Alloviroidin, the Naturally Occurring Toxic Isomer of the Cyclopeptide Viroidin†

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Received November 5, 1985; Revised Manuscript Received January 8, 1986

ABSTRACT: A novel toxic cyclopeptide from *Amanita suballiacea* (Murr.) mushrooms that possesses structural features similar to viroidin is described. This peptide, alloviroidin, is identical with viroidin in mass, affinity for actin, and all amino acids except for one. The single discernible difference between the two peptides exists in the configuration at carbon 4 of the 4,5-dihydroxyleucine residues, as shown by a combination of chemical modification and magnetic resonance experiments. The configuration of this residue in viroidin is similar to that of phalloidin and is 2*S*,4*R*, while that in alloviroidin is established to be 2*S*,4*S*. This peptide is thus unique in its hydroxylation pattern among both the virotoxins and phallotoxins and may be an intermediate for more highly hydroxylated virotoxins, such as viroisin.

Toxic mushrooms of the genus *Amanita* produce at least three classes of cyclopeptide toxins, the amatoxins, phallotoxins, and virotoxins (Wieland, 1983). The amatoxins are known to specifically inhibit eukaryotic DNA-dependent RNA polymerase II, causing cessation of transcription and, hence, death of target cells. The phallotoxins have high affinity for actin, and their ability to perturb the equilibrium between the F and G forms is considered to be responsible for their toxic effects [for review, see Wieland (1983)]. The distribution of these toxins in a variety of mushrooms has been previously described (Wieland, 1983; Preston et al., 1982; Mullersman & Preston, 1982; Little & Preston, 1984).

The virotoxins, initially discovered in *Amanita virosa*, are monocyclic peptides resembling the bicyclic phallotoxins in both general amino acid sequence and affinity for actin (Faulstich et al., 1980). However, the virotoxins are chemically different in that they possess 2,3-*trans*-3,4-dihydroxy-L-proline, D-serine, and 2′-(methylsulfonyl)-L-tryptophan in lieu of *cis*-4-hydroxy-L-proline, L-cysteine, and L-tryptophan linked to L-cysteine through a sulfide bridge, respectively, in the phallotoxins. Both classes of toxins also contain hydroxylated (2*S*)-leucine as either the 4,5-dihydroxy- or 4,5,5′-trihydroxy-substituted amino acid. One of the phallotoxins, i.e., phalloin, contains 4-hydroxyleucine (Wieland, 1983). The chirality of the dihydroxylated leucines in both series, for the virotoxin VD[1] and the phallotoxin PD, is 2*S*,4*R* (Wieland, 1983; Faulstich et al., 1980).

We have previously described (Little & Preston, 1984) the discovery of a novel virotoxin from *Amanita suballiacea* and now report on its structure and biological properties. This new toxin, designated alloVD, is shown to be an isomer of VD and contains (2*S*,4*S*)-4,5-dihydroxyleucine, a configuration unique to both series of toxins. The possible role of this modified leucine in the hydroxylation of leucines in these peptides is discussed.

Materials and Methods

Materials. Water used for these experiments was deionized and glass-distilled, and that for HPLC was filtered (0.2-μm GVWP, Millipore). All solvents were HPLC-grade unless stated otherwise. DNase I was purchased from Sigma. Phalloidin was prepared as described by Little and Preston (1984).

Virotoxin Preparation and Modification. Virotoxins were purified from extracts of *A. suballiacea* as described (Little & Preston, 1984) and quantitified by assuming a molar absorptivity of 15 500 M^{-1} cm^{-1} at 278 nm as for 2-skatyl ethyl sulfone (Faulstich et al., 1968). Typical preparations had absorbance ratios at 278 nm/245 nm of 6.8 or greater. The identities of VS and VD were established with standards graciously provided by H. Faulstich.

Modifications using sodium periodate and sodium borohydride were as follows. An aqueous solution of 4 mM toxin was incubated at room temperature for 20 min with 1 equiv of $NaIO_4$. Ten equivalents of ethylene glycol was then added, and the products were resolved by HPLC. The α-aminolevulinic acid residue in the peptides was reduced to γ-hydroxynorvaline with sodium borohydride for 90 min at 0 °C with 5 equiv of sodium borohydride/equiv of toxin (1 mM aqueous). Products of this reduction were resolved as above for oxidation products derived from $NaIO_4$.

Oxidation of VS to [Asp[7]]-VS was as above except that 2 equiv of $NaIO_4$ was used. Resolution of the desired product was achieved by chromatography on Sephadex LH-20 in methanol (0.9 × 45 cm column).

