THE JOURNAL OF BIOLOGICAL CHEMISTRY

Vol. 267, No. 29, Issue of October 15, pp. 20701-20705, 1992
Printed in U.S.A.

Heterodimeric Structure of the Spider Toxin ω-Agatoxin IA Revealed by Precursor Analysis and Mass Spectrometry*

(Received for publication, April 10, 1992)

Ameurfina D. Santos‡§¶, Julita S. Imperial‡, Tanuja Chaudhary‖, Ronald C. Beavis‖, Brian T. Chait‖, John P. Hunsperger‡, Baldomero M. Olivera‡, Michael E. Adams, and David R. Hillyard‡ ‡‡**

*From the ‡Departments of Biology and Pathology, University of Utah, Salt Lake City, Utah 84112, the §Institute of Chemistry, University of the Philippines, Diliman, Quezon City 1101, Philippines, ‖Rockefeller University, New York, New York 10021, and the **Departments of Entomology and Neuroscience, University of California, Riverside, California 92521*

We report the first molecular characterization of a precursor sequence for a small, Ca^{2+} channel blocking, peptide spider toxin, ω-agatoxin IA. By integrating information generated from a molecular genetic approach using agatoxin cDNAs with data provided from mass spectrometry of the mature toxin, we were able to deduce the likely mechanisms by which the toxin precursor peptide is processed to its mature heterodimeric form. A particularly interesting feature of the prepropeptide is the occurrence of two glutamate-rich sequences interposed between the signal sequences, the major peptide toxin, and the minor toxin peptide. Excision of the more distal glutamate-rich region appears to be signaled by flanking arginine residues but likely occurs only after a disulfide linkage has formed between the major and minor chains of the mature toxin. Our molecular genetic approach toward characterizing this toxin will allow us to quickly generate a series of spider sequences from which mature toxin structures can be deduced and eventually expressed. Additionally, this approach will provide insights into the evolutionary divergence observed among spider peptide toxins.

Spiders contain a diverse array of toxic compounds in their venoms. There are at least three distinctive chemical classes that have been chemically characterized. First are the organic polyamine compounds, such as the α-agatoxins and argiotoxins; these have been shown to block glutamate receptor channels. Second are large proteins such as α-latrotoxin from black widow spider venom (molecular mass = 120 kDa) (Kiyatkin *et al.*, 1990). Perhaps the largest and most diverse group of spider toxins are the small, disulfide-rich polypeptides, typically 40–90 amino acids in length. Such small polypeptide toxins have been described in a variety of spiders including *Atrax robustus* (Sheumack *et al.*, 1985; Brown *et al.*, 1988), *Hololena curta* (Stapleton *et al.*, 1990; Quistad *et al.*, 1991), *Plectruerys tristis* (Branton *et al.*, 1987), and *Agelenopsis aperta*.

In the funnel web spider, *Agelenopsis aperta*, a large variety of small polypeptide toxins are present. Two general classes have been identified, the μ-agatoxins (Skinner *et al.*, 1989), which activate Na^{2+} channels at the insect neuromuscular junction (Adams *et al.*, 1989), and the ω-agatoxins. The ω-agatoxins specifically inhibit voltage-sensitive Ca^{2+} channels (Adams *et al.*, 1990; Scott *et al.*, 1990; Mintz *et al.*, 1991, 1992; Venema *et al.*, 1992). Four different classes of ω-agatoxins have been described, each with divergent biochemical characteristics and pharmacological specificity (Olivera *et al.*, 1991; Adams *et al.*, 1992). The first of these to be characterized, ω-Aga-IA,[1] is a potent antagonist of presynaptic Ca^{2+} channels at insect neuromuscular junctions (Bindokas and Adams, 1989; Adams *et al.*, 1990; Bindokas *et al.*, 1991).

The primary structure of ω-Aga-IA was reported to be an unblocked, 66-amino acid peptide (Adams *et al.*, 1990; see Fig. 1). Unlike virtually every peptide toxin described previously, ω-Aga-IA appeared to contain 9 cysteine residues. Since cysteine residues typically are involved in disulfide bonding, the presence of an odd number suggested that one of these residues might be present in a reduced form. However, alkylation experiments carried out to detect free sulfhydryl moieties were consistently negative. Furthermore, mass spectrometry studies indicated the association of an undefined moiety that is removed upon exposure of the peptide to reducing conditions (Chaudhary *et al.*, 1991).

