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The Amino Acid Sequence of Viscotoxin A2 from the European Mistletoe (*Viscum album* L., Loranthaceae)

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The amino acid sequences of peptides obtained by tryptic and chymotryptic digestion of Viscotoxin A2 permit deduction of the following amino acid sequence for this protein:

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                  |   |
Lys-Ser-Cys-Cys-Pro-Asn-Thr-Thr-Gly-Arg-Asn-Ile-Tyr-Asn-Thr-
 1   2   3   4   5   6   7   8   9  10  11  12  13  14  15

|                                                   |
Cys-Arg-Phe-Gly-Gly-Gly-Ser-Arg-Glu-Val-Cys-Ala-Ser-Leu-Ser-Gly-
16  17  18  19  20  21  22  23  24  25  26  27  28  29  30  31

|                                   |
Cys-Lys-Ile-Ile-Ser-Ala-Ser-Thr-Cys-Pro-Ser-Tyr-Pro-Asp-Lys
32  33  34  35  36  37  38  39  40  41  42  43  44  45  46
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Viscotoxin is a mixture of pharmacologically active, small, basic proteins isolated from the European mistletoe, *Viscum album* L.[1,2] One component of this mixture is Viscotoxin A2, which can be isolated from Viscotoxin by chromatographic methods as previously described.[3,4] Viscotoxin A2 is composed of 46 amino acid residues and has a molecular weight of 4833.[4] This paper describes the determination of the amino acid sequence of Viscotoxin A2.

EXPERIMENTAL

Materials and apparatus

Viscotoxin A2 was obtained either as the oxidized product Viscotoxin A*ox2* by chromatography of oxidized Viscotoxin on cellulose phosphate and DEAE-cellulose,[3] or as the native material by chromatography of Viscotoxin on SE-Sephadex.[4]

Enzymes. Trypsin, chymotrypsin, leucine aminopeptidase, carboxypeptidases A and B. The sources and treatments of these enzymes have been described previously.[5]

Dansyl chloride (1-dimethylamino-naphthalene-5-sulfonyl chloride) was obtained from Sigma or Pierce Chemical Co. The substance was dissolved in acetone, filtered to remove insoluble hydrolysis products, and the solution evaporated to dryness *in vacuo* in a rotary evaporator. The dry material was dissolved in acetone to a concentration of 10 mg/ml, and the solution stored in a glasstoppered bottle over silica gel in the dark.

Sephadex® G-10, G-15, and G-25, Pharmacia Fine Chemicals, Uppsala, Sweden.
SE-Sephadex® C-25, Pharmacia Fine Chemicals, Uppsala, Sweden.
pH-stat, Radiometer titrator TTT1c with titrigraph SBR2c. Radiometer, Copenhagen, Denmark.

Methods

Performic acid oxidation of Viscotoxin A2 was performed at 0°C as described by Hirs.[6]

Digestion with trypsin and chymotrypsin. 220 mg oxidized Viscotoxin A2 was digested with trypsin at pH 8.5 and 37°C for 8 h in the pH-stat, using a previously described procedure.[5] The final enzyme : protein ratio was 1 : 30 (w/w). The same technique was used for digestion of 195 mg oxidized Viscotoxin A2 with chymotrypsin at pH 8.5 and 37°C for 5 h at a final enzyme : protein ratio of 1 : 30 (w/w).

Separation of tryptic peptides. The tryptic hydrolysate was subjected to gel filtration on a column (1 × 500 cm) of Sephadex G-25, with 10 % acetic acid as eluent. The column consisted of a tube made of polyvinyl chloride, suspended in a vertical position between two floors in the laboratory. 5 ml fractions were collected, and their O.D. at 280 nm determined. Two peaks were obtained. The peptides of these peaks were separated by chromatography on Dowex 1-X2 following the procedure of Schroeder *et al.*[7] with previously described modifications.[5] The eluate from the Dowex 1-X2 chromatography was analyzed for content of peptides by thin-layer chromatography, of 60 μl aliquots from each third fraction of the eluate. Plates, 20 × 40 cm, coated with a 0.25 mm layer of Silica gel G (Merck) were used. The plates were developed with propanol : conc. ammonia : water (60 : 6 : 34), dried, and sprayed with ninhydrin reagent (0.05 g ninhydrin, 50 ml ethanol, 2 ml collidine and 15 ml acetic acid) and heated 5 min at 105°C. Fractions containing pure peptides were pooled, concentrated *in vacuo* and desalted on Sephadex G-10 or G-15 with 10 % acetic acid as eluent, lyophilized and dissolved in 3 ml 10 % acetic acid, and stored in the refrigerator until further investigated.