Chromatography. Chromatography on TLC plates (Merck, SG60 with F254 indicator) was carried out in 2-butanol–ethyl

† This work was supported by IFAS Experiment Station CRIS Project FLA-MCS-02183-BI and LET Project 8063 for Plant Genetic Improvement and represents Journal Series No. 6835 of the University of Florida Institute of Food and Agricultural Sciences Experiment Station.

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[1] Abbreviations: VS, viroisin; VD, viroidin; alloVD, alloviroidin; TLC, thin-layer chromatography; HPLC, high-performance liquid chromatography; PD, phalloidin; Me_4Si, tetramethylsilane; NMR, nuclear magnetic resonance; FABMS, fast atom bombardment mass spectrometry; FT, Fourier transform.

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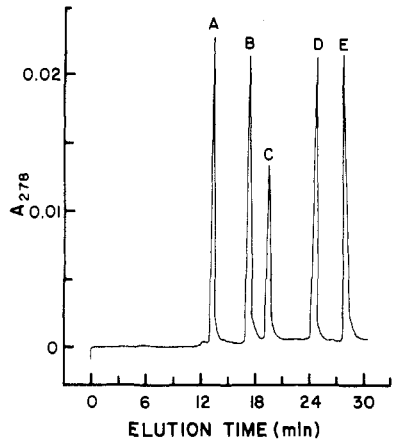

FIGURE 1: Separation of native and modified virotoxins was achieved by reverse-phase HPLC on a Du Pont Zorbax column (9.4 × 25 cm) with 19% CH_3CN. Absorbance at 278 nm of the eluate was continuously monitored with a Gilson Holochrome (1-cm flow cell). Peaks A–C are VS, alloVD, and VD, respectively. Peak D represents the derivative formed upon treating either alloVD or VD with $NaIO_4$. Peak E is the product of treating peak D with $NaBH_4$.

acetate–water, 14:12:5, with visualization by fluorescence attenuation. Reverse-phase HPLC was performed as described (Little & Preston, 1984).

Amino Acid Analysis. Pure peptides were hydrolyzed as described (Faulstich et al., 1980) and resolved into component amino acids on a JEOLCO 6AH dual-column amino acid analyzer. Detection utilized postcolumn derivatization with ninhydrin.

Spectroscopy. Proton NMR spectra were acquired at 25 °C on a Nicolet NT-300 NMR spectrometer operating at 300 MHz in the FT mode, with a 7-μs pulse and 6-s delay between pulses. Water was removed from dried peptides by several additions of absolute ethanol, which were then removed in vacuo. Peptides were then dissolved in 100% dimethyl-d_6 sulfoxide (Aldrich) with 1% Me_4Si at concentrations of approximately 0.05 M.

Carbon-13 spectra were acquired on an NT-300 operating at 75.46 MHz for ^{13}C with the INEPT pulse sequence of Morris and Freeman (1979).

Fast atom bombardment mass spectra (FABMS) were acquired on a Kratos MS-50 mass spectrometer fitted with a Kratos DS-55 data system. Spectra were scanned at 30 s/decade with a resolution of 2000. A neutral 6-kV xenon beam provided ionization.

Ultraviolet spectra were recorded in water on a Beckman 25 dual-beam spectrophotometer with a 1-cm cuvette.

Interaction of Native and Modified Virotoxins with F-Actin. Native virotoxins and those derived by modification with $NaIO_4$ and $NaBH_4$ treatments were assessed for their interaction with F-actin by the assay of Mullersman and Preston (1982). Final concentrations of F-actin and DNase I were 0.5 and 1.6 μg/mL, respectively, and those of virotoxins typically ranged from 1.3 to 13 nM. Rabbit muscle actin was purified by the method of Pardee and Spudich (1982).

RESULTS

Resolution and Amino Acid Analyses. The resolution of native and modified virotoxins by HPLC is shown in Figure 1. When either alloVD (peak B) or VD (peak C) is treated with a molar equivalent of $NaIO_4$, a single peak (peak D) is observed, the latter of which is itself converted to only one peak (peak E) by reduction with $NaBH_4$. Samples derived from peaks A–E, when subjected to TLC analysis as described under Materials and Methods, showed single components (detected