We report here both the precursor structure of ω-Aga-IA and a modified structure for the mature toxin. By using a cDNA cloning approach, an ω-Aga-IA clone was identified from an *A. aperta* cDNA library and sequenced. This is the first small polypeptide toxin precursor from spiders to be reported. The structure of the toxin precursor, supported by mass spectroscopy and sequencing data, revealed that the mature ω-agatoxin IA consists of two polypeptide chains, which are formed from the precursor by excision of an internal heptapeptide.

EXPERIMENTAL PROCEDURES

Materials—Oligonucleotide probes and primers were synthesized on an Applied Biosystems 380B synthesizer. Nitrocellulose blotting membranes and [γ-^{32}P]ATP were purchased from Amersham. The FastTrack mRNA isolation kit was purchased from Invitrogen. Sequencing reactions were performed with Sequenase Version 2 from United States Biochemical.

RNA Preparation—Venom ducts hand-dissected from 200 adult *A. aperta* spiders were frozen in liquid nitrogen, ground to powder, and extracted in an SDS/proteinase K solution (FastTrack mRNA iso-

* This work was supported in part by National Institute of Health Grants GM22737 (to B. M. O.) and NS24472 (to M. E. A.) and RR00862 (to B. T. C.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) M95540.

¶ Supported by a fellowship from the Philippine American Education Foundation.

‡‡ To whom all correspondence should be addressed.

[1] The abbreviations used are: ω-Aga-IA, ω-agatoxin IA; SDS, sodium dodecyl sulfate; HPLC, high-performance liquid chromatography; kb, kilobase(s).

AKALPPGSVCDGNES[DCKCYG]KWHKCRCPWKW...
HFTGEGPCTCEKGMKHTCITKLHCPNKAEWGLDW

FIG. 1. **The previously determined amino acid sequence of ω-agatoxin IA.** It is demonstrated in this article that this sequence represents only one of two polypeptide chains in the mature toxin. The sequences corresponding to the oligonucleotide probes used to identify ω-Aga-IA cDNA clones are *boxed*.

lation kit from Invitrogen). Poly(A)$^+$ RNA was subsequently batch-annealed to oligo(dT) cellulose, washed, and eluted from a spin column.

Vector—The polylinker region of plasmid pGEM 3Zf+ was extended to include five additional sites by cloning into the *Eco*RI and *Kpn*I sites a synthetic oligomer sequentially encoding *Eco*RI, *Sac*I, *Eco*RV, *Not*I, *Sph*I, *Nsi*I, *Stu*I, *Bgl*II, and *Kpn*I. The extension was created in order to flank cDNA inserts on each side with six to eight restriction sites, thereby facilitating subsequent manipulations with any useful clone. The modified plasmid is designated pDH52.

Vector-primer Preparation—Cesium gradient-purified plasmid pDH52 was passed over a Bio-Gel A150m column, cleaved with *Kpn*I, and T-tailed with terminal transferase to a mean length of 72 ± 8 bases. Following a second cleavage with *Bam*HI and removal of the free T-tail by agarose gel electrophoresis, a vector-primer was generated which allows cDNA inserts to be unidirectionally and coordinately oriented with respect to lacZα.

cDNA Library Construction—First strand cDNA synthesis was initiated with 5 mg of methylmercury-denatured poly(A)$^+$ RNA annealed to 1 pmol of vector-primer in the presence of AMV reverse transcriptase. Second strand synthesis was performed essentially according to the RNase H/Pol I protocol of Okayama and Berg (1982), and the product was blunt-ended with T4 polymerase. Following protection of all *Eco*RI sites with *Eco*RI methylase, the blunt termini of the vector-cDNA were ligated to *Eco*RI linkers d(pCGGAATTCCG) which earlier had been partially ligated to the 5-mer level. Cohesive ends were then generated with *Eco*RI, and the product was electrophoresed in low melting temperature agarose. Six discrete size fractions of cDNA were recovered by CTAB purification (Langridge *et al.*, 1980). The recovered fractions of vector-cDNA molecules were individually recircularized, ligated, and electroporated into MC1061 (F′ lac I^q, Tn5) yielding a multiply size-fractionated library composed of 3.0×10^6 independent clones.

Synthesis of Oligonucleotides—All oligonucleotides used as probes for screening the cDNA library and primers for sequencing were synthesized using the Applied Biosystems 380B synthesizer. One probe was used for screening; four primers for sequencing.

