

The amino acid sequence of toxin Rp_{III} from the sea anemone, *Radianthus paumotensis*

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The amino acid sequence of the sodium channel toxin Rp_{III} from the sea anemone *Radianthus paumotensis* has been determined. The protein is homologous with five analogous toxins from three anemone species, and is most similar to a less toxic protein, Rp_{II}, from the same organism. Twelve residues are conserved in all six toxins, one of which is an arginine residue thought to be essential for toxicity. The others (Cys, Gly, Pro and Trp) tend to be conserved in other sets of homologous proteins to maintain functional folds. Comparisons of the sequences suggest the existence of two separate but related classes of toxins common to the three species of anemone.

Na⁺ channel toxin; Amino acid sequence; Sequence homology; (Sea anemone)

1. INTRODUCTION

Four toxins (Rp_I-Rp_{IV}) have been isolated from the sea anemone *Radianthus paumotensis* [1]. Each slows the inactivation of the sodium channel in mice and prolongs the action potential. These toxins are 48-49-residue polypeptides that cross-react immunologically among themselves, but not with analogous toxins from other sea anemones.

The most abundant toxin from *R. paumotensis* is designated Rp_{III}. Its toxicity toward mice is much greater than that of Rp_{II} [1]; however, only Rp_{II} has been described at the level of primary and secondary structure [2]. Its amino acid sequence is homologous with toxins of two other sea anemones, *Anthopleura xanthogrammica* [3] and *Anemonia sulcata* [4-6].

This report describes the determination of the amino acid sequence of Rp_{III} and a comparison of its structure with that of other anemone toxins.

2. MATERIALS AND METHODS

The toxin Rp_{III} was prepared in the laboratory of Dr Michel Lazdunski, Université de Nice. It was

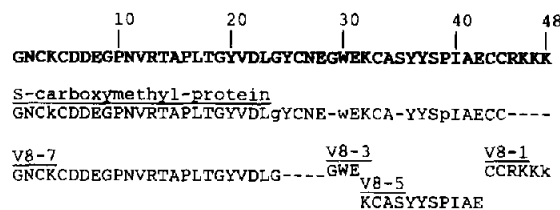


Fig.1. Summary of the proof of sequence of Rp_{III}. The sequences of the specific peptides are given in one-letter code below the summary sequence (bold type). The four peptides were generated by the action of *S. aureus* V8 protease. A dash indicates an unidentified amino acid. A lower-case letter indicates a tentative identification. Lys-48 is only tentatively assigned, largely on the basis of the composition of the whole protein and NMR data.

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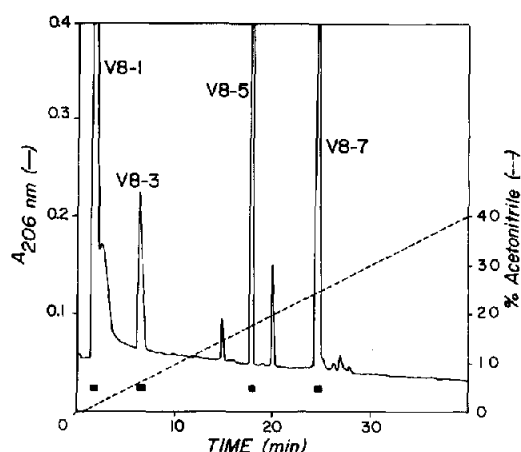


Fig.2. Separation of peptides after digestion of 10 nmol CM-protein with *S. aureus* V8 protease. The flow rate was 2.0 ml/min through a SynChropak RP-P column. Pooled peptides V8-1, V8-3, V8-5 and V8-7 were analyzed in detail (fig.1). The composition of the fraction eluting at 20 min indicated that it contained a small amount of a peptide comprising residues 9–28.

reduced and carboxymethylated as described [2]. Amino acid compositions were determined with a Waters Picotag system [7] or with a Dionex D-500 analyzer. The CM-protein was cleaved at glytamy residues with *Staphylococcus aureus* V8 protease (Miles) in 0.1 M ammonium bicarbonate buffer, pH 7.8, for 4 h at 37°C, using a substrate/enzyme ratio of 30:1 (w/w). The peptides were separated on SynChropak RP-P columns (4.1 × 250 mm) with a linear gradient from 0.1% aqueous trifluoroacetic acid to 40% acetonitrile (containing 0.08% trifluoroacetic acid) over 40 min. The peptides and intact *S*-carboxymethyl-protein were analyzed with a Beckman 890C sequencer, phenylthiohydantoin were identified by HPLC, and sequences were analyzed with SEARCH and ALIGN programs, all previously described [8].