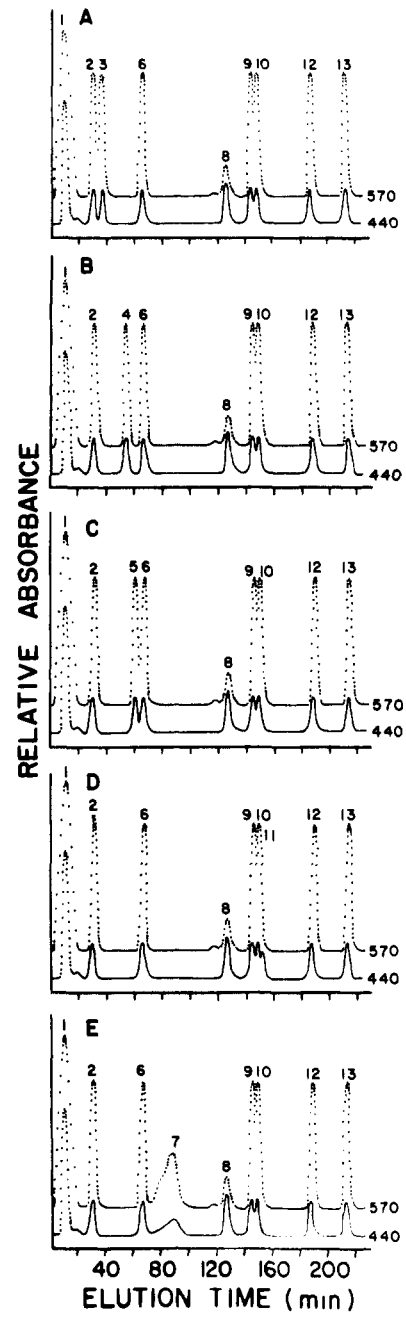

FIGURE 2: Amino acid profiles for peaks A–E of Figure 1 are given in panels A–E, respectively. Hydrolyzed peptides were resolved into amino acids as described in the text. The identities of peaks 1–13 are as follows: (1) acidic and neutral amino acids, (2) basic degradation product of 2′-(methylsulfonyl)tryptophan, (3) trihydroxyleucine aminolactone, (4) (2*S*,4*S*)-dihydroxyleucine aminolactone, (5) (2*S*,4*R*)-dihydroxyleucine aminolactone, (6) NH_4^+, (7) γ-hydroxynorvaline aminolactone, (8) dihydroxyproline, (9) serine, (10) threonine, (11) α-aminolevulinic acid, (12) alanine, and (13) valine. Assignments were made with authentic VS, VD, PD, demethylphalloin, and dihydroxyproline standards.

by quenching of the F254 fluor) with R_f values of 0.64, 0.70, 0.70, 0.71, and 0.72, respectively. The oxidation of VS (peak A) with 2 molar equiv of $NaIO_4$ led to a single component with an R_f of 0.38 in this system. The amino acid analysis of this latter derivative indicated it to be [Asp^7]-VS, and the lower mobility observed upon the TLC analysis was consistent with the presence of an ionizable carboxyl group. The similarity in migration of native and modified alloVD and VD in this system underscores the need for and the resolution achieved by HPLC fractionation.

Figure 2 presents the chromatographic separation of the

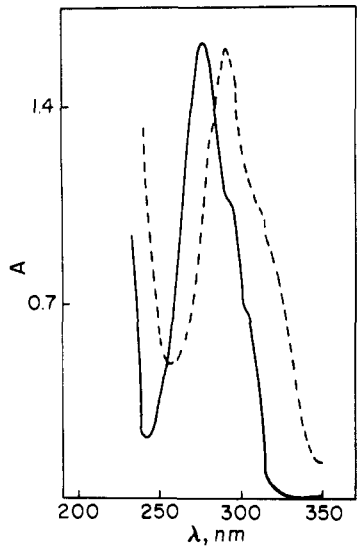

FIGURE 3: Ultraviolet spectra in water for alloVD (104 μM) were recorded at pH 7 (solid line) or, after the addition of dilute NaOH, at pH 11 (dashed line).

Table I: Summary of Measured Masses Determined by FABMS

compd	mass calcd	mass exptl
viroisin	912.35	912.43
alloviroidin	896.35	896.52
viroidin	896.35	896.56

amino acids derived upon acid hydrolysis of the peptides in Figure 1. An inspection of these profiles shows that the treatment with $NaIO_4$ alters only a basic component (the dihydroxyleucine aminolactones) in alloVD (panels B and D) and VD (panels C and D). The subsequent reduction of the compound in peak D (Figure 1) to yield that in peak E (Figure 1) converts the α-aminolevulinic acid moiety to γ-hydroxynorvaline (panels 2D and 2E). These experiments prove that 4,5-dihydroxyleucine is a component of alloVD since the periodate oxidation yields the expected methyl ketone (and 1 mol equiv of CH_2O, data not shown); further, since a single peak (D in Figure 1) results from peaks B and C (Figure 1) by this treatment, which would eliminate chirality at carbon 4 of the 4,5-dihydroxyleucine, alloVD and VD must be isomers differing only in configuration at this carbon. Thus, the possibility of alloVD being an isomer of 2*R* configuration is unlikely. As expected, the elution positions of the (2*S*,4*S*)- or (2*S*,4*R*)-dihydroxyleucine aminolactones from panels B and C of Figure 2 correlate well to those reported by Wieland et al. (1968) for synthetic dihydroxyleucines of these configurations.