Library Screening—Samples from appropriate cDNA size fractions were replica-plated onto nitrocellulose membranes and prepared for colony hybridization (Hanahan and Meselson, 1980). The nucleotide sequences of mixed 17-mer probes were inferred from amino acid sequence information determined from purified toxin preparations. Probes were 5′-end-labeled with [γ-^{32}P]ATP and T4 polynucleotide kinase. To identify individual clones containing the ω-Aga-IA peptide sequence, probe 190 was used. Probe 190 is a 32-mix 17-mer oligonucleotide 5′CCRTARCAYTTRCARTC3′ (R = A or G, Y = C or T) which is complementary to the cDNA sequence corresponding to six amino acids of the ω-Aga-IA peptide. About 3500 colonies were plated out from each of three size fractions of the cDNA library, and duplicate nitrocellulose filter lifts were prehybridized in 3 M tetramethylammonium chloride, 0.1 M phosphate buffer, pH 6.8, 1 mM EDTA, 5 × Denhardt's, 0.6% SDS, and 100 μg/ml denatured salmon sperm DNA at 48 °C for 3 h. The filters were then hybridized for 40 h at 48 °C in the same solutions with the addition of 16 pmol of probe 190 which were 5′-end-labeled with [γ-^{32}P]ATP by T4 polynucleotide kinase. The filters were washed 4 times: 2 × with 3 M tetramethylammonium chloride, 50 mM Tris, 0.2% SDS, first for 15 min at room temperature followed by 1 h at 50 °C; and twice more with 2 × SSC, 0.1% SDS, both at room temperature. The filters were then exposed to x-ray film using intensifying screens at −70 °C for 24 h. Well-isolated positive clones on both duplicate filters were picked.

Sequencing—Five μg of cesium gradient-purified plasmid was denatured in 0.4 M NaOH for 0.5 h at 37 °C and neutralized by adjusting to 0.4 M ammonium acetate. The DNA was ethanol-precipitated, resuspended in 8 ml of H_2O, and annealed with 2 pmol of sequencing primer by heating to 65 °C for 5 min and cooling slowly to 30 °C. Labeling and termination reactions were performed as specified by the protocol accompanying the Sequenase Version 2.0 enzyme.

Dideoxy sequencing was performed on alkali-denatured supercoiled plasmid DNA purified by centrifugation to equilibrium in CsCl-ethidium bromide gradients. A Sequenase Version 2.0 sequencing kit of United States Biochemical was used following the manufacturer's protocol.

Peptide Isolation and Characterization—ω-Aga-IA was HPLC-purified from whole *A. aperta* venom (purchased from Spider Pharm, Black Canyon City, AZ) as previously described (Adams *et al.*, 1990). Native peptide was concentrated to dryness using a Speed-Vac (Savant Instruments) and submitted to the University of California Riverside Biotechnology Instrumentation Facility for Edman sequence analysis. Sequencing was carried out using an Applied Biosystems, Inc. instrumentation which included a Model 475-A liquid-pulsed sequenator, on-line phenylthiohydantoin analysis on the 120A HPLC, and data collection and reduction with a Model 900-A computer and Applied Biosystems' version 2.42 software. Applied Biosystems' phenylthiohydantoin standards, chemicals, and reagents were used throughout. All sequence calls were confirmed by visual examination of the chromatograms.

Mass Spectrometry—Mass spectrometric measurements were performed on the ^{252}Cf plasma desorption and matrix-assisted laser desorption time-of-flight mass spectrometers constructed at the Rockefeller University and described previously (Chait *et al.*, 1981; Chait and Field, 1984; Beavis and Chait, 1990a, 1990b). Samples were prepared for measurement by ^{252}Cf plasma desorption mass spectrometry by absorption of approximately 1 nmol of toxin in 0.1% trifluoroacetic acid to a thin layer of nitrocellulose as described previously (Chait and Field, 1986). Samples were prepared for matrix-assisted laser desorption mass spectrometry by applying 1 pmol of the toxin in a saturated solution of 3-methoxy-4-hydroxycinnamic acid (in 30% acetonitrile, 0.1% trifluoroacetic acid) to a metal probe tip, drying the solution with a stream of cool air, and inserting the probe into the mass spectrometer (Beavis and Chait, 1989). The mass accuracies of both techniques were approximately 100 ppm.

