Diversity of folds in animal toxins acting on ion channels

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INTRODUCTION

Detailing the structures and functions of the various animal toxins provides several interesting research avenues in terms of protein engineering and therapeutic potential [1–3]. First, it appears pivotal to unravel the molecular determinants by which structurally specific animal toxins are able to recognize, often with high affinity, the diverse ion channel types if one plans to benefit from their pharmacological properties. Because toxins generally display a large array of ion channel targets, of which only one may be of therapeutic value [e.g. K,1.3 (voltage-gated K channel 1.3) as a therapeutic target for immune suppression] [4], a detailed examination of their folds and molecular determinants offers potential to alter their pharmacological selectivity, specificity and potency. Although this identification step of toxin molecular determinants is crucial, it reveals itself as a complex task with regard to the diversity of toxin folds identified so far. Second, toxins are structured by a high number of disulphide bridges (from two to five) in relation to their backbone length, thereby conferring some rigidity to the molecules, a stabilization of their secondary structures, as well as a relative resistance to denaturation (heat, acid/alkali, detergents, etc.). Despite this peptide rigidity, there are some flexible domains, that are more or less conserved, that could be diverted from their usual molecular roles – such as ionic selectivity, structure and/or cell function. Alongside the huge molecular and functional diversity of ion channels, a no less impressive structural diversity of animal toxins has been indicated by the discovery of an increasing number of polypeptide folds that are able to target these ion channels. Indeed, it appears that these peptide toxins have evolved over time on the basis of clearly distinct architectural motifs, in order to adapt to different ion channel modulating strategies (pore blockers compared with gating modifiers). Herein, we provide an up-to-date overview of the various types of fold from animal toxins that act on ion channels selective for K⁺, Na⁺, Ca²⁺ or Cl⁻ ions, with special emphasis on disulphide bridge frameworks and structural motifs associated with these peptide folds.

Key words: animal toxin, disulphide bridging, ion channel, structural motif, toxin fold.

FOLDS OF TOXINS ACTING ON K⁺ CHANNELS

Animal toxins acting on K⁺ channels have been isolated from the venom of numerous animal species, such as marine cone snails, spiders, scorpions, sea anemones and snakes [4,10,11]. The toxins characterized hitherto contain between 22 and 60 amino acid residues, and can be cross-linked by between two and four disulphide bridges. As shown in Figure 1, we have classified these peptide toxins into eight categories depending on their type of fold [7–9,12–16]. These folds contain between two and four well defined elements of secondary structure (see Table 1), and range...
Table 1  Folds found in animal toxins

<table>
<thead>
<tr>
<th>Structure/fold/motif</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>3₁₀</td>
<td>Right-handed helix of the 3₁₀ type (three amino acid residues per turn of helix)</td>
</tr>
<tr>
<td>α</td>
<td>Right-handed helix of the 3.₆₁₃ type (3.6 amino acid residues per turn of helix); also termed α-helix</td>
</tr>
<tr>
<td>αα</td>
<td>Two consecutive α-helices within a peptide or protein structure</td>
</tr>
<tr>
<td>3₁₀α</td>
<td>Helix of the 3₁₀ type followed by an α-helix within a peptide or protein structure (from N- to C-terminus)</td>
</tr>
<tr>
<td>β</td>
<td>Strand of a β-sheet</td>
</tr>
<tr>
<td>ββ</td>
<td>Two-stranded β-sheet (anti-parallel)</td>
</tr>
<tr>
<td>βββ</td>
<td>Three-stranded β-sheet (anti-parallel)</td>
</tr>
<tr>
<td>β₁β₂β₁β₂</td>
<td>Two consecutive (1 and 2) β-sheets (anti-parallel) within a peptide or protein structure (the first is a two-stranded β-sheet and the second is a three-stranded β-sheet)</td>
</tr>
<tr>
<td>αββ</td>
<td>Combination of an α-helix (N-terminal) followed by a two-stranded β-sheet (C-terminal)</td>
</tr>
<tr>
<td>βαββ</td>
<td>First strand (N-terminal), α-helix, second and third (C-terminal) strands of a three-stranded β-sheet</td>
</tr>
<tr>
<td>α/β scaffold</td>
<td>Architectural motif in which a helical structure is connected to a β-sheet by two disulphide bridges</td>
</tr>
</tbody>
</table>