Ultraviolet, NMR, and Mass Spectroscopy. The neutral and alkaline UV spectra of alloVD in Figure 3 confirm the presence of a 2′-(methylsulfonyl)tryptophan nucleus for this isomer (Faulstich et al., 1980). These spectra possess the strong inflections at 293 and 305 nm typical of a sulfone (Faulstich & Wieland, 1968). Since alloVD was not observed to undergo β-elimination upon exposure to alkaline conditions and because the positions of a methyl group in the ^1H NMR (3.34 ppm) and ^{13}C NMR (44.89 ppm) spectra correspond to those seen in VS and VD, alloVD must also possess the 2′-(methylsulfonyl)tryptophan nucleus.

The calculated and experimental masses obtained from the low-resolution FABMS pseudomolecular ions, M + H^+, for VS, alloVD, and VD are compiled in Table I. The calculated masses for VS and VD are in good agreement with the corresponding experimental values. The results of the amino acid analyses above and the essentially identical masses observed for VD and alloVD limit the number of possible structures that might be ascribed to alloVD, exclude the possibility of acid-labile adducts (i.e., acetyl esters) of alloVD, and thus support its proposed structure (see Figure 6). In addition, the absence of similar acid-labile adducts for VS and VD is indicated.

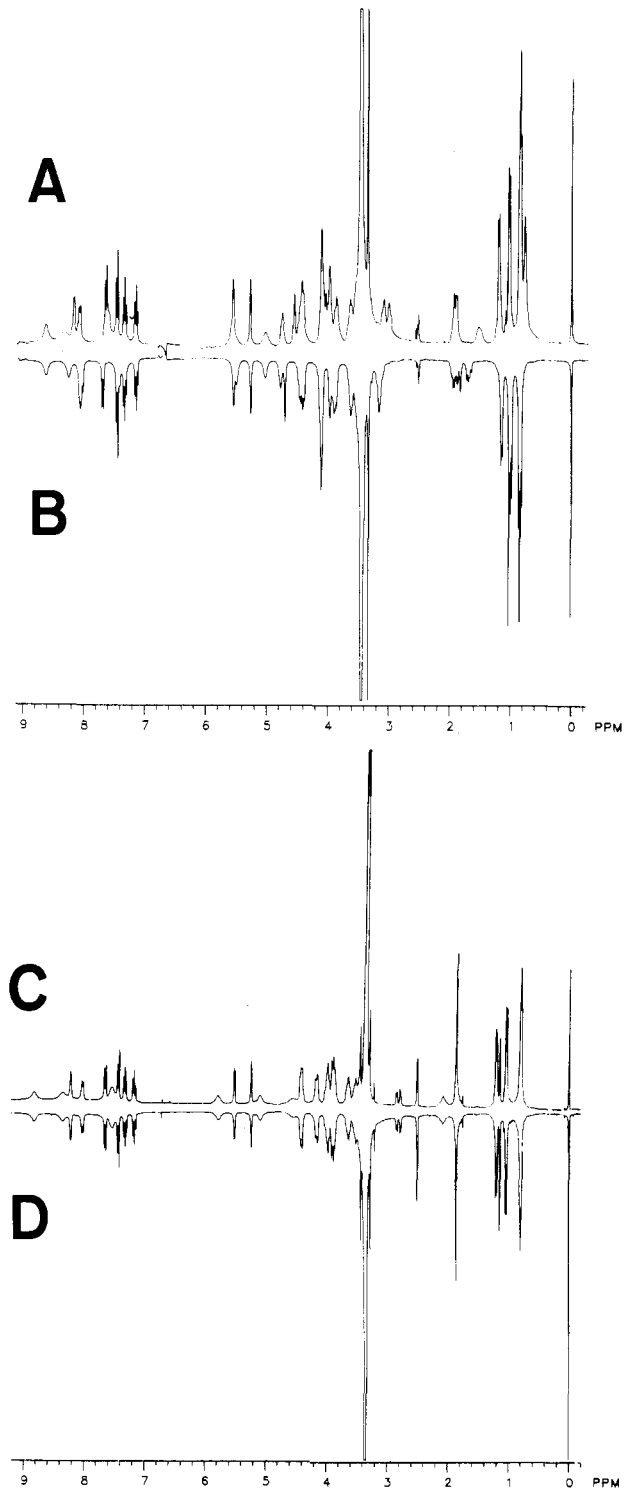

FIGURE 4: Proton NMR spectra of alloVD (A) and VD (B, inverted plot) are presented in comparison with $NaIO_4$-treated alloVD (C) and $NaIO_4$-treated VD (D, inverted plot). Spectra were acquired on 50 mM samples (alloVD and VD, 40 acquisitions) or 3 mM samples ($NaIO_4$-treated toxin, 400 acquisitions). Shifts in ppm are relative to the Me_4Si standard. The singlet at 1.146 ppm (C and D) is an artifact with variable appearance. The indolyl NH resonances at 12 ppm are not shown.

Table II: Carbon-13 Chemical Shifts for Carbons 4 and 5 of 4,5-Dihydroxyleucines in VS, VD, and AlloVD

carbon	chemical shift (ppm) VS	VD	alloVD
4	73.47	71.56	71.31
5	64.86	68.93	69.31
5′	64.26	24.27	23.50

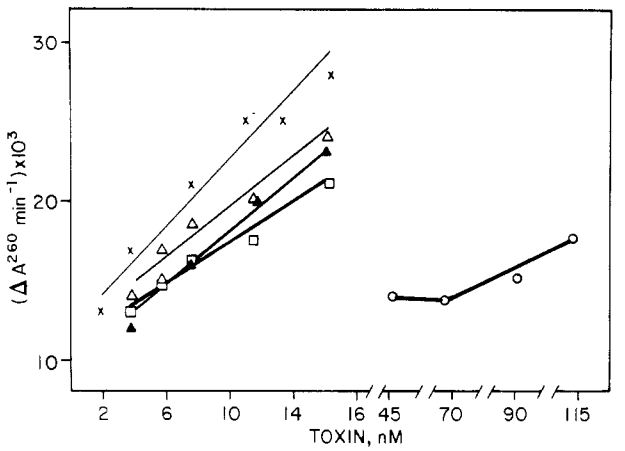

FIGURE 5: Interaction of virotoxins with actin was compared to PD as described in the text. Identities of toxins are PD (×), VS (squares), VD (open triangles), alloVD (closed triangles), and [Asp^7]-VS (open circles).

