THE 1.3-Å STRUCTURE OF A SCORPION NEUROTOXIN

A MEMBRANE-BINDING PROTEIN

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Scorpion venoms contain small basic proteins that are responsible for the neurotoxic activities of the venoms. The complete amino acid sequences of about 20 scorpion toxins have been determined along with partial sequences for several others. These toxins display considerable variation in amino acid composition, but they generally consist of 60–70 amino acids and are cross-linked by four disulfide bridges. The amino acid sequences display a number of common features, including the same general locations of the eight cysteine residues, similar disulfide bridging patterns, and the location of several invariant or conserved residues. To obtain information about the three-dimensional structures of these proteins we have initiated a series of crystallographic studies of toxins from venom of *Centruroides sculpturatus* Ewing, a scorpion native to the Arizona desert.

METHOD

The variant-3 scorpion toxin was prepared as described (1). The crystallization techniques were the same as outlined (2) except that the seeded solution was equilibrated by vapor diffusion against unbuffered 66% (vol/vol) 2-methyl-2,4-pentanediol (MPD) in water. The structure at 3-Å resolution was solved by MIR phases determined from two heavy atom derivatives, K_2PtCl_6 and K_2IrCl_6 (3). Data for native and derivative crystals were collected with a Picker FACS-1 diffractometer (Picker Corp., Cleveland, OH) at room temperature using an ω step scan procedure and nickel-filtered CuKα radiation. Atomic coordinates were measured from a Kendrew wire model constructed to match the 3-Å electron density map. The structure has been refined at 1.8-Å[1] and 1.3-Å resolution by constrained-difference Fourier methods (4) and by free-atom block-diagonal least-squares.

[1]Almassy, R. J., J. C. Fontecilla-Camps, S. E. Ealick, F. L. Suddath, and C. E. Bugg. The 1.8 Å structure of variant-3 scorpion neurotoxin from *Centruroides Sculpturatus* Ewing. In preparation.

RESULTS

A tracing of the α-carbon backbone is shown in Fig. 1 *a*. The most prominent secondary structural features are two and a half turns of α-helix and a three-strand stretch of antiparallel β-sheet which runs parallel to the α-helix. The helix is connected to the middle strand of the β-sheet by two disulfide bridges. A third disulfide bridge is located nearby. Several loops extend out of this dense core of secondary structure. The largest loop is joined to the COOH-terminus of the molecule by the fourth disulfide bridge. The overall shape of the molecule resembles a right-hand fist: the α-helix runs along the knuckles of the fist; the β-sheet lies along the second and third joints of the fingers; the thumb is defined by two short loops that are composed of residues 16–21 and residues 41–46; the wrist corresponds to the COOH-terminal stretch of residues 52–65 and a loop composed of residues 1–14; and the second joint of the little finger is near the NH_2-terminus of the molecule. The β-sheet structure forms a large flat surface, which contains most of the conserved amino acids in the various scorpion toxins that have been sequenced. This surface also contains an impressive array of exposed hydrophobic residues, including seven of the eight aromatic amino acids in the toxin.

A space-filling model of the structure, as viewed toward the conserved-hydrophobic surface, is shown in Fig. 1 *b*.

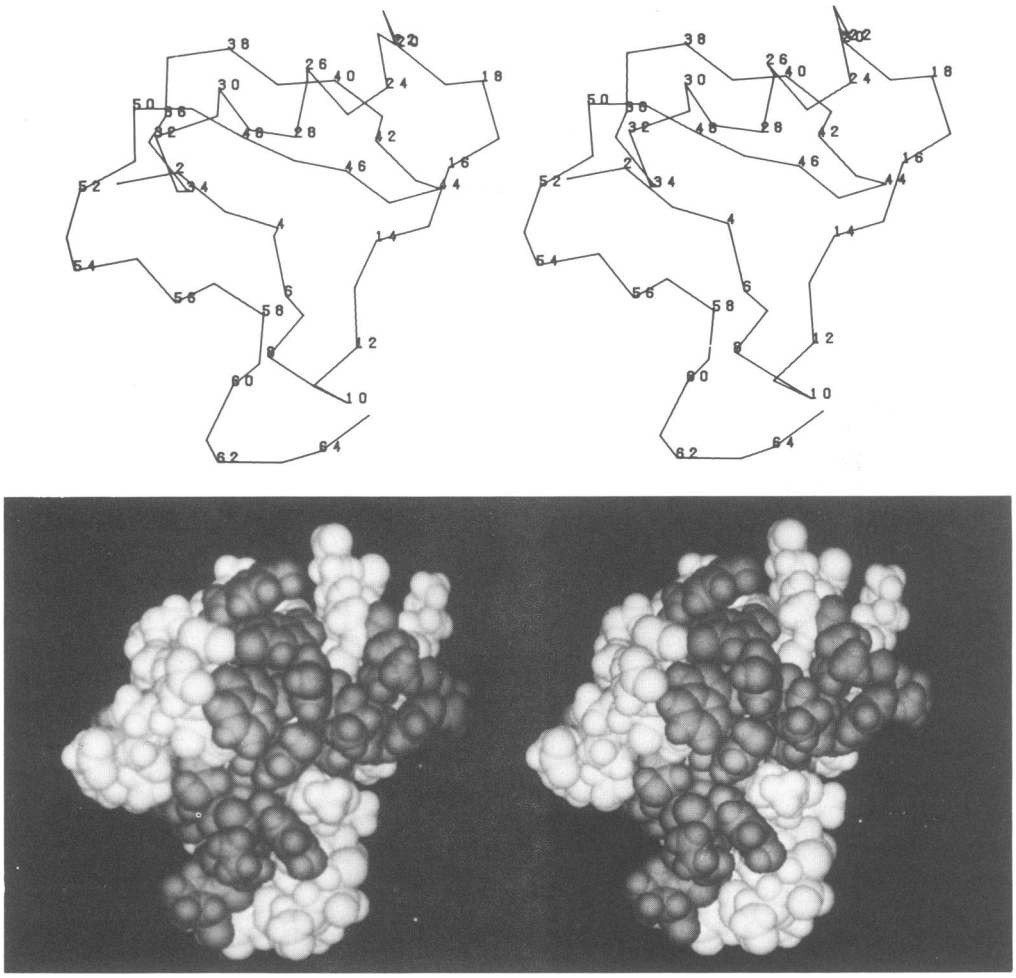

FIGURE 1 Stereo-drawing of the variant-3 structure as viewed toward the conserved-hydrophobic side of the molecule (looking into the palm of the right-handed first model). (*a*) The α-carbon backbone with labels on the even-numbered residues. (*b*) A space-filling model in which hydrophobic residues (Ala, Ile, Leu, Phe, Pro, Thr, Trp, Tyr and Val) are darkened.

The hydrophobic amino acids form a continuous band that spans the entire length of the molecule. The patch of conserved residues penetrates into this hydrophobic band and includes most of the protuberance of the left of the molecule in Fig. 1 *b*, along with tryptophan-47 and tyrosine-4. Lysine-13 can be seen jutting out of the hydrophobic band immediately above tyrosine-58. Most of the scorpion neurotoxins have a lysine or an arginine residue at either position 13 or position 58, and there is some evidence that a basic group in this region is required for toxicity. The hydrophobic surface of the molecule is fairly smooth and even. In contrast, the surface on the other side of the molecule mainly contains hydrophilic amino acids and is relatively rough, with many crevices and protuberances. It appears likely that the hydrophobic-conserved surface shown in Fig. 1 is involved in interactions of scorpion toxins with sodium channels of excitable membranes.[2]

REFERENCES

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