Separation of chymotryptic peptides. The chymotryptic hydrolysate was separated by chromatography on Dowex 1-X2 as described by Schroeder *et al.*[7] and in a previous communication.[5] The separation was followed by determination of the ninhydrin color of aliquots of the fractions after alkaline hydrolysis, as described earlier.[5]

Peptide maps. The purity of isolated peptides was checked by peptide mapping, as described previously.[5]

Preparative thin-layer chromatography was performed as previously described.[5]

Quantitative amino acid analysis. Peptides were hydrolyzed with constant boiling HCl at 110°C, in sealed evacuated tubes as described by Hirs *et. al.*[8] Following hydrolysis, the samples were immediately taken to dryness *in vacuo* in a rotary evaporator, and the amino acids determined with an automatic amino acid analyzer according to Spackman *et al.*,[9] as modified by Samuelsson.[10]

Amino acid sequence analysis. The previously used modification of the Edman degradation [5] was used also in this work, with the following changes: the benzene washing of the initially formed phenylthiocarbamate was omitted, and the excess of phenylisothiocyanate was instead removed by heating the lyophilized sample at 50°C in vacuum for 30 min.[11]

Detection of amino terminal amino acids by dansylation. Dansylation was performed on 0.01 μmol samples of peptides by the procedure of Gros and Labouesse.[12] Following hydrolysis of the samples, the liberated dansylamino acid was identified by thin layer chromatography according to Stehelin and Duranton.[13]

Digestion with carboxypeptidases and LAP. Peptides were digested with these enzymes as previously described.[5]

Partial acid hydrolysis of peptide OT4. 5 μM of the peptide was hydrolyzed with 1.0 ml 0.1 N HCl at 100°C for 22 h. The sample was taken to dryness *in vacuo*, in a rotary evaporator, dissolved in 1 ml 0.01 M NaOAc buffer of pH 4.3, and applied to a column (1 × 9 cm) of SE-Sephadex previously equilibrated with 0.01 M NaOAc buffer of pH 5.0. The column was eluted with 110 ml of the buffer used for equilibration and then with 140 ml of 0.1 M NaOAc buffer, pH 5.0. Finally the eluent was changed to 0.1 M NaOAc of pH 5.0 and containing NaCl to a concentration of 1 M. Fractions of 5 ml were collected and their O.D. at 280 nm determined. Fractions corresponding to UV-absorbing peaks were pooled, desalted on Sephadex G-10 with 10 % acetic acid as eluent, lyophilized and dissolved in 3 ml 2 % acetic acid.

RESULTS AND DISCUSSION

Separation and amino acid sequences of chymotryptic peptides

The result of chromatography of a chymotryptic hydrolysate of oxidized Viscotoxin A2 on a column of Dowex 1-X2 is illustrated in Fig. 1. Four main

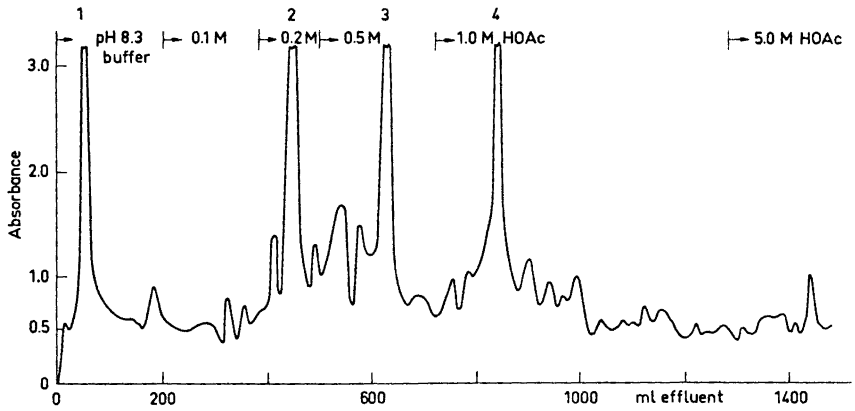

Fig. 1. Chromatography of a chymotryptic hydrolysate of 195 mg of oxidized Viscotoxin A2 on Dowex 1-X2. The composition of the liquid flowing into the mixing chamber is indicated in the upper part.

peaks were obtained, numbered 1 – 4 in the figure. Peptide mapping showed peaks 2 and 4 to represent pure peptides. They were designated as peptides *OC1* and *OC4*. Peak 1 as well as the small unnumbered peaks in the chromatogram were found to contain mixtures of peptides, and amino acid analyses showed the yields of these peptides to be small (in the range 1 – 2.5 μmol, compared to 7 – 15 μmol of the main peptides). They were therefore not investigated further.