An examination of the ^{1}H NMR spectra of VD and alloVD in Figure 4 reveals several interesting features. Viroidin (Figure 4B) is seen to possess a CH_3 group from the dihydroxyleucine at 1.025 ppm that is nearly identical in shift with that in PD (1.030 ppm, not shown) but different from alloVD (0.77 ppm, Figure 4A). On the basis of ^{13}C NMR studies, the carbon of this methyl group in alloVD (Table II) is also shielded (likely by the leucine carbonyl) relative to that in VD by 0.77 ppm. These data support the postulation of similar C-4 configurations for VD and PD and a different configuration for alloVD. In these same proton spectra, other differences in chemical shift are noticeable, including the dihydroxyleucine C-3 protons (1.4–1.9 ppm), the alanine and threonine NH resonances (7.95–8.25 ppm), and other regions as well. In contrast, the spectra for the $NaIO_4$-treated alloVD and VD (Figure 4C,D) show none of the aforementioned differences and appear identical in line shape and chemical shift. Thus, we conclude that since the elimination of a single chiral center has rendered the spectra of Figure 4C,D indistinguishable and since no amino acid sequence differences between the two peptides were found, VD and alloVD must be isomeric in the configuration about carbon 4 of their respective 4,5-dihydroxyleucines.

Biological Properties of AlloVD. To address whether this difference in configuration might lend a noticeable difference in actin binding, we compared the interactions of the virotoxins and PD. Figure 5 shows that native virotoxins, including alloVD, have high affinity for actin, as measured by this assay, and appear nearly as effective as PD in releasing DNase I from inhibition by G-actin. This appears consonant with data from Turcotte et al. (1984) and Faulstich et al. (1980), who examined the interaction of virotoxins other than alloVD with actin by other methods. Interestingly, modification of VS to [Asp^7]-VS reduces the apparent interaction by at least 10-fold, while VD treated with $NaIO_4$ alone, or subsequently with $NaBH_4$, yields a curve that is similar to the unmodified compound (not shown).