Figure 1  Molscript representations of the 3-D structures of reference animal toxins acting on K⁺ channels

Different types of fold of toxins from various animal species are shown. Helical structures are shown in red (α-helix) or orange (3₁₀ helix), strands of β-sheet are blue, and disulphide bridges are green. The Cα peptide backbone trace is depicted in yellow. The toxin N- and C-terminal extremities are indicated. The name of the toxin is provided, along with the Protein Data Bank (PDB) code in parentheses. All of the 3-D structures of toxins presented originate from experimental data obtained in the original publications.

from a combination of β-strands {βββ type, e.g. the marine cone snail toxin κ-PVIIA (Figure 1a) [12,17], α-helices {two variants of the αα type, e.g. scorpion κ-hefutoxin 1 (Figure 1b) [7] and sea anemone BgK (Figure 1c) [13]; and one 3₁₀αα type, e.g. sea anemone ShK (Figure 1d) [14]}, or a mixture of both secondary structures {3₁₀ββ, αββ, βαββ, and 3₁₀ββαα types; examples illustrated are spider hanatoxin 1 [15], scorpion maurotoxin [8], scorpion charybdotoxin [9], and snake dendrotoxin I [16] (Figures 1e–1h)}. As mentioned, there are some variants in the same combination of secondary structures. Indeed, the αα type of fold can be subdivided into two families: (i) the so-called ‘helical hairpin-like’ motif, in which two α-helices are arranged in an anti-parallel manner [7], and (ii) the ‘helical cross-like’ motif, in which one α-helix is disposed perpendicular to the other [13]. The more complex 3₁₀αα arrangement is also referred to as the ‘helical capping’ motif, since one α-helix caps the other two
Diversity of folds in animal toxins acting on ion channels

Figure 2 Structural data and corresponding pharmacology of reference animal toxins acting on K\textsuperscript{+} channels

Primary structures, the relative positioning of secondary structures, and half-cystine pairings of the toxins are shown. The colour coding is as indicated in Figure 1, except that disulphide bridge arrangements are shown as black plain lines. The animal species, peptide chain lengths, corresponding types of toxin fold and known pharmacological targets are also indicated. Filled squares above amino acid lettering indicate the residues that are thought to comprise the functional dyad or triad. The questionmark highlights a putatively functionally important residue. O and Z represent 4-trans-L-hydroxyproline and pyroglutamic acid residues respectively. The star indicates a toxin with a carboxyl-amidated C-terminus. IK, intermediate-conductance Ca\textsuperscript{2+}-activated K\textsuperscript{+} channels; SK, small-conductance Ca\textsuperscript{2+}-activated K\textsuperscript{+} channels; BK, big-conductance Ca\textsuperscript{2+}-activated K\textsuperscript{+} channels.