Peak 3 contained two main peptides, which were separated by preparative thin-layer chromatography. These peptides were designated as peptides *OC2* and *OC3*.

The amino acid composition of the four chymotryptic peptides is presented in Table 1. These peptides account for the total amino acid composition of Viscotoxin A2.

Amino acid sequence of peptide OC1. The results of 10 steps of Edman degradation of this peptide are presented in Table 2. After the 10th step, the presence of free leucine was demonstrated by dansylation. These results give the following amino acid sequence for peptide *OC1*: *Gly-Gly-Gly-Ser-Arg-Glu-Val-Cys(O_3H)-Ala-Ser-Leu.* Digestion of peptide *OC2* with LAP gave no welldefined results. The identification of the -Glu- residue as glutamic acid was performed in connection with determination of the position of the disulfide bridges (to be published elsewhere). A peptide fragment was isolated, which

Table 1. Amino acid composition of chymotryptic peptides from oxidized Viscotoxin A2.

Amino acid	*OC1*	*OC2*	*OC3*	*OC4*	Sum	Oxidized Viscotoxin A2
Lysine		1.0		2.0	3.0	3
Arginine	0.8	1.0	0.9		2.7	3
Cysteic acid	0.9	2.0	1.0	1.9	5.8	6
Aspartic acid		1.9	1.0	1.0	3.9	4
Threonine		1.7	1.0	1.0	3.7	4
Serine [a]	2.1	1.0		3.7	6.8	7
Glutamic acid	1.0				1.0	1
Proline		1.0		1.8	2.8	3
Glycine	2.8	1.0		1.1	4.9	5
Alanine	1.0			0.9	1.9	2
Valine	0.9				0.9	1
Isoleucine		0.7		1.9[b]	2.6	3
Leucine	1.1				1.1	1
Tyrosine		0.4		0.7	1.1	2
Phenylalanine			1.1		1.1	1
Yield, μmol from about 32 μmol Viscotoxin A2	6.7	15	15	15		

[a] Corrected for 10 % loss during hydrolysis.
[b] After hydrolysis for 72 h.

contained this residue as *N*-terminal amino acid. The fragment was obtained by digestion of native Viscotoxin A2 with pepsin, trypsin, and chymotrypsin.

Amino acid sequence of peptide OC2. This peptide was taken through 10 steps of Edman degradation, the results of which are presented in Table 3. Assuming normal chymotryptic specificity the following partial amino acid

Table 2. Edman degradation of peptide *OC1*.

Amino acid	Residues after step No.										
	0	1	2	3	4	5	6	7	8	9	10
Arginine	0.8	×	1.0	×	1.0	*0.7*	×	0.6	×	0.6	×
Cysteic acid	0.9	1.0	1.0	1.1	1.0	0.9	0.9	1.0	*0.7*	0.8	0.8
Serine	2.1	1.8	1.8	1.8	*1.6*	1.6	1.5	1.4	*a*	1.4	*1.0*
Glutamic acid	1.0	0.9	0.9	1.0	1.0	1.1	*0.7*	0.7	*a*	0.7	0.7
Glycine	2.8	*2.1*	*1.5*	*1.0*	1.0	1.1	1.0	1.0	1.1	1.1	1.0
Alanine	1.0	1.0	1.0	1.0	1.0	0.9	0.9	1.0	1.0	*0.7*	0.7
Valine	0.9	1.0	1.0	1.0	1.0	0.9	1.1	*0.7*	0.7	0.7	0.6
Leucine	1.1	0.9	1.0	1.0	1.0	1.0	1.1	0.9	1.0	0.9	0.8

× Not determined.
[a] Not determined due to technical error.

Table 3. Edman degradation of peptide *OC2*.

Amino acid	Residues after step No.										
	0	1	2	3	4	5	6	7	8	9	10
Lysine	1.0	*0*	0	0	0	0	×	×	×	×	×
Arginine	1.0	1.0	1.0	1.0	1.0	1.0	×	×	×	×	*0.7*
Cysteic acid	2.0	2.1	2.1	*1.8*	*1.1*	0.9	0.8	0.8	0.8	0.8	0.8
Aspartic acid	1.9	1.8	1.9	1.9	1.9	1.9	*1.5*	1.4	1.3	1.3	1.1
Threonine	1.7	1.7	1.7	1.7	1.8	1.8	1.7	*1.3*	*0.9*	0.9	0.8
Serine	1.0	1.1	*0.8*	0.6	0.5	0.4	0.5	0.3	0.3	0.3	0.3
Proline	1.0	1.0	1.0	1.0	0.9	*0.7*	0.5	0.4	0.4	0.4	0.4
Glycine	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.9	0.9	*0.7*	0.6
Isoleucine	0.7	0.7	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.7	0.7
Tyrosine	0.4	0.6	0.7	0.7	0.7	0.7	0.7	0.7	[a]	0.6	[a]

