunits. Experiments with subtype-specific antibodies reveal that most of the channels in the brain contain K\(_{1,2}\) (80%) or K\(_{1,1}\) (50%) subunits (24). α-Dendrotoxin blocks K\(_{1,1}\) and K\(_{1,2}\) channels with almost equal affinity (IC\(_{50}\) values ~20 nM), and has greater than 10 times less affinity for other cloned channels (25). Therefore, in view of such complexity, the present competition data have to be considered with caution. From curves similar to those shown in Fig. 3, we deduced IC\(_{50}\) values for all variants. These values are compiled in Table II. The correlation coefficient for fitting the data points was calculated from the Hill equation, y = R\(_ma\)x/[1 + (X/IC\(_{50}\))^H]. As can be seen from Table II, a value close to 1 was obtained in all cases. Moreover, preliminary competition experiments performed with cloned K\(_{1,2}\) channels and a number of the variants exhibiting a lower inhibitory capacity, as compared to native BgK, nicely paral-
led those reported in Table II. Therefore, we anticipate that data obtained with brain synaptosomes mostly reflect the functional importance of residues implicated in the capacity of BgK to bind to K_{1,2} potassium channels. Nevertheless, in order not to overinterpret our data, we considered only the relative inhibitory capacity of the variants, without any attempt to deduce their binding affinities.

Competition experiments, shown in Table II, revealed that mutation of lysine 25 into alanine is associated with the largest affinity decrease. This mutation, however, caused no significant change in the secondary structure of the toxin, as inferred from the circular dichroism spectra of the variant which is quite similar to the spectrum of the native toxin (Fig. 4). We conclude, therefore, that the low competition ability of this mutant is not due to distortions in the toxin structure but to the absence of the lysine side chain and possibly to the loss of its positive charge. Upon mutations of three other residues, Phe^6, Tyr^26, and Thr^33, BgK was a less potent inhibitor, since its competition capacity decreased by factors of 46, 38, and 19, respectively. However, while the circular dichroism spectra of the first two variants were indistinguishable from that of the native toxin, the spectrum of the T33A variant showed some distortions (data not shown), which could not be readily interpreted but which might reflect some structural perturbations. Additionally, the inhibition curve with the T33A variant (see Fig. 3) was not quite parallel to those observed with the native BgK or other variants. In addition, synthesis of this variant was particularly difficult to achieve, leading to a relatively low yield of recovery. Therefore, all these observations suggest that introduction of an alanine at position 33 may be associated with structural perturbations, which might account for the substantial decrease in inhibitory activity of BgK. As a result, one cannot safely propose that the side chain of residue 33 is implicated in the binding of the toxin to the BgK target. Therefore, in the absence of further data, only the side chains of Lys^25, Phe^6, and Tyr^26 are concluded to be involved in the functional site of BgK. Mutations at two positions, Ser^23 and His^13, also induced approximately 8- and 6-fold decrease in competition capacity. Although relatively low, these values might also reflect involvement of these residues in the binding site of BgK. Mutations at the other positions either had no effect on the affinity of BgK or caused changes in inhibitory capacity that are lower than 3-fold. These side chains are not considered, therefore, as actors in the recognition capacity of BgK for dendrotoxin-sensitive binding sites.

In summary, alanine-scanning-based experiments indicated that three, and perhaps five, residues of BgK belong to the surface by which the toxin interacts with the channels.

**DISCUSSION**

Potassium channels constitute a major target for various toxins produced by venomous animals from distinct phyla, including cnidaria (26), arthropods (10), and reptiles (27). Thus,

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3 A. Lecoq, J. Cotton, M. Dauplais, C. L. C. de Medeiros, E. G. Rowan, A. L. Harvey, and A. Ménez, unpublished data.
FIG. 3. Inhibition of $^{125}$I-labeled dendrotoxin to rat brain synaptosomes by various concentrations of the wild type BgK and four variants in which an alanine was introduced in place of Phe$^6$, Ser$^{23}$, Tyr$^{26}$, and Thr$^{33}$. Nonspecific binding was approximately 10% of the total binding.