The amino acid sequences, secondary structures and disulphide bridge frameworks of representative animal toxins of each reference fold are illustrated in Figure 2. In the examples shown, only the relative positioning of the secondary structures within the toxin amino acid sequence is conserved. For each type of fold, peptide chain length and disulphide bridge organization can vary, depending on the toxin under consideration, as described below. Figure 2 also provides some basic information on the K\textsuperscript{+} channel subtype(s) that is (are) preferentially targeted by the representative toxin examples. In spite of the existence of toxins with different folds, able to recognize various Kv channel subtypes, it has been demonstrated that these peptides may have in common some key molecular determinants, such as a functional dyad [10,13,22] that contributes to blocking efficacy, at least in the case of toxins that act through an ion channel pore-blocking mechanism. The position of these key amino acid residues within the toxin primary structure is also shown in Figure 2. The typical dyad is composed of a Lys residue entering by its side chain into the ion channel pore and an aromatic (Tyr or Phe) or aliphatic (Leu) amino acid residue [10,13,23], separated by a distance of approx. 7 Å. The Lys residue is positioned in the centre of a ring composed of the carbonyl groups of four equivalent acidic
residues (Asp or Glu) [22,24,25], each belonging to one of the four α-subunits that form a functional K⁺ channel. The critical aromatic or aliphatic residue of the functional dyad reportedly interacts – via hydrophobic forces – with a cluster of aromatic residues (Tyr and Trp), generally belonging to a unique K⁺ channel α-subunit. Despite the existence of multiple toxin folds, the dyads of these toxins are spatially superimposed [1,13]. It is noteworthy that, in marine cone snail κ-PVIIA (βββ), sea anemone BgK (αα) and snake dendrotoxin I (310ββα), a triad has been highlighted rather than a dyad (Figure 2) [13,17,26]. In toxins possessing triads, two equivalent hydrophobic residues (e.g. Tyr, Phe or Trp) can functionally substitute for each other to compose, together with the pivotal Lys residue, the actual functional dyad. This suggests that toxins displaying triads instead of dyads may adopt more than a single docking position over the ion channel. Of note, the systematic presence of either a dyad or a triad in toxins with unrelated folds suggests that their functional effects could rely mainly on pore-blocking mechanisms [13]. However, an exception to the rule is provided by scorpion toxin Tc32 [27], which apparently lacks a functional dyad but behaves as a potent blocker of a K⁺ channel. In fact, dyads are unlikely to represent the sole molecular determinants of toxins involved in binding to ion channels [28]. It has been proposed that a ring of basic residues may also play a pivotal role in this function, in line with the concept of a multi-point attachment of the toxin on to its ion channel target [22,24]. Recently, the 3-D (three-dimensional) structure of the nine-residue toxin contrypphan-Vn (PDB code 1N3V), isolated from the venom of the marine cone snail Conus ventricosus, has been reported [29]. Unlike other K⁺ channel-acting toxins belonging to any of the eight families of toxin folds, contrypphan-Vn is folded according to a type IV turn and a type I β-turn that are connected by a unique disulphide bridge. Despite its minimal structure, this toxin also exhibits a dyad (Lys-6 and Trp-8, located at the correct distance from each other), the functionality of which remains to be verified experimentally. Potentially, this toxin structure may thus form a ninth class of toxin folds.

**FOLDS OF TOXINS ACTING ON Na⁺ CHANNELS**

To date, eight different folds have been described of animal toxins acting on Na⁺ channels (Figure 3). The ‘simplest’ folds include three families based on β-sheet structures {ββ, βββ and ββββ types, respectively illustrated by spider huwentoxin-IV (Figure 3a) [20], spider ACTX-Hi:OB4219 (Figure 3b) [30] and sea anemone ATX Ia (Figure 3c) [31]}, and one family based on α-helical structures {helical hairpin αα type, represented by marine worm B-IV (Figure 3d) [32]}. More complex types of folds include ββ310 {e.g. spider δ-atractotoxin-Hv1 (Figure 3e) [21]}, βαββ {e.g. scorpion AahII (Figure 3f) [33]}, αβββ {e.g. snake crotamine (Figure 3g) [34]}, and βααββα {e.g. scorpion Bj-xtrIT (Figure 3h) [35]} types. The ββ, βββ and helical-ended βββ310 types of fold are three variants of the ICK architectural motif [19,36]. It is noteworthy that three out of the eight types of fold presented here – βββ, helical hairpin αα and βαββ – are also representative of toxin folds that recognize K⁺ channels (see

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**Figure 3** Molscript representations of the 3-D structures of reference animal toxins acting on Na⁺ channels

Different types of fold of toxins from various animal species are shown. See the legend of Figure 1 for further details.
Diversity of folds in animal toxins acting on ion channels

Figure 4  Structural data and corresponding pharmacology of reference animal toxins acting on Na\(^+\) channels

Primary structures, relative positioning of secondary structures, and half-cystine pairings of the toxins are shown. See the legend of Figure 2 for further details. Nav, voltage-gated Na\(^+\) channels (mammalian except where indicated).

