Vol. 169, No. 1, 1990 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
May 31, 1990 Pages 51-56

STRUCTURES OF PARALYTIC ACYLPOLYAMINES FROM THE SPIDER AGELENOPSIS APERTA

Gary B. Quistad[1], Somchai Suwanrumpha[2], Mary Ann Jarema[2], Michael J. Shapiro[2], Wayne S. Skinner[1], Gene C. Jamieson[1], Alfred Lui[1],and Emil W. Fu[2]

[1]Sandoz Crop Protection Corporation
975 California Avenue
Palo Alto, California 94304

[2]Sandoz Research Institute
East Hanover, New Jersey 07935

Received April 9, 1990

The structures are given for five paralytic acylpolyamines from the venom of the funnel web spider, Agelenopsis aperta. The acyl moieties are derived from (3-indolyl)acetic acid, (4-hydroxy-3-indolyl)acetic acid, and 4-hydroxybenzoic acid. The polyamine portions of the toxins are novel. Three toxins (AG_{489}, AG_{505}, and AG_{452}) contain 1, 5, 9, 13, 18, 22-hexaazadocosane which is unique as a natural polyamine because of its length and hydroxylation at the 5-aza position. The polyamine portions of two other α-agatoxins (AG_{488} and AG_{504}) are unusual also, containing guanidinooxy moieties.

The recent availability of venom from numerous spider species has prompted a keen interest in venom constituents as selective modulators of distinct ion channels in both vertebrates and insects (1,2). Paralytic toxins blocking glutamate receptors have been identified from several spiders, including Argiope (3-5) and Nephila (6,7). The α-agatoxins from the funnel web spider, Agelenopsis aperta, are acylpolyamines causing reversible paralysis and a block of postsynaptic glutamate-sensitive receptor channels in insects (8). The paralytic α-agatoxins complement the slower lethal activity of polypeptides (μ- and ω-agatoxins) in this venom which affect presynaptic voltage-sensitive ion channels (9,10). We report here the structures of five α-agatoxins from A. aperta venom.

Abbreviations: Fast atom bombardment (FAB), distortion enhancement by polarization transfer (DEPT), chemical ionization (CI), ultra-violet (UV), mass spectrometry (MS), nuclear magnetic resonance (NMR), correlated spectroscopy (COSY), heteronuclear correlated spectroscopy (HETCOR).

0006-291X/90 $1.50

Materials and Methods

Venom from Agelenopsis aperta was purchased from Spider Pharm (Black Canyon City, AZ). The α-agatoxins were purified by reversed-phase liquid chromatography as described previously (10).

Individual α-agatoxins were analyzed by mass spectroscopy using three separate instruments: 1) VG 7070, FAB with xenon in thioglycerol matrix; 2) Hewlett-Packard 5985 for FAB using xenon with glycerol matrix or direct-inlet CI with ammonia; and 3) Finnigan TSQ-70 for MS/MS by FAB with sample in glycerol.

Nuclear magnetic resonance spectra of AG_{489} and AG_{488} were obtained on a Bruker 500 MHz instrument. The samples were dissolved in dimethyl sulfoxide for ^{1}H and ^{13}C spectra. AG_{488} was analyzed further by DEPT and AG_{489} was studied using COSY and HETCOR.

Results

Hydroxylamine Toxins. Our early structural analysis centered on AG_{489}, the most abundant acylpolyamine in this venom. Most of the structure could be assigned readily from mass spectral analysis (particularly CI with NH_3). The ultra-violet spectrum suggested an indole chromophore which mass spectrometry indicated to arise from an indoleacetyl moiety. Hydrolysis of AG_{489} gave no amino acids, but rather a linear 3,3,3,4,3-polyamine (1,5,9,13,18,