3. RESULTS

The results of Edman degradation of the intact carboxymethyl protein and of four digestion products are summarized in fig.1. Together these analyses provide an overlapping set of data that describes the amino acid sequence of Rp_{III}.

Table 1
Amino acid compositions^a

| Peptide Residues | V8-1 ^b 43–47 | V8-3 29–31 | V8-5 32–42 | V8-7 1–28 | Rp _{III} | |
|---------------------|----------------------------|---------------|---------------|--------------|--------------------------|----------|
| | | | | | Composition ^c | Sequence |
| Asp/Asn (D/N) | | | | 4.4 (6) | 5.8 | (6) |
| Glu/Gln (E/Q) | 0.2 (0) | 1.0 (1) | 1.1 (1) | 2.0 (2) | 4.1 | (4) |
| Cys (C) | 1.7 (2) | | 0.8 (1) | 1.2 (3) | 4.7 | (6) |
| Ser (S) | 0.2 (0) | | 2.0 (2) | | 2.0 | (2) |
| Gly (G) | 0.4 (0) | 1.2 (1) | | 4.0 (4) | 4.6 | (5) |
| Arg (R) | 1.0 (1) | | | 1.0 (1) | 2.0 | (2) |
| Thr (T) | | | | 1.6 (2) | 2.0 | (2) |
| Ala (A) | | | 2.0 (2) | 1.2 (1) | 3.1 | (3) |
| Pro (P) | | | 1.2 (1) | 2.1 (2) | 2.6 | (3) |
| Tyr (Y) | | | 2.0 (2) | 1.7 (2) | 4.1 | (4) |
| Val (V) | | | | 1.5 (2) | 2.0 | (2) |
| Ile (I) | | | 1.0 (1) | | 1.0 | (1) |
| Leu (L) | | | | 1.8 (2) | 2.0 | (2) |
| Lys (K) | 1.9 (2) | | 0.9 (1) | 0.8 (1) | 4.9 | (5) |
| Trp (W) | | N.D. (1) | N.D. (0) | N.D. (0) | 1.0 | (1) |
| Yield (%) | 65 | 72 | 66 | 34 | | |

^a Residues/molecule by amino acid analysis or (in parentheses) from the sequence. N.D., not determined

^b Determined with a Dionex D-500 analyzer; all other peptides were analyzed in the Picotag system

^c From Schweitz et al. [1]

Amino-terminal analysis of the intact carboxy-methyl protein placed 44 residues, but with three tentative assignments and two unidentified amino acids. These placements were made in peptides generated by the action of *S. aureus* V8 protease on Rp_{III} (fig.2, table 1). The cleavage between residues 8 and 9 was incomplete, providing V8-7 as a major product. The assignment of V8-1 to the C-terminus is confirmed by its lack of a C-terminal glutamic acid.

Degradation of V8-1 identified Lys-46 and Lys-47, but an additional Lys suggested by the composition of the whole protein (table 1) may have been largely washed from the spinning cup in the previous cycle. NMR studies by Dr David Wemmer (personal communication) confirmed the presence of five Lys in the protein. However, the amino acid composition of V8-1 indicated only two Lys, or a total of four in the protein. In view of these conflicting data we tentatively conclude that the original protein has five Lys and that some has been lost from the carboxyl terminus so that the material in hand includes a mixture of chains with two and with three Lys at the carboxyl terminus.

4. DISCUSSION

The amino acid sequence of Rp_{III} is very similar to that of Rp_{II} (fig.3). Thirty-one of the 48 amino acid residues are in identical positions.