× Not determined.
[a] Not determined due to technical error.

sequence can be deduced for this peptide: *Lys-Ser-Cys(O_3H)-Cys(O_3H)-Pro-Asx-Thr-Thr-Gly-Arg-(Asx, Ile)-Tyr*. As lysine has been demonstrated to be the amino terminal amino acid of Viscotoxin A2, this peptide is the amino terminal fragment of the protein.

Amino acid sequence of peptide OC3. Three steps of Edman degradation of this peptide are presented in Table 4. The presence of free phenylalanine was demonstrated after completion of the fourth step. Thus, the amino acid sequence of peptide *OC3* is: *Asx-Thr-Cys(O_3H)-Arg-Phe*.

Amino acid sequence of peptide OC4. Table 5 shows the results of 9 steps of Edman degradation of this peptide. The following partial amino acid sequence can be deduced: *Ser-Gly-Cys(O_3H)-Lys-Ile-Ile-Ser-Ala-Ser-(Thr, Cys(O_3H), 2 Pro,Ser,Tyr,Asx,Lys)*.

The presence of an Ile-Ile bond is assumed from the low yields of isoleucine when the peptide is hydrolyzed under normal conditions (24 h at 110°C) and the fact that no decrease in any amino acid is demonstrated in step 4, but a 50 % decrease in isoleucine is found in the 5th step.

Table 4. Edman degradation of peptide *OC3*.

Amino acid	Residues after step No.			
	0	1	2	3
Arginine	0.9	×	×	0.8
Cysteic acid	1.0	1.0	1.0	*0.6*
Aspartic acid	1.0	*0.4*	0.4	0.4
Threonine	1.0	1.0	*0.7*	0.5
Phenylalanine	1.1	1.2	1.1	1.0

× Not determined.

Table 5. Edman degradation of peptide *OC4*.

Amino acid	Residues after step No.									
	0	1	2	3	4	5	6	7	8	9
Lysine	2.0	×	2.0	2.0	*1.1*	1.0	1.0	1.0	1.0	×
Cysteic acid	1.9	2.0	2.2	*1.5*	1.4	1.4	1.3	1.4	1.2	1.3
Aspartic acid	1.0	1.2	1.2	1.2	1.0	1.0	1.0	1.0	1.1	1.1
Threonine	1.0	1.1	1.1	1.0	1.0	0.9	1.0	0.9	0.9	0.8
Serine	3.7	*2.9*	2.8	2.7	2.5	2.5	2.5	*2.0*	1.9	*1.4*
Proline	1.8	2.1	1.7	1.8	1.7	1.8	1.8	1.8	1.8	1.7
Glycine	1.1	1.2	*0.6*	0.5	0.4	0.4	0.4	0.4	0.4	0.4
Alanine	0.9	1.2	1.0	1.0	1.0	1.0	0.9	0.9	*0.5*	0.5
Isoleucine	1.9[a]	1.3	0.9	0.9	0.8	*0.8*	*0.4*	0.5	0.4	0.5
Tyrosine	0.7	0.6	0.7	0.7	0.8	0.7	0.7	0.8	0.8	[b]

× Not determined.
[a] After hydrolysis for 72 h.
[b] Not determined due to technical error.

Isolation and amino acid sequences of tryptic peptides

The tryptic hydrolysate of oxidized Viscotoxin A2 was separated into two fractions, by gel filtration on Sephadex G-25. These fractions were then chromatographed on a column of Dowex 1-X2 and the separation followed by thin-layer chromatography of the fractions of the eluate. Five pure peptides were obtained. They were designated as peptides *OT1*, *OT2*, *OT3*, *OT4*, and *OT5*. The amino acid compositions of these peptides are presented in Table 6. Besides these main peptides there were also a number of peptides, the yields of which were low (1 – 2 μmol compared to 15 – 22 μmol of the main peptides). These peptides were not further investigated.

Amino acid sequence of peptide OT1. 8 steps of Edman degradation of this peptide are presented in Table 7. Digestion with carboxypeptidases A and B liberated arginine followed by glycine, threonine, and asparagine. The following partial amino acid sequence can be deduced for this peptide: *Lys-Ser-Cys(O_3H)-Cys(O_3H)-Pro-Asn-Thr-(Thr,Gly)-Arg.* This sequence corresponds to the sequence of the first 10 amino acids of peptide *OC2*.