Figure 1) [30,32,33]. Generally, toxins acting on Na\(^+\) channels [37,38] have longer peptide chain lengths than toxins acting on K\(^+\) channels. Additionally, they clearly possess more than one mechanism of action, and can often be classified as gating modifiers. For instance, several different pharmacological sites for toxins have been demonstrated on voltage-gated Na\(^+\) channels [37]. However, only a limited amount of experimental data are available with regard to the critical amino acid residues involved in the bioactivity of these toxins on Na\(^+\) channels. This situation probably arises from the difficulty in performing systematic structure–function studies with long-chain toxins that are difficult to produce by a recombinant or chemical route [39]. It has been proposed that these toxins may bind on to their target channel via a hydrophobic interacting surface composed of aliphatic and aromatic amino acid residues [37,40,41].

Amino acid sequences, secondary structures and disulphide bridge arrangements of representative animal toxins of each type of fold are pictured in Figure 4. For each toxin with a particular type of fold, the relative order of secondary structure appearance is conserved. In contrast with the short-chain animal toxins, only few peptides acting on Na\(^+\) channels have been fully characterized from the standpoints of 3-D structure and half-cystine pairing pattern [37]. Toxins acting on Na\(^+\) channels are cross-linked by between three and four disulphide bridges. Figure 4 also shows that voltage-gated Na\(^+\) channels are the main targets of the representative toxins.

FOLDS OF TOXINS ACTING ON Ca\(^{2+}\) CHANNELS

Toxins acting on Ca\(^{2+}\) channels can be classified into four types of fold (Figure 5). These include peptide folds of the \(\beta\beta\) [e.g. bug Ptu1 (Figure 5a) [42]], \(\beta\alpha\beta\) [e.g. cone snail \(\omega\)-conotoxin GVIA (Figure 5b) [43]], \(\beta\beta\beta\) [e.g. snake FS2 (Figure 5c) [44]] and \(\alpha\alpha\beta\) types. Although not produced by an animal species, KP4 is presented here to illustrate the structural complexity of some toxins acting on Ca\(^{2+}\) channels [46]. It is the sole toxin in this group reported to contain \(\alpha\)-helical structures as well as \(\beta\)-sheets. From the various folds, three toxin architectures can be distinguished: ICK (\(\beta\beta\) or \(\beta\beta\beta\)), three-finger (\(\beta\alpha\beta\)) and \(\alpha\beta\) sandwich (\(\beta\alpha\alpha\beta\)). The \(\beta\beta\) type of fold is found in some toxins acting on Na\(^+\) channels [20], whereas the \(\beta\beta\beta\) type is found in some toxins specific for either K\(^+\) or Na\(^+\) channels [12,30]. The three-finger toxins are found mainly in snake venoms (cobras, mambas, kraits and sea snakes). They contain between four and five disulphide bridges, with four being conserved in all toxins. Therefore...
three-finger toxins display three β-stranded loops that extend from a central core containing the four conserved disulphide bridges. Of note, the three-fingered fold is not limited to elapid or hydrophid toxins.

Owing to the relative complexity of the fold associated with the α/β sandwich motif [45], one can anticipate the existence of other types of fold for toxins acting on Ca²⁺ channels. For instance, the 63-residue kurtoxin [5] isolated from the scorpion *Parabuthus transvaalicus* and acting on low-voltage-gated Ca²⁺ channels shares marked structural similarities with scorpion α-toxins [37] acting on Na⁺ channels (βαββ type) and is expected to fold according to the α/β scaffold. If this is confirmed experimentally, kurtoxin would represent the first member of an additional family of toxin folds. Interestingly, the marine cone snail ω-conotoxin GVIA [43], which acts as an ion channel pore blocker, contains a functional dyad composed of Lys-2 and Tyr-13 [48] that is similar to those of toxins acting on K⁺ channels [13]. Not all toxins active on Ca²⁺ channels are pore blockers, and many were found to affect ion channel gating properties as well. Again, this is the case for kurtoxin, which slows down the inactivation rates of both voltage-gated Ca²⁺ and Na⁺ channels [5]. The cross-reactivity of kurtoxin is of interest and further suggests that the βαββ type of fold is common to certain toxins that may act on K⁺, Na⁺ and/or Ca²⁺ channels. This is also the case for scorpion chlorotoxin [49], which reportedly acts on Cl⁻ channels (see next section). This observation suggests a remarkable adaptation of this family of toxin folds to ion channels of different ionic selectivity. Some key structural features of the reference toxins are shown in Figure 6. Note that the number of toxin disulphide bridges varies between three and five. Biological targets shown here are voltage-gated Ca²⁺ channels, but endoplasmic reticulum-based Ca²⁺ channels (such as the ryanodine-sensitive receptor) have also been identified as molecular targets [50].