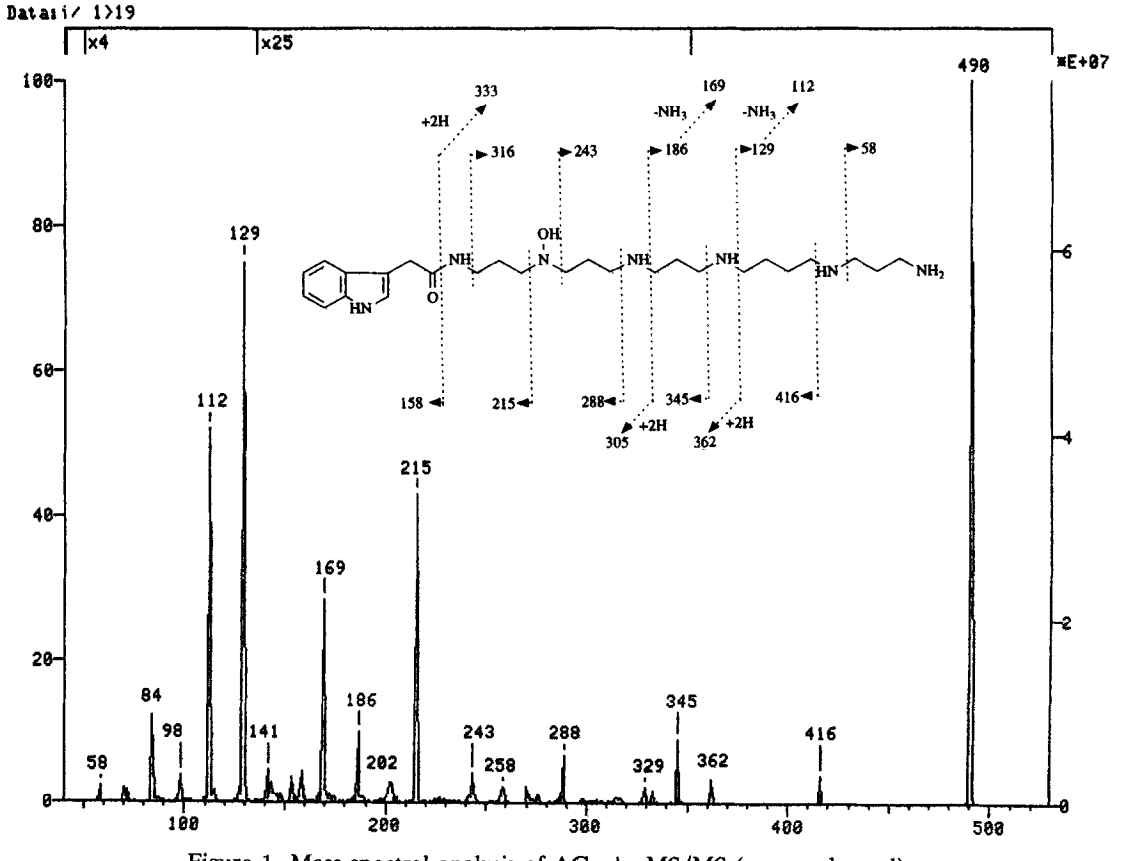

Figure 1. Mass spectral analysis of AG_{489} by MS/MS (xenon, glycerol).

22-hexaazadocosane). The structure of the 3,3,3,4,3-polyamine was deduced from mass spectral analysis (CI and FAB) of its perbenzoylated derivative (molecular mass = 940) prepared under Schotten-Baumann conditions. A synthetic sample of this hexamine was available for chromatographic comparison to hexamine from the native toxin. Thus, AG_{489} is an acylpolyamine with a 3,3,3,4,3-polyamine acylated at a terminal nitrogen by (3-indolyl)acetic acid. The FAB mass spectrum of AG_{489} indicated that the polyamine moiety contained a rather labile oxygen which was not evident by CI (NH_3) analysis and which was lost during hydrolysis. The labile oxygen on the polyamine could also be removed by reduction with $TiCl_3$ (AG_{489} converted to derivative with mass = 473).

To complete the structural assignment for AG_{489}, we needed to determine the placement of the additional oxygen (i.e 16 atomic mass units). Since AG_{452} contained the same oxygenated polyamine as AG_{489}, it was chosen as a synthetic target. Three compounds containing 4-hydroxybenzoic acid were synthesized for comparison to native AG_{452}: with 3,3,3,4,3-polyamine alone, and the corresponding hydroxamate and terminal hydroxylamine analogs. Each of these three standards was chromatographically similar to native AG_{452}, but none was identical.