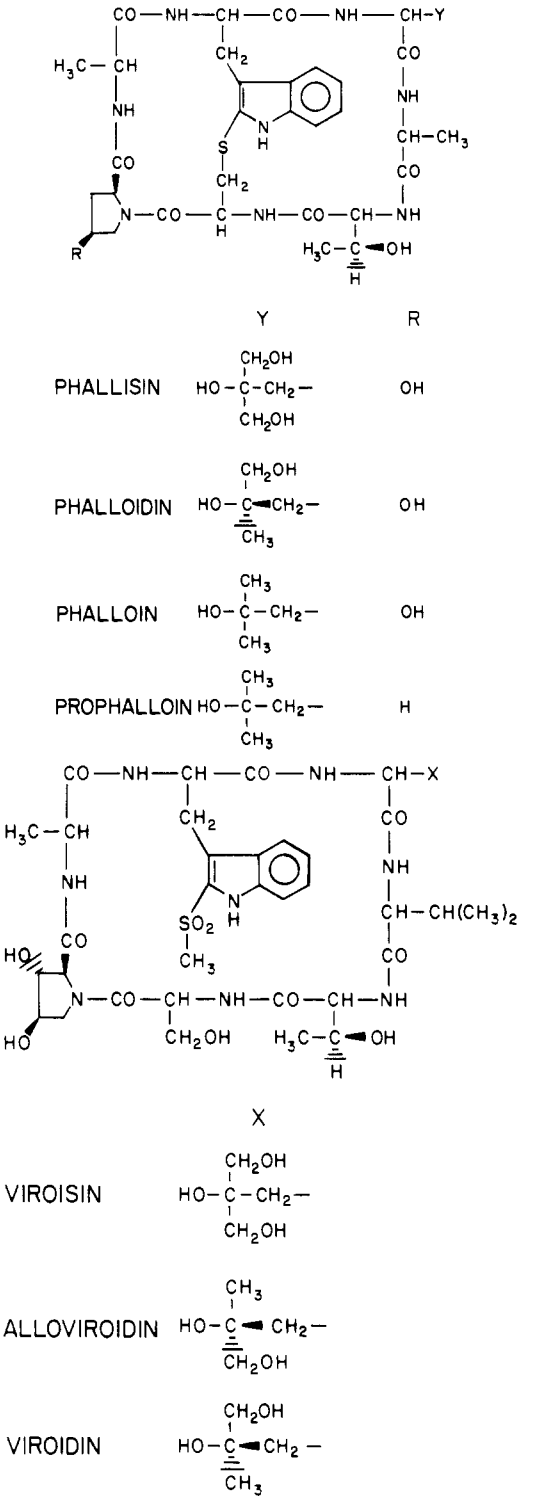

FIGURE 6: Structural comparisons of select phallotoxins and virotoxins.

The actin-binding properties of alloVD were reflected in vivo. Doses injected ip of 1–2 nmol/g alloVD or 3–4 nmol/g PD resulted in 50% mortality in white mice in 3–6 h. An examination at postmortem suggested toxicity typical of phallotoxin poisoning, i.e., an engorgement of the livers with blood (Wieland, 1983). This further supports a 2*S* configuration for the leucine in alloVD since phallotoxins of a 2*R* configuration are nontoxic up to 64 nmol/g (Munekata et al., 1979).

Discussion

Structure and Conformation. The structures of alloVD, VD, and VS are compared to relevant members of the phallotoxin series in Figure 6. To date, neither a phallotoxin

analogue of alloVD, i.e., PD containing a (2*S*,4*S*)-4,5-dihydroxyleucine, nor a phalloin or prophalloin analogue in the virotoxin series is known. Moreover, although dihydroxyleucine-containing phallotoxins of 2*R* configuration may be synthesized by recyclization of PD (Munekata et al., 1979) and such nontoxic derivatives have an affinity for actin approximately 150-fold lower than PD, the 2*R* configuration in nature for phallotoxins or virotoxins has not been reported. It is possible that only the 2*S* configuration is conserved in natural derivatives of both series and that derivatives possessing fewer hydroxy groups in the leucine residues, i.e., phalloin and an analogous pre-VD molecule, may serve as precursors for molecules of greater hydroxylation, such as phallisin and VS, respectively. If this is true and if phallotoxin hydroxylation occurs independent of virotoxin hydroxylation, then hydroxylating enzymes that convert phalloin to PD may have prochiral specificity in choosing the 5- or 5′-carbon. In contrast, since alloVD (4*S*) appears in the virotoxin series along with VD (4*R*), it is possible that hydroxylating enzymes in this series, which might use a pre-VD substrate, lack stringent prochiral specificity. Of course, if a phallotoxin analogue of alloVD is ever discovered, it would imply that enzymes mediating these hydroxylations in both series of toxins lack prochiral specificity. The test of this hypothesis and others awaits the development of culture conditions for *Amanita* that permit syntheses of these molecules.