| Affinity variations of refolded alanine mutants | $IC_{50}$ (nM) | Correlation coefficient | Mutant/WT |
|-----------------------------------------------|----------------|------------------------|-----------|
| WT                                           | 3.6 (±0.6)    | 0.99                   | 1         |
| V1A                                          | 1.6 (±0.1)    | 0.99                   | 0.4       |
| R3A                                          | 7.7 (±0.9)    | 0.99                   | 2.1       |
| W3A                                          | 2.6 (±0.4)    | 0.99                   | 0.7       |
| F6A                                          | 167 (±48)     | 0.90                   | 46        |
| K7A                                          | 5.7 (±0.6)    | 0.99                   | 1.6       |
| E8A                                          | 1.5 (±0.1)    | 0.99                   | 0.4       |
| T9A                                          | 1.6 (±0.3)    | 0.99                   | 0.4       |
| R12A                                         | 3.6 (±0.3)    | 0.99                   | 0.9       |
| H12A                                         | 20 (±3)       | 0.99                   | 5.6       |
| K15A                                         | 5.5 (±1.4)    | 0.99                   | 1.5       |
| S16A                                         | 2.8 (±0.3)    | 0.99                   | 0.8       |
| L17A                                         | 2.3 (±0.2)    | 0.99                   | 0.6       |
| N19A                                         | 6.5 (±1)      | 0.99                   | 1.8       |
| R21A                                         | 4.6 (±1)      | 0.99                   | 1.3       |
| T22A                                         | 2.5 (±0.5)    | 0.99                   | 0.7       |
| S23A                                         | 30 (±6.9)     | 0.96                   | 8.3       |
| Q24A                                         | 1.5 (±0.3)    | 0.99                   | 0.4       |
| K25A                                         | >300          |                        | >90       |
| Y26A                                         | 100 (±33)     | 0.95                   | 28        |
| R27A                                         | 5.1 (±1.8)    | 0.98                   | 1.4       |
| N29A                                         | 4.2 (±0.8)    | 0.99                   | 1.2       |
| K32A                                         | 5.5 (±0.5)    | 0.99                   | 1.5       |
| T33A                                         | 70 (±9.2)     | 0.99                   | 19        |
| E35A                                         | 3.0 (±0.2)    | 0.99                   | 0.8       |
| L36A                                         | 6.4 (±0.8)    | 0.99                   | 1.8       |

FIG. 4. Circular dichroism spectra of synthetic BgK (- - -) and K25A mutant (---). Spectra were recorded at 20°C with a 15–20 nM concentration.
ing that the target of BgK also includes a highly conserved region of the channels, possibly the P-region. In the absence of further data on channel regions that are recognized by these toxins, one way to understand the molecular basis associated with their common capacity to inhibit the binding of dendrotoxin consists in comparing the sites by which these toxins recognize their targets.

With a view toward identifying the functionally important residues of BgK, we submitted the toxin to an alanine-scanning experiment and compared the ability of all the synthetic variants to inhibit the binding of dendrotoxin membranes from rat brain synaptosomes. Twenty-five positions out of 37 have been modified. Therefore, if one excepts the six half-cystines, which are likely to play a structural role, nearly 80% of the positions of BgK have been individually explored regarding their possible involvement in the binding of the toxin to dendrotoxin-sensitive sites in rat brain synaptosomes. Our data showed that introduction of an alanine at five positions, i.e. Lys25, Phe6, and Tyr26, and, to a lesser extent, at His13 and Ser23, caused a decrease in the ability of the toxin to compete with dendrotoxin for its specific binding sites, without changing the secondary structure of the toxin. Moreover, preliminary and unpublished data performed with cloned K+ channels and a number of BgK variants parallel those obtained with rat brain synaptosomes, strongly supporting the view that the residues Lys25, Phe6, and Tyr26, and perhaps His13 and Ser23, are involved in the functional site of BgK. Of these residues, however, mutation at Lys25 most dramatically affected the capacity of BgK to inhibit dendrotoxin binding, suggesting that this particular lysine is the major binding actor of BgK. This finding agrees with recent observations made with ShK, a homologous K+ channel-blocking toxin from the sea anemone S. helianthus whose lysine 22, which corresponds to Lys25 in BgK, plays a critical binding role toward the same channels (38). Previously, ChTX was submitted to site-directed mutagenesis, and the residues by which the toxin binds to the voltage-sensitive Shaker K+ channel were identified (8, 39). Clearly, Lys27 was the most critical residue, with four neighboring amino acids (Tyr26, Met29, Asn30, and Arg34) whose mutation also affected the affinity of ChTX to the channel. Thus, the functional residues of ChTX form a homogeneous area located on the flat β-sheet face that is exposed to solvent (8, 39) and that covers approximately 200 Å² (18 × 12 Å). Although BgK has no β-sheet structure, it nevertheless possesses a flat surface of similar size (21 × 9 Å), which is formed by the edge of the 9–16 helix and its two flanking loops. Strikingly, this surface harbors the functional residues of BgK (see Fig. 5A). Therefore, BgK and ChTX possess comparable flat surfaces, which include a similar small number of energetically important residues, among which a lysine is the major binding actor (Fig. 5B).