RESULTS

Construction of A. aperta Library; Identification of ω-Aga-IA cDNA Clones—A cDNA library (3.0×10^6 independent clones) was prepared from *A. aperta* venom duct poly(A)$^+$ RNA as described under "Experimental Procedures." The library was size-fractionated into six size classes.

A mixed 17-mer oligonucleotide probe specific for ω-Aga-IA was labeled and used to probe the 0.40–0.45- and 0.45–0.55-kb size fractions of the cDNA library. Approximately 4% of all cDNA clones in these size fractions hybridized to the probe; eight independent clones were analyzed further by a partial sequence analysis. Six of these appeared to have identical sequences; the other two encoded a minor variant precursor form of ω-Aga-IA (which will be described elsewhere).[2] Three of the six major variant clones of ω-Aga-IA were completely sequenced; all encoded the same open reading frame.

Sequence of the ω-Aga-IA Precursor—The ω-Aga-IA cDNA clones encoded a 112 amino acid polypeptide; both the nucleotide sequence and deduced amino acid sequence are shown in Fig. 2. Fig. 2 shows a schematic analysis of the different regions in the polypeptide precursor for ω-Aga-IA. The *underlined* region in Fig. 2 corresponds to the amino acid sequence obtained when purified ω-Aga-IA was initially biochemically characterized. Clearly, however, the predicted precursor sequence of ω-Aga-IA includes three other distinctive regions. The N terminus encodes a typical signal sequence (*bracketed*). This is followed by a short hydrophilic region, highly enriched in glutamate residues. At the C-terminal end are 10 additional amino acids which were not detected after the original toxin had been reduced and alkylated and the purified product had been sequenced (Adams *et al.*, 1990). The most noteworthy features of the C-terminal extension are that it contains a small glutamate-rich region flanked by 2 arginine residues and a C-terminal cysteine residue. Thus, together with the

[2] A. D. Santos and D. R. Hillyard, unpublished observation.

66-amino acid polypeptide that was sequenced after toxin purification, the C-terminal fragment would yield a 76-amino acid precursor with 10 Cys residues; these could presumably form five disulfide bonds.

However, the previous Edman sequencing results (Adams *et al.*, 1990) indicated that a proteolytic event must have occurred following Trp-66, since this residue was found at the C-terminal end of the reduced and carboxymethylated toxin. If the Cys residue on the C-terminal decapeptide (or some fragment derived from it) were linked by a disulfide bond to the main chain, such a disulfide-bonded peptide fragment would have been lost during the reduction and carboxymethylation procedures. In order to investigate this possibility, both the native and reduced toxin were analyzed by mass spectrometry. In addition, Edman sequencing was carried out on the native toxin.

Mass Spectrometry of ω-Aga-IA—Native ω-Aga-IA, purified by reversed-phase HPLC was subjected to ^{252}Cf plasma desorption mass spectrometry. The spectrum (shown in Fig. 3) indicated a molecular mass (*MM*) of 7791.4 Da, which is 303.7 Da higher than that predicted from the previously published (Adams *et al.*, 1990) Edman sequencing data (7487.7 Da). A search for modification(s) of the peptide using plasma desorption mass spectrometry of the trypsin and cyanogen bromide cleavage products of ω-Aga-IA showed no altered residues and confirmed the Edman sequencing data. However, since all of the enzymatic and chemical procedures used previously were preceded by a reduction step to open up the tightly cross-linked polypeptide, we hypothesized that the reduction may have removed a 304.7-Da component present on the native material. The PD mass spectrum of dithiothreitol reduced ω-Aga-IA was, however, too weak to be informative.

Further analysis with matrix-assisted UV laser desorption time-of-flight mass spectrometry confirmed the results obtained with plasma desorption mass spectrometry, but gave much higher quality mass spectra (see Fig. 4). Thus, we were able to obtain an intense mass spectrum from dithiothreitol-reduced ω-Aga-IA. The reduced material had a molecular mass of 7495.3 Da, matching the value predicted from the amino acid sequence of the major chain (7495.8 Da) and demonstrating the removal of a component with a mass of 304.7 Da. This mass value exactly matches the predicted mass of the C-terminal tripeptide of the precursor, Ser-Pro-Cys. Thus, it appears that the sulfhydryl in the native peptide is not free. Loss of 303.7 Da following exposure of the peptide to reducing conditions is consistent with the hypothesis that the C-terminal tripeptide of the precursor sequence (SPC) is linked to the major 66 amino acid chain by a disulfide bond, and that the heptapeptide sequence RSEESER was excised.