The data above also provide some information on structure–conformation relationships for the virotoxins. Although virotoxins may be considered as analogues of monocyclic derivatives of phallotoxins, the latter, derived by Raney nickel reduction of phallotoxins, are nontoxic even when the resulting dethiophallotoxin is appended with the corresponding methylsulfonyl group (Wieland, 1983). Other studies have stressed the importance of the additional 3′-OH of dihydroxyproline and the D-serine residues in assisting the binding of virotoxins to actin (Kahl et al., 1984). Thus, while the aromatic group in PD is known to shield the alanine residue adjacent to hydroxyproline and yield an upfield methyl group (0.78 ppm; Patel et al., 1973) in the ^{1}H NMR spectrum, the corresponding methyl group of alanine in VD and alloVD is not shielded and appears at 1.18–1.20 ppm. Therefore, it seems likely, given the free rotations possible about the indole carbon 2 and, by inference, the lack of a rigid solution conformation resembling PD, that the virotoxins may indeed bind to actin by induced fit as postulated (Faulstich et al., 1980), perhaps assisted by the hydrogen-bonding potential of the serine and dihydroxyproline hydroxy groups. A comparative examination of models of PD and VS shows this to be possible. However, extensive decoupling experiments (data not shown) have not firmly provided us with a conformation of the virotoxins in solution; perhaps such a conformation may be elucidated by a combination of X-ray diffraction analysis and 2-D proton NOE experiments.

The [Asp^7]-VS, derived from VS by $NaIO_4$ treatment, proved to be a useful derivative in several regards. First, the assignment of the ^{13}C chemical shifts in Table II was greatly facilitated by the loss of select carbons. Second, this derivative showed a lesser affinity for actin, which may be related to the negative charge existing at physiological pH in the aspartyl residue; such an analogue in the phallotoxin series has apparently not been similarly examined (Wieland, 1983). Third, since the ^{1}H NMR and CD spectra of [Asp^7]-VS show no overt differences compared to those of the other virotoxins, this derivative is likely to have similar allowed conformations and because it readily crystallized from aqueous MeOH, may be an ideal candidate for X-ray analysis. Lastly, a conjugable carboxyl group in [Asp^7]-VS may permit the construction of appropriate immunogens for antibody production or of affinity matrices.

Chemotaxonomy and Other Peptides. Although virotoxins were initially discovered in and presumed unique to *A. virosa* (Faulstich et al., 1980), we have shown that these compounds exist in *A. subalbiacea* as well (Little & Preston, 1984). Of the virotoxins (1.3 mg/g of dry weight tissue) in *A. subalbiacea*, VS represents approximately 75% of the total, alloVD and VD each represents 15 and 9–10%, respectively, and [Ala^1]-VD can be found in levels of less than 1%. Importantly, there do not appear to be any virotoxins containing a sulfoxyindole chromophore in this species, even though such do exist in *A. virosa* (Faulstich et al., 1980). This difference in addition to the accumulation of acidic and neutral toxins (Little & Preston, 1984) serves to distinguish *A. subalbiacea*.

We have discovered an interesting family of cyclic peptides in a species of mushroom tentatively identified as *Amanita pseudoverna* (Murr.) that deserve mention due to their similarity to phallotoxins and virotoxins (M. Little, T. Romeo, and J. F. Preston, unpublished results). The neutral member of this group elutes between the positions of VS and α-amanitin on a Sephadex LH20 (water) column and the two acidic species elute with β-amanitin. Following acid hydrolysis, (2*S*,4*R*)-4,5-dihydroxyleucine, 3,4-dihydroxyproline, threonine (or β-hydroxyaspartic acid in the acidic species), cysteic acid, and 2 equiv of alanine were detected. The chromophore in these species has a λ_{max} of 284 nm, with no inflections at a greater wavelength, is reactive with diazotized sulfanilic acid and hence phenolic, and is rapidly lost upon alkaline treatment, suggesting a β-elimination reaction. These compounds react immediately with cinnamaldehyde and HCl to yield deep purple spots on a chromatogram. Thus, a structure suggesting a bicyclic-modified virotoxin or phallotoxin is easily proposed. Although a sulfoxide or sulfone is unlikely, the oxidation state of the sulfur remains to be ascertained; it is possible that these molecules may be virotoxin precursors and hence possess a methylated sulfur atom as postulated by Faulstich et al. (1980). Finally, it is interesting to note that *A. pseudoverna* accumulates acidic and neutral amatoxins and viroisin but no phallotoxins, further implicating this family as possible intermediates in the proposed pathway of virotoxin biosynthesis (Faulstich et al., 1980).