Ultimately, the missing 16 mass units were assigned by mass spectral analysis (Figure 1). Collisionally induced fragmentation of MH^+ from AG_{489} gave a series of ions consistent with our proposed structure (e.g. m/z = 288). MS/MS analysis of AG_{505} and AG_{452} gave the same placement of the oxygen. The positions of the hydroxyl group on the aroyl moieties of AG_{505} and AG_{452} (also AG_{504}) were deduced from UV spectra (including 2nd derivative

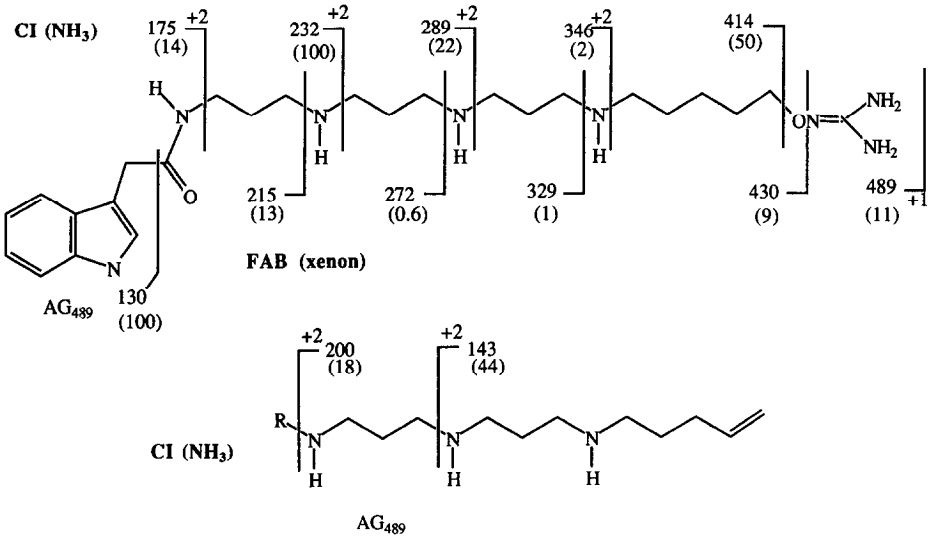

Figure 2. Mass spectral analysis of AG_{488} by chemical ionization (NH_3) and fast atom bombardment (xenon, glycerol, relative intensities of m/z values in parentheses).

analysis). A synthetic argiotoxin (AR_{659}) containing a 4-hydroxyindolyl moiety was available for comparison of UV spectra with AG_{504} and AG_{505} (4).

Guanidinooxy Toxins. The structures of AG_{488} and AG_{504} were deduced primarily from mass spectra (e.g. Figure 2). High-field NMR of AG_{488} was consistent with the assigned structure. In particular, DEPT analysis clearly indicated a $-CH_2O-$ unit.

Discussion

The hydroxylamine α-agatoxins (Figure 3, AG_{489}, AG_{505}, and AG_{452}) instantly paralyze larval lepidopteran insects when injected (e.g. ED_{50} = ca. 60 μg/g for continuous paralysis of 3rd stadium M. sexta lasting 1 hr). Flaccid paralysis by these toxins immobilizes prey long enough for peptide μ-agatoxins to exert a lethal effect. The guanidinooxy toxins are less paralytic in our injection assay using larval M. sexta (10). Four synthetic analogs of AG_{452} were also bioassayed for structure-activity relationships. Paralytic activity decreases when the polyamine hydroxyl is removed or moved to the 1- or 22-aza positions. Shortening the polyamine chain by even one terminal aminopropyl group reduces bioactivity as noted for philanthotoxins from the solitary wasp, Philanthus triangulum (11). The minimal requirements for paralytic action are a polyamine at least the size of spermine which is acylated with an aromatic acid (cf 7,12).