Amino Acid Sequencing of Native ω-Aga-IA—Previous peptide sequence analyses of ω-Aga-IA (Adams *et al.*, 1990) were performed consistently on the reduced, alkylated peptide. Since the combined data from precursor cDNA sequencing and mass spectrometry indicate that this procedure may have resulted in loss of the presumed native dimeric character of the molecule, we subjected the native toxin to N-terminal sequence analysis without the intervention of reducing conditions. Results of this analysis yielded the following amino acid assignments through the first 4 cycles of Edman sequencing: cycle 1, A+S; cycle 2, K+P; cycle 3, A; cycle 4, L (Table I). Higher molar ratios for A and K on the first two sequence cycles probably resulted from the presence of reducing reagents in the sequencing protocols, leading to a greater loss of the tripeptide during the intervening wash cycles. The lack of a Cys residue registered on the cycle 3 of the sequence was expected, since no reduction and alkylation was performed.

These data are consistent with the presence of two N-terminal sequences coming from a major chain (66 amino acids) and a minor chain (3 amino acids) processed from the precursor molecule. The combined data taken from cDNA sequencing, Edman sequencing, and mass spectrometry thus indicates that the mature biologically active ω-Aga-IA is a 69-amino acid heterodimer consisting of a major chain (66 amino acids) and a minor chain (3 amino acids), with an interchain disulfide linkage.

```
GAA TTA ATG AAT ACA AAG ATG ATG AAG TTT GTC GTA TTT CTT GCT TGT TTG TTT GTT GCT  60
                        [met met lys phe val val phe leu ala cys leu phe val ala  14

GCG CAT TCA TTT GCA GTT GAA GGC GAG GAA GAA TAT TTT GAA GCT GAA GTG CCA GAA TTA  120
ala his ser phe ala]val glu gly glu glu glu tyr phe glu ala glu val pro glu leu  34

GAA AGA GCA AAA GCT TTG CCT CCA GGT TCT GTA TGT GAC GGA AAT GAG TCC GAT TGC AAA  180
glu arg ala lys ala leu pro pro gly ser val cys asp gly asn glu ser asp cys lys  54

TGT TAT GGC AAA TGG CAT AAG TGC CGC TGC CCA TGG AAG TGG CAC TTT ACT GGA GAA GGA  240
cys tyr gly lys trp his lys cys arg cys pro trp lys trp his phe thr gly glu gly  74

CCA TGT ACT TGC GAG AAG GGA ATG AAG CAC ACC TGC ATC ACC AAG CTT CAT TGT CCA AAT  300
pro cys thr cys glu lys gly met lys his thr cys ile thr lys leu his cys pro asn  94

AAA GCC GAA TGG GGT CTT GAT TGG CGT AGC GAG GAG TCT GAA AGA AGT CCT TGT TAA ATG  360
lys ala glu trp gly leu asp trp arg ser glu glu ser glu arg ser pro cys stop     112

TTG TCT GAA AGA AGT CCT TGT CCA AGT TTT AAT TGC AAT AAA TAT TCA AGC AAT TGT TAA  420

AAA AAA AAA AAA                                                               432
```

FIG. 2. **cDNA sequence of clones encoding the 112 amino acid ω-Aga-IA precursor.** Both the nucleotide and predicted amino acid sequence are indicated. The previously determined amino acid sequence is *underlined*.

DISCUSSION

In this work we report the precursor structure for ω-agatoxin IA, a small polypeptide spider toxin which blocks Ca^{2+} channels at the insect neuromuscular transmission as well as in mammalian sensory neurons (Bindokas and Adams, 1989; Bindokas *et al.*, 1991; Scott *et al.*, 1990). Our characterization of the ω-Aga-IA precursor is based upon sequence analysis of cDNA clones isolated from an *A. aperta* venom duct cDNA library. In the course of determining the toxin precursor structure we were also able to deduce the structure of the mature, biologically active toxin.