If one assumes that these lysines play a similar binding role in BgK and ChTX, their superimposition can be readily associated with superimposition of Tyr26 in BgK with Tyr36 in ChTX, two aromatic residues that play an important binding function in the toxins (Fig. 6, top). The distances that separate the Ca of lysines from the center of the benzene rings of the tyrosines are 6.6 ± 1.0 Å. The common capacity of the two toxins to recognize potassium channels is therefore associated with the conservation of a similar diad of functional residues, a lysine and a close aromatic residue. The other functional residues are different in the two toxins and do not form any evident superimposable pattern. Remarkably, however, if one rotates ChTX 90° around a central axis localized near the crucial lysine, Phe6 of BgK superimposes with Tyr36 in ChTX (Fig. 6, bottom). Thus, by virtue of the four-fold symmetrical organization of the channel (40), Phe6 in BgK is likely to interact with the same aromatic binding site as Tyr26, but on another monomer of the channel.

Similar diads are present in toxins that are homologous to BgK and ChTX. Thus, the diad Lys25-Tyr26 is conserved in the three known K+ channel-blocking toxins from sea anemones (Fig. 7). The situation is slightly more complex with K+ channel-blocking scorpion toxins. Thirteen K+ channel blocking scorpion toxins are presently known. They have been previously divided into four subfamilies (41). Three of these subfamilies possess the same Lys27-Tyr36 diad as in charybdotoxin. In contrast, all toxins forming the fourth subfamily, i.e. the two kalitoxins and the three agitoxins (AgTX1–3), have a threonine at position 36. Strikingly, all these toxins uniquely possess a phenylalanine at position 24 or 25, located on the exposed face of the β-sheet. Mutational studies of a member of this family (AgTX3) showed that this phenylalanine is involved in the binding to the Shaker potassium channel (42). Interestingly, the diad Phe25-Lys27 in AgTX3 can be superimposed on the diad

FIG. 5. A, stereoview of a structure of BgK and its functional residues. Lys25, whose mutation caused the larger decrease in inhibitory capacity, is shown in red. Tyr26 and Phe6, whose mutations caused a decrease in inhibitory activity by factors higher than 10, are shown in orange. His13 and Ser23, whose mutations caused the lowest effect, are in yellow. Note that these residues occupy a flat surface defined by an edge of the longest helix and the two sandwiching loops. The homogeneous surface that is covered by these residues is also shown in B, where the toxin atoms are represented in CPK. The color code of the functional residues is the same as in A.
Lys\textsuperscript{27}–Tyr\textsuperscript{36} in ChTX provided the \(\beta\)-sheets of the two toxins form a 90° angle. We suggest, therefore, that Tyr\textsuperscript{36} in toxins of three subgroups of scorpion toxins and Phe\textsuperscript{25} in the remaining toxins occupy the same binding site on the four-fold symmetrical channel, but on different monomers. Thus, the common capacity of sea anemone and scorpion toxins to recognize K\textsuperscript{+} channels is associated with the conservation of a functional diad, composed of an essential lysine assisted by a 6.6 ± 1.0 Å distant aromatic residue whose precise nature (Tyr or Phe) and location may differ from one toxin to another.

Why is such a diad conserved among two families of functionally similar but structurally unrelated proteins? In scorpion toxins, the positively ammonium group of the lysine of the diad may mimic K\textsuperscript{+} ions entering the pore, occluding the ion pathway (39, 43, 44). A similar situation that takes place in the functional lysine of the toxins from sea anemones. ChTX coordinates come from the Protein Data Bank.

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