**FOLD OF TOXINS ACTING ON Cl⁻ CHANNELS**

A unique toxin fold (βαββ) has been described for the 36-residue chlorotoxin [49] (Figure 7) and the 35-residue Bm-12 [51], both from scorpion. These short-chain toxins are cross-linked by four disulphide bridges and display the classical α/β scaffold [18].

The scarcity of characterized toxins acting on Cl⁻ channels must be due to the fact that only a few pharmacological studies have focused on these channels.

**TOXIN FOLDS AND DISULPHIDE BRIDGE FRAMEWORKS**

Animal toxins not only possess well-defined folds, but are also highly reticulated peptides in relation to their size. Toxin disulphide bridges vary in number from two to five. The half-cystine pairings of the toxin contribute to the stabilization and rigidity of its fold. Indeed, the resulting decrease in toxin backbone flexibility is thought to increase the efficacy of channel modulation through strict spatial positioning of toxin residues that are key to interaction with the ion channel. The disulphide bridge pattern of a toxin is not necessarily linked to a particular type of fold, and vice versa. For instance, sea anemone ShK from *Stichodactyla helianthus* [14], BgK from *Bunodosoma granulifera* [13] and snake dendrototoxin I from *Dendroaspis polyplepis* [16], all acting on K⁺ channels, exhibit similar C1–C6, C2–C4 and C3–C5 half-cystine pairing patterns, although they fold differently (3ωαωα, 3ωωωω and 3ωβωω types respectively). For three-disulphide-bridged toxins of various animal species, all acting on different ion channel targets, an identical pattern of disulphide bridges can be found, indicating that peptide reticulation is independent of the animal species, biological target and type of fold. When considering the C1–C4, C2–C5 and C3–C6 pattern, which is the most highly represented reticulation among the various toxin folds, the following examples are found: (i) spider huwentoxin-IV, acting on Na⁺ channels, displays a ββ type of fold with an ICK motif [20], (ii) scorpion charrybotoxin, acting on K⁺ channels, has a βαββ type of fold with an αβ scaffold [9], and (iii) the marine cone snail ω-conotoxin GVIA, acting on Ca²⁺ channels, possesses a ββ type of fold, also with an ICK motif [43]. A similar observation is valid for toxins reticulated by four disulphide bridges, e.g. C1–C4, C2–C6, C3–C7 and C5–C8: (i) spider δ-atracotoxin-Hv1, acting on Na⁺ channels, shows a helical ended βββ3αβ type of fold with an ICK motif [21], whereas (ii) scorpion chlorotoxin, acting on Cl⁻ channels, displays a βαββ type of fold with an αβ scaffold [49]. Conversely, for a defined toxin architectural motif, several patterns of disulphide bridging may occur. For example, in the
Diversity of folds in animal toxins acting on ion channels

Table 1: Summary of structural data and corresponding pharmacology of reference toxins acting on Ca\textsuperscript{2+} channels

<table>
<thead>
<tr>
<th>Toxin</th>
<th>Structural data</th>
<th>Fold</th>
<th>Main target</th>
</tr>
</thead>
<tbody>
<tr>
<td>PuI (34-mer) <em>Petrius tardis</em></td>
<td>AEBDCIAAGACGFDTCMKPCNNPRAKGGYVYKCL</td>
<td>( \beta \beta ) (ICK)</td>
<td>Ca\textsuperscript{2+} (N-type)</td>
</tr>
<tr>
<td>o-GVIA (27-mer) <em>Coma geographica</em></td>
<td>KSOGSSCSQ7YSVCCSNOYTKRCY*</td>
<td>( \beta \beta \beta ) (ICK)</td>
<td>Ca\textsuperscript{2+} (N-type)</td>
</tr>
<tr>
<td>FS2 (60-mer) <em>Dendroaspis polyepis</em></td>
<td>RICYSHKSLPRATKTVCTCFLMYRMFICREYIERSGCGTAMFWYCCNGORCNK</td>
<td>( \beta \beta \beta \beta \beta ) (three-finger motif)</td>
<td>Ca\textsuperscript{2+} (L-type)</td>
</tr>
<tr>
<td>KP4 (105-mer) <em>Ustilago maydis</em></td>
<td>LGINCRGSSCQGSLHGNLVRDQACGNQTMCPGERRAKCVGTNISAYVQS</td>
<td>( \beta_1 \alpha \alpha \beta_2 \beta_1 ) (a/b sandwich motif)</td>
<td>Ca\textsuperscript{2+} (L-type)</td>
</tr>
</tbody>
</table>