The organization of the precursor structure of ω-agatoxin IA is consistent with a prepropeptide format. After processing, the mature toxin comprises two polypeptide chains, a larger fragment of 66 amino acids which is disulfide-bonded to a

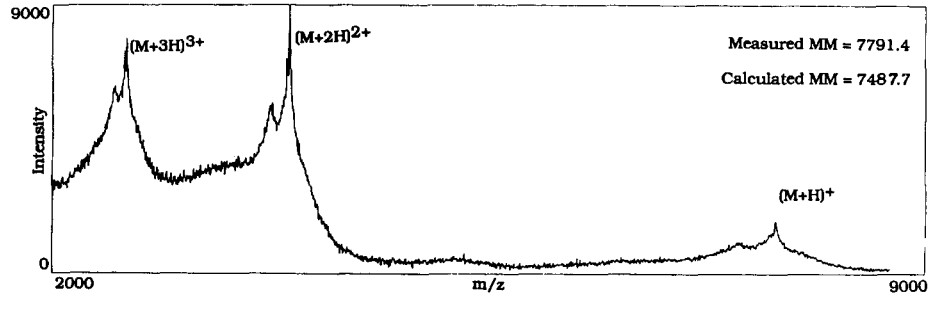

FIG. 3. **^{252}Cf plasma desorption mass spectrum of native ω-Aga-IA.** The spectrum indicates a dominant species with molecular mass (*MM*) of 7791.4 Da, a value 303.7 Da higher than the mass of 7487.7 Da calculated from the sequence.

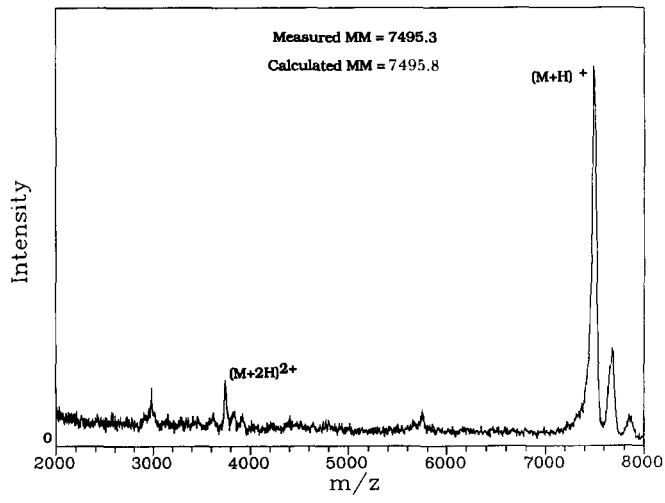

FIG. 4. **Matrix-assisted laser desorption mass spectrum of ω-Aga-IA that has been subjected to reduction by dithiothreitol.** The mass spectrum shows a dominant species with molecular mass (*MM*) of 7495.3 Da, resulting from the loss from the native toxin of an entity with mass 305 Da and the addition of 9 hydrogens to the half-cysteine residues.

TABLE I

N-terminal sequence analysis of native ω-Aga-IA

The theoretical initial yield was 43.7 pmol.

Cycle no.	Amino acid	Yield[a]
1	ALA/SER	66.3/39.0
2	LYS/PRO	95.5/49.1
3	ALA/–[b]	114.7/—
4	LEU/–	114.2/—

[a] Yield uncorrected for background.
[b] Cys was not reduced and alkylated.

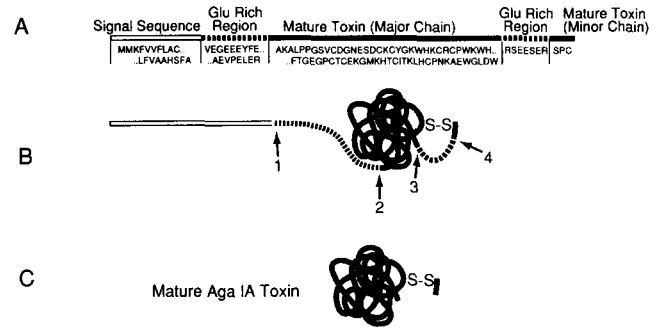

FIG. 5. **Diagrammatic representation of ω-Aga-IA precursor and the presumed processing steps to the mature toxin showing functional domains, including signal sequence (*black bar*), Glu-rich sequence (*striped bar*), major chain (*shaded bar*), excised domain (*striped bar*), and minor chain (*shaded bar*).** In addition to the signal peptidase, at least one other proteolytic activity which cleaved at Arg residues bordering Glu-rich regions would be required. It is possible that other proteolytic activities (such as aminopeptidases) participate in processing.

small tripeptide with sequence Ser-Pro-Cys. Our cDNA sequencing data, together with supporting biochemical evidence, indicate that the mature toxin is formed by proteolytic excision of both the N-terminal region of the ω-Aga-IA precursor, as well as an internal peptide toward the C terminus. The processing of precursor to mature toxin requires at least two proteolytic enzymes, *i.e.* a signal peptidase, as well as a protease specific for arginine residues. A summary of the presumed processing steps is shown in Fig. 5.