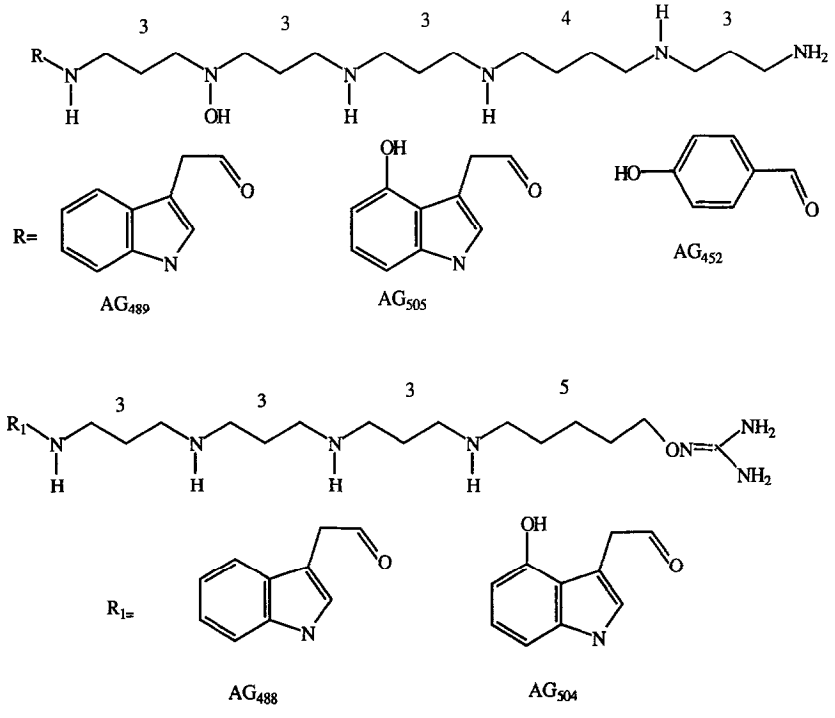

Figure 3. Structures of acylpolyamines in venom from Agelenopsis aperta.

The assigned structures of the α-agatoxins are based on spectral analysis. The complementary fragmentation patterns from mass spectral analysis by CI (NH_3) and FAB were particularly useful in defining the positions of nitrogens in the polyamine chain (e.g. Figure 2). The most difficult structural feature was designating the site of an additional labile oxygen on the linear polyamine of AG_{489}, AG_{505}, and AG_{452}. Since the oxygen could reside on any of six nitrogens, synthesis of all possible structures was a formidable task. NMR spectra of AG_{489} at 500 MHz were insufficient to assign the hydroxylation site unambiguously although it could be determined that hydroxylation did not occur at the 1- and 22-aza positions. Ultimately, MS/MS of AG_{489} gave the strongest case for a hydroxyl at the 5-aza position.

Spider venom is a good source of novel polyamines. In the case of A. aperta, the acylpolyamines reported here represent about 80% of the total polyamine component. Shorter polyamines such as spermine, cadaverine, and putrescine are not prevalent in this venom. The hydroxylamine functionality of AG_{489}, AG_{505}, and AG_{452} is highly unusual for natural products and its contribution to the mode of paralytic action for these α-agatoxins is intriguing, but awaits further study. These same toxins contain 1,5,9,13,18,22-hexaazadocosane (a 3,3,3,4,3-polyamine). This hexamine is the longest natural polyamine chain we are aware of and the next longest is homocaldopentamine, a 3,3,3,4-polyamine from bacteria. The guanidinooxy moiety of AG_{488} and AG_{504} is novel for natural polyamines derived from animals although this functional group occurs in nonprotein amino acids such as canavanine from leguminous plants.

Polyamines are ubiquitous intracellular organic cations with important roles in regulating numerous aspects of metabolism, including DNA synthesis, cell differentiation, and proliferation. Polyamine derivatives have broad biological activities such as chelating of iron (siderophores), cytotoxicity, and antibiotic effects (13). Urinalysis of polyamines has been utilized as a diagnostic test for malignant tumors and for chemotherapy evaluation (14). The recent identification of numerous acylpolyamines in arthropod venoms portends a general role for polyamines as paralytic toxins. This report suggests that spider venom is a reservoir of novel polyamine structures.

Acknowledgments. We thank J. P. Li and R. L. Carney for synthetic support, D. A. Schooley for encouragement, and M. E. Adams for early studies.

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