Four other toxins from two different sea anemones have similar action on sodium channels but differ in their toxicity toward target organisms. The homology of all of these toxins is evident from

comparison of their amino acid sequences (fig.3). Only twelve invariant residues are observed in all six toxins, including the six half-cystines that suggest identical disulfide pairs and similar three-dimensional structure. Arg-13 is probably essential for the toxic interaction (see below). The others, three Gly, one Pro and one Trp, are all residues that tend to be highly conserved in other sets of homologous proteins where common folding modes are maintained.

Alignment scores among the six toxins (table 2) indicate that Rp_{III} resembles the other *R. paumotensis* toxin, Rp_{II}, more than any of the other toxins. In addition, the *A. sulcata* toxins resemble each other and the *A. xanthogrammica* toxin more than they do the Rp toxins. These relationships are also evident in fig.3 where asterisks indicate the residues common to anemone toxins from species other than *R. paumotensis*. Thus, it appears that there are two distinct structural subclasses within this family of homologous toxins. Nonetheless, the basic secondary structure of Rp_{II} [2] is conserved among toxins of the other two organisms [9].

The common toxicity of the six toxins should relate directly to common structural features. Arg-13 (Rp_{III} numbering) is reported to be essential in As_{II} [10] and is conserved in all six toxins. The carboxyl group originally found to be essential for toxicity in As_{II} [10] is most likely to be the side chain carboxyl of Asp-8 because Asp-6 is replaced by a Lys in As_I [6] and the α -carboxyl is displaced in As_V and in the Rp toxins. However, in Rp_{III} the critical residue is replaced by Glu (fig.3). It will be of interest to compare the three-dimensional structure of this region of Rp_{III} with that of Rp_{II} [2].

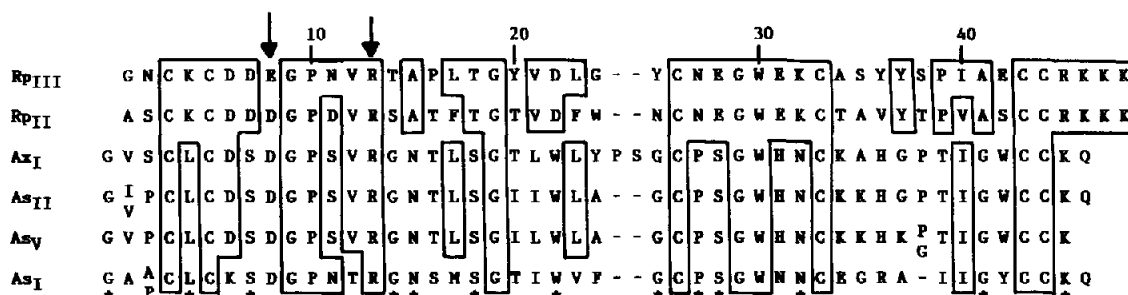


Fig.3. Comparison of the sequences of sea anemone toxins [2-6]. The abbreviations of table 2 are used. Boxed residues are identical with those in Rp_{III}. (*) Residues identical in the *Anthopleura* and *Anemonia* toxins but different from the *Radianthus* toxins. Arrows identify homologous residues that may be essential for toxicity (see text).

Table 2

Sequence similarities of sea anemone toxins^a

| | Rp _{III} | Rp _{II} | Ax _I | As _{II} | As _V |
|------------------|-------------------|------------------|-----------------|------------------|-----------------|
| Rp _{II} | 15.8 | | | | |
| Ax _I | 9.0 | 9.5 | | | |
| As _{II} | 8.1 | 9.5 | 15.8 | | |
| As _V | 8.4 | 10.7 | 20.4 | 17.2 | |
| As _I | 7.0 | 6.9 | 12.5 | 14.4 | 11.4 |

^a Comparisons were made by the ALIGN program and are expressed as alignment scores. An alignment score of greater than 3 (standard deviations) corresponds to a probability of less than 0.0014 that a randomly generated sequence of the same composition would produce a better score. Toxins Rp are from *Radianthus paumotensis* [2], As are from *Anemonia sulcata* [4-6] and Ax is from *Anthopleura xanthogrammica* [3] (cf. fig.3)

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