A novel feature of the predicted precursor of ω-agatoxin IA is the presence of two short acidic regions, one between the signal sequence and the N terminus of the major chain, and the second between the major chain and the tripeptide at the C terminus. The presence of two glutamate-rich sequences in the ω-Aga-IA precursor raises the obvious question of function. One possible explanation is that these regions serve as part of the recognition site for proteolytic cleavage, since the 3 arginine residues all border glutamate-rich regions. It should be noted that such glutamate-rich regions have previously been described in other small peptide precursors (Richter *et al.*, 1990); in those cases as well, flanking arginine residues appear to be signals for proteolytic cleavage. Whether or not these glutamate-rich regions serve other functions is not known at the present time. Some other possibilities might be folding of the highly positively charged mature toxin, or to promote condensation of the toxin into secretory granules.

Since ω-Aga-IB, like ω-Aga-IA, is known to contain 9 cysteine residues (Adams *et al.*, 1990) a processing pattern similar to that described for ω-Aga-IA can be anticipated, leading to a dimeric mature toxin form. The first spider toxin to be described as a presynaptic antagonist of insect neuromuscular transmission, *Hololena* toxin (Bowers *et al.*, 1987) also appears to be dimeric. However, all other spider toxins, the Type II, Type III, and Type IV ω-agatoxins, the μ-agatoxins, as well as the *Plectruerys* toxins (Branton *et al.*, 1987) appear to be monomers.

It is interesting to compare the precursor structure elucidated here with those of the various other disulfide-rich polypeptide toxins. Apart from the internal peptide excision, the ω-Aga-IA precursor exhibits some features that are intermediate between the α-neurotoxins of snakes, and the smaller conotoxins from cone snail venom. While the mature region of snake toxin precursors immediately follows the signal sequence (see for example Tamiya *et al.*, 1985), the corresponding region in cone snails follows both a signal sequence and a long intervening region (Woodward *et al.*, 1990; Colledge *et al.*, 1992). In ω-Aga-IA, the signal sequence precedes a relatively short intervening region, enriched with glutamate residues. An analysis of other spider toxin precursors has shown that the presence of a short glutamate-rich region between the signal sequence and the mature toxin may be a general characteristic of small polypeptide precursors from spider venom ducts.[3]

Our molecular approach to analyzing spider toxins has considerable potential. Not only is there the possibility of elucidating precursor structure from toxins that have already been biochemically characterized, but the availability of clones should eventually permit efficient expression of the toxin. This approach can also solve problems associated with acquiring adequate quantities of minor constituent toxins, or of chromatographically separating closely related molecular species whose pharmacological properties can be very distinct. Furthermore, a molecular approach avoids the tedious tasks of milking spiders and purifying toxins from the venom. It also obviates the need to maintain large colonies of spiders since, in principle, only a few specimens will be required. By using a molecular approach to characterize and express spider toxins, these valuable ligands for receptors and ion channels can be made much more accessible to the scientific community. Finally, a systematic molecular analysis of spider toxin genes will eventually provide a rich data base for insights into the molecular evolutionary mechanisms which helped to generate the great diversity of spider species.

[3] D. Hillyard, V. Monje, A. Santos, and M. Adams, unpublished results.

REFERENCES

Adams, M. E., Bindokas, V. P., Hasegawa, L., and Venema, V. J. (1990) *J. Biol. Chem.* **265,** 861–867

Adams, M. E., Bindokas, V. P., and Venema, V. J. (1992) in *Neurotox 91. The Molecular Basis of Drug and Pesticide Action* (Duce, I. R., ed) Elsevier Science Publishers, Amsterdam, in press

Beavis, R. C., and Chait, B. T. (1989) *Rapid Commun. Mass Spectrom.* **3,** 432–435

Beavis, R. C., and Chait, B. T. (1990a) *Anal. Chem.* **62,** 1846–1850

Beavis, R. C., and Chait, B. T. (1990b) *Proc. Natl. Acad. Sci. U. S. A.* **87,** 6873–6877