ACKNOWLEDGMENTS

We are grateful to H. Faulstich and J.-U. Kahl for the generous gifts of authentic VS and VD and of dihydroxyproline, respectively. We thank J. E. Gander, under whose direction the proton NMR spectra were acquired in the IFAS NMR Facility. We thank S. Hurst and B. Parten for amino acid analyses and Edman degradation, W. S. Brey for ^{13}C spectra, and J. E. Mullersman for inspiring discussions. We thank D. Huseman for preparing all figures and Barbara Cannon for expertly typing the manuscript.

Registry No. Viroidin, 53568-33-5; alloviroidin, 101470-22-8; phalloidin, 17466-45-4; viroisin, 74113-57-8.

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Ion-Conducting Channels Produced by Botulinum Toxin in Planar Lipid Membranes

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Received June 28, 1985; Revised Manuscript Received January 13, 1986

ABSTRACT: The interaction of botulinum neurotoxin (Botx) with planar lipid membranes was studied by measuring the ability of the toxin to form ion-conducting channels. Channel formation was pH dependent. At physiological pH, Botx formed no channels, whereas at pH 6.6, the toxin formed channels with a unit conductance of 12 pS in 0.1 M NaCl. The rate of channel formation increased with decreasing pH, reaching a maximum at pH 6.1, and then decreased at lower values of pH. The channels, once formed, were permanent entities in the membrane throughout the course of an experiment and fluctuated between an open and a closed state. The rate of channel formation depended upon the square of the toxin concentration, suggesting an aggregation step is involved in channel formation. The data were consistent with the hypothesis that Botx enters cells through endocytosis, followed by its release into the cytoplasm at low pH.

Botulinum toxin (Botx), an exotoxin produced by the bacterium *Clostridium botulinum*, is the etiological agent in botulism poisoning [for comprehensive reviews, see Simpson (1981) or Sugiyama (1980)]. Although there are seven distinct serotypes (designated A–G), they have the same general molecular properties. The neurotoxins are single-chain proteins with a molecular weight around 150 000. In most cases, the toxins produced in culture are purified in the "nicked" form, having been cleaved by protease(s) to constitute chains of M_r 50 000 (light chain) and M_r 100 000 (heavy chain). The chains are held together by a disulfide bond and noncovalent interactions, and there is evidence to suggest that nicking is necessary for the full expression of toxicity. While the nature of the receptor for Botx has not been defined, it may consist in part of a ganglioside.

It has been known for some time that the primary action of botulinum toxin is to inhibit the release of acetylcholine from the presynaptic membrane of the neuromuscular junction (Burgen et al., 1949). Unfortunately, little else is known about the molecular events that lead to intoxication. In the last few years, indirect evidence has suggested that the attack of Botx proceeds by several steps (Simpson, 1981). The first step is binding of the toxin to a receptor on the plasma membrane outer surface (Kitamura et al., 1980; Dolly et al., 1984). The second step seems to involve entry of the toxin into the cell, where it inhibits the release of acetylcholine through an unknown third step. The entry mechanism is as yet unclear, but an intriguing possibility was recently raised by Simpson (1984) when he showed that lysosomotropic amines, such as chloroquine, can protect the nerve from attack by Botx. This protection is similar to the protective action of amines from attack by other bacterial toxins, such as diphtheria toxin (Dorland et al., 1981; Leppla et al., 1980) and *Pseudomonas* A exotoxin (Fitzgerald et al., 1980). The similarity led Simpson to suggest that the mechanisms of entry of the toxins might be the same, i.e., that botulinum toxin may enter cells by a mechanism analogous to diphtheria toxin.

Diphtheria toxin enters mammalian cells by the process of receptor-mediated endocytosis (Middlebrook et al., 1978; Dorland et al., 1979). This involves the endocytosis of the receptor-bound toxin, followed by an escape from the endocytic compartment triggered by the low intravesicular pH. This escape, which arises from transport of diphtheria toxin across the membrane, occurs simultaneously with insertion of the toxin into the membrane and formation of an ion-conducting pore (Donovan et al., 1981, 1982; Zalman & Wisnieski, 1984). The actual transport of the toxic fragment of the molecule has been suggested to be through that pore (Boquet et al., 1976; Kagan et al., 1981). Were Botx to enter neurons by a similar mechanism, it would also be expected to form ion-conducting pores under conditions which prevail inside endocytic vesicles.

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0006-2960/86/0425-2872$01.50/0