Figure 6 Structural data and corresponding pharmacology of reference toxins acting on Ca\textsuperscript{2+} channels

Primary structures, relative positioning of secondary structures, and half-cystine pairings of the toxins are shown. See the legend of Figure 2 for a detailed description. The numbering of strands of each \( \beta \)-sheet structure is also shown.

Figure 7 Structural information and corresponding pharmacology of a reference toxin (scorpion) acting on Cl\textsuperscript{–} channels

(a) Molscript representation of the 3-D structure of chlorotoxin (see the legend of Figure 1 for details). (b) Primary structure, relative positioning of secondary structures, and half-cystine pairings of chlorotoxin. The same colours codes as in (a) are used, except that disulphide bridging is shown in black plain lines. The animal species, peptide chain length, corresponding type of toxin fold and pharmacological target are also indicated.

case of the ICK motif, the following peptide reticulations have been demonstrated: (i) C1–C4, C2–C5 and C3–C6 (\( \omega \)-conotoxin GVIA, hanatoxin 1, \( \kappa \)-PVIIA and huventoxin-IV), (ii) C1–C4, C2–C6, C3–C7 and C5–C8 (\( \delta \)-atracotoxin-Hv1), and (iii) C1–C4, C2–C5, C3–C8 and C6–C7 (ACTX-Hi:OB4219). Another example deals with Pi1 [52] and maurotoxin [53], two scorpion toxins (\( \alpha \)/\( \beta \)scaffold) that possess an \( \alpha / \beta \) scaffold, but which differ in their disulphide bridge arrangements (C1–C5, C2–C6, C3–C7 and C4–C8 compared with C1–C5, C2–C6, C3–C4 and C7–C8 respectively) [54,55]. However, the helical hairpin (\( \alpha \)\( \alpha \)) toxin fold is obligatorily linked to a specific disulphide bridge organization (e.g. C1–C4 and C2–C3 in the case of the two-disulphide-bridged scorpion \( \kappa \)-hefutoxin 1 [7], and C1–C8, C2–C7, C3–C6 and C4–C5 in the case of the four-disulphide-bridged marine worm toxin B-IV [32]).

**TOXIN FOLDS AND CONSENSUS AMINO ACID SEQUENCES**

Toxin architectures (e.g. ICK motif or \( \alpha / \beta \) scaffold) are often associated with the presence of consensus amino acid sequences, such as \( CX_3.CX_4.CX_5.CX_6.CX_7.CX_8. \) (ICK motif) [19] or \( X.CX.CX.CX,(G/A/S)XCX.CXCX_8 \) (standard structural motif...
for α/β scaffold [18]) and its variant X,CX,CX,CX,-G(A/S)XXC,CX,X, found in short-chain scorpion toxins cross-linked by four disulfide bridges (variant structural motif for α/β scaffold). Conotoxins, which target a great variety of ion channels (K+, Na+ and Ca2+), display only a few conserved structural motifs. These are categorized on the basis of their particular patterns of half-cystine arrangements – as follows: (i) the four cysteine/two-loop framework (CC-C-C), found in the α-conotoxins that target nicotinic acetylcholine receptors, (ii) the six cysteine/three-loop framework (CC-C-C-CC), found in the μ-conotoxins acting on Na+ channels, and (iii) the sixcysteine/four-loop framework, which has two variants: C-C-C-CC (the most abundant of all frameworks), found in δ-, ω- (e.g. ω-GVIA) and κ-conotoxins (e.g. κ-PVIIA), targeting Na+, Ca2+ and K+ channels respectively, and CC-C-C-C, found in μ-PmIVA/B that targets Na+ channels [56].