Bindokas, V. P., and Adams, M. E. (1989) *J. Neurobiol.* **20,** 171–188

Bindokas, V. P., Venema, V. J., and Adams, M. E. (1991) *J. Neurophysiol.* **66,** 590–601

Bowers, C. W., Phillips, H. S., Lee, P., Jan, Y. N., and Jan, L. Y. (1987) *Proc. Natl. Acad. Sci. U. S. A.* **84,** 3506–3510

Branton, W. D., Kolton, L., Jan, Y. N., and Jan, L. Y. (1987) *J. Neurosci.* **7,** 4195–4200

Brown, M. R., Sheumack, D. D., Tyler, M. I., and Howden, M. E. H. (1988) *Biochem. J.* **250,** 401–405

Chait, B. T., and Field, F. H. (1984) *J. Am. Chem. Soc.* **106,** 1931–1938

Chait, B. T., and Field, F. H. (1986) *Biochem. Biophys. Res. Commun.* **134,** 420–426

Chait, B. T., Agosta, W. C., and Field, F. H. (1981) *Int. J. Mass Spectrom. Ion Physics* **39,** 339–366

Chaudhary, T., Adams, M. E., Venema, V. J., Beavis, R. C., and Chait, B. T. (1991) *Proceedings of the 38th ASMS Conference on Mass Spectrometry and Allied Topics*, Tucson, AZ, June 3–8, 1990, pp. 283–284

Colledge, C. J., Hunsperger, J. P., Imperial, J. S., and Hillyard, D. R. (1992) *Toxicon*, in press

Hanahan, D., and Meselson, M. (1980) *Gene (Amst.)* **10,** 63–67

Kiyatkin, N. I., Dulubova, I. E., Chekhovskaya, I. A., and Grishin, E. V. (1990) *FEBS Lett.* **270,** 127–131

Langridge, J., Langridge, P., and Bergquist, P. L. (1980) *Anal. Biochem.* **103,** 264–271

Mintz, I. M., Venema, V. J., Adams, M. E., and Bean, B. P. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88,** 6628–6631

Mintz, I. M., Venema, V. J., Swiderek, K. M., Lee, T. K., Bean, B. P., and Adams, M. E. (1992) *Nature* **355,** 827–829

Okayama, H., and Berg, P. (1982) *Mol. Cell Biol.* **2,** 161–170

Olivera, B. M., Imperial, J. S., Cruz, L. J., Bindokas, V. P., Venema, V. J., and Adams, M. E. (1991) *Ann. N. Y. Acad. Sci.* **635,** 114–122

Quistad, G. B., Reuter, C. C., Skinner, W. S., Dennis, P. A., Suwanrumpha, S., and Fu, E. W. (1991) *Toxicon* **29,** 329–336

Richter, K., Egger, R., Negri, L., Corsi, R., Severini, C., and Kreil, G. (1990) *Proc. Natl. Acad. Sci. U. S. A.* **87,** 4836–4839

Scott, R. H., Dolphin, A. C., Bindokas, V. P., and Adams, M. E. (1990) *Mol. Pharmacol.* **38,** 711–718

Sheumack, D. D., Claassens, R., Whiteley, N. M., and Howden, M. E. H. (1985) *FEBS Lett.* **181,** 154–156

Skinner, W. S., Adams, M. E., Quistad, G. B., Kataoka, H., Cesarin, B. J., Enderlin, F. E., and Schooley, D. A. (1989) *J. Biol. Chem.* **264,** 2150–2155

Stapleton, A., Blankenship, D. T., Ackermann, B. L., Chen, T.-M., Gorder, G. W., Manley, G. D., Palfreyman, M. G., Coutant, J. E., and Cardin, A. D. (1990) *J. Biol. Chem.* **265,** 2054–2059

Tamiya, T., Lamouroux, A., Julien, J. F., Grima, B., Mallet, J., Fromagest, P., and Menez, A. (1985) *Biochemie* **67,** 185–189

Venema, V. J., Swiderek, K. M., Lee, T. D., Hathaway, G. M., and Adams, M. E. (1992) *J. Biol. Chem.* **267,** 2610–2615

Woodward, S. R., Cruz, L. J., Olivera, B. M., and Hillyard, D. R. (1990) *EMBO J.* **9,** 1015–1020