CONCLUDING REMARKS AND PERSPECTIVES

We have surveyed the various types of fold found in animal toxins that are able to recognize, bind to and modulate ion channels of different ionic selectivity. To date, we estimate that animal toxins may be classified into 14 different families of toxin folds. In view of the tremendous number of toxins that remain to be structurally characterized from the venoms of diverse animal species, we would reasonably expect the number of different toxin folds described to increase dramatically in the forthcoming years. Indeed, it is observed that the venom of each animal species is a very rich source of molecules exhibiting different folds. From this overview, it may appear that analysing toxins of certain animal species has provided a greater number of peptide folds. However, this observation is likely to have resulted from a historical bias, since the venoms of some animal species have been studied to a far greater extent than others. The main reasons for such a bias are the ease with which the venom can be collected and the quantity of material produced, along with considerations such as health priorities within different countries, economic value, etc. It is more likely that none of the toxin folds will be definitively associated with a particular animal species. What emerges conclusively from our structural analysis of animal toxins is that peptides that possess a similar type of fold can exert their action on several types of ion channels, the most representative examples being provided by peptides belonging to the large family of conotoxins (e.g. κ-PVIIA and α-conotoxin GVIA). Other examples are scorpion kurtoxin [5] and kurtoxin-like I (KLI) peptides [6], which are active on both voltage-gated Na+ and T-type Ca2+ channels. These examples of cross-reactivity might represent the rule rather than the exception. Indeed, most toxins have been characterized on the basis of a limited number of pharmacological targets, underscoring the likelihood that many other true physiological targets remain to be discovered [57]. However, it should be noted that we have been unable so far to identify a group of toxins sharing the same fold and able to modulate the four types of ion channel considered here. We assume that this is just a matter of time, as the list of characterized toxins is growing exponentially. Conversely, it is now clear that a particular ion channel can be targeted by toxins that possess unrelated folds. In view of this, one should note that the action of toxins with unrelated folds on the same channel implies distinct interaction sites. This would be expected for toxins differing in their modes of action, e.g. ion channel pore blockers compared with gating modifiers. The ability of structurally divergent toxins to recognize a particular ion channel relies on the equivalent spatial distributions of amino acid residues that are key to the toxin–channel interaction. Therefore, when considering the interaction of a toxin with its ion channel target, what clearly matters most is the spatial distribution of these key amino acid residues of the toxin, rather than its type of fold. To illustrate this point, it was reported that the functional dyads of the sea anemone toxin BgK (helical cross-like αα) and the scorpion charybdotoxin (βαββ), or other K+, channel-activating scorpion toxins, are spatially superimposed [13]. Furthermore, it was also shown that spider δ-atracotoxin-Hv1 (ββββ333) competed off the binding of scorpion α-toxins (βαββ), presumably by binding to similar – or partially overlapping – receptor site 3 of the voltage-gated Na+ channels [58] of excitatory cells. A similar competition for binding to Na+ channels was reported for scorpion α-toxins and sea anemone toxins [59].

Finally, there is no striking correlation between toxin fold, disulfide bridge framework and pharmacology. Where the fold and disulfide bridge organization play crucial roles is in the spatial distribution of residues key to toxin pharmacology. Improvement of ion channel recognition by toxins (selectivity, affinity, potency) can be achieved through slight or more marked alterations in toxin structures [60,61]. Therefore more structure–activity relationship studies on animal toxins will be required to detail the intimate molecular basis of the interaction between toxins and ion channels. This is an immediate and important issue with regard to the therapeutic potential of toxins and structural analogues thereof for producing immunosuppressant agents [4], or treating chronic pain [62] or various human disorders such as multiple sclerosis [63], cancer, diabetes, cardiovascular and neurological diseases [3,61].

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Received 3 December 2003; accepted 16 December 2003
Published as BJ Immediate Publication 16 December 2003, DOI 10.1042/BJ20031860