Amino acid sequence of peptide OT2. This peptide was taken through 4 steps of Edman degradation, the results of which are presented in Table 8. Free arginine was demonstrated to be present after completion of the 5th degradation step. The amino acid sequence of peptide *OT2* is: *Phe-Gly-Gly Gly-Ser-Arg.* As Viscotoxin A2 contains only one residue of phenylalanine, this sequence forms an overlap between peptides *OC3* and *OC1*.

Amino acid sequence of peptide OT3. The results of 5 steps of Edman degradation are presented in Table 9. Free arginine was demonstrated to be present after completion of the 6th degradation step. Digestion with LAP showed the presence of asparagine. The amino acid sequence of peptide *OT3* is:

Table 6. Amino acid composition of tryptic peptides from oxidized Viscotoxin A2.

Amino acid	OT1	OT2	OT3	OT4	OT5
Lysine	1.0			1.1	
Arginine	1.0	1.1	1.1		0.9
Cysteic acid	2.3		1.2	1.1	2.2
Aspartic acid	1.1		1.9	1.0	1.0
Threonine	1.9		0.8	0.9	1.8
Serine	1.0	0.9		2.9	0.9
Glutamic acid					
Proline	1.0			1.9	1.0
Glycine	1.1	3.1			1.1
Alanine				0.9	
Valine					
Isoleucine			0.9	1.9[a]	
Leucine					
Tyrosine			0.7	0.9	
Phenylalanine		1.0			
Yield, μmol from about 33 μmol oxid. Viscotoxin A2	20	20	15	22	3

[a] After hydrolysis for 72 h.

Asn-Ile-Tyr-Asn-Thr-Cys(O_3H)-Arg. This sequence forms an overlap between the chymotryptic peptides *OC2* and *OC3*.

The amino acid sequence of peptide OT4. 11 steps of Edman degradation are presented in Table 10. Dansylation confirmed that the amino terminal residue is isoleucine. Digestion with carboxypeptidase B liberated lysine, and digestion with a mixture of carboxypeptidases A and B liberated lysine and

Table 7. Edman degradation of peptide *OT1*.

Amino acid	Residues after step No.							
	0	1	2	3	4	5	6	7
Lysine	1.0	*0.4*	×	×	×	×	×	×
Arginine	0.9	0.9	×	×	×	×	×	×
Cysteic acid	2.3	2.0	2.0	*1.3*	*1.0*	1.0	1.0	1.0
Aspartic acid	1.1	1.0	[a]	1.0	1.0	1.0	*0.6*	0.6
Threonine	1.9	1.9	1.7	1.8	1.7	[a]	1.7	*0.8*
Serine	1.0	1.1	*0.4*	0.3	0.3	0	0	0
Proline	1.0	1.1	1.1	1.1	0.9	*0.5*	0.6	0.5
Glycine	1.1	1.1	1.0	1.2	1.0	1.1	0.9	0.9

× Not determined.
[a] Not determined due to technical error.

Table 8. Edman degradation of peptide *OT2*.

Amino acid	Residue after step No.				
	0	1	2	3	4
Arginine	1.1	×	×	×	×
Serine	0.9	0.8	0.8	0.8	0.8
Glycine	3.1	2.9	*2.4*	*1.5*	*0.6*
Phenylalanine	1.0	*0*	0	0	0

× Not determined.

Table 9. Edman degradation of peptide *OT3*.

Amino acid	Residues after step No.					
	0	1	2	3	4	5
Arginine	1.1	×	×	×	×	×
Cysteic acid	1.2	1.3	1.2	1.2	1.2	1.0
Aspartic acid	1.9	*1.1*	1.0	1.1	*0.4*	0.2
Threonine	0.8	0.8	0.7	0.9	1.0	*0.3*
Isoleucine	0.9	0.9	*0.1*	0.1	0.1	0.1
Tyrosine	0.7	0.5	0.7	*0.1*	0.1	0.1

× Not determined.

Table 10. Edman degradation of peptide *OT4*.

Amino acid	Residues after step No.											
	0	1	2	3	4	5	6	7	8	9	10	11
Lysine	1.0	1.1	×	×	×	×	×	×	×	×	×	×
Cysteic acid	1.1	1.2	1.1	1.1	1.0	1.0	1.0	*0.7*	0.7	0.5	0.6	0.4
Aspartic acid	1.0	1.1	1.0	0.9	1.0	1.0	1.0	1.0	1.0	1.0	0.9	0.9
Threonine	1.0	1.0	0.9	0.9	0.8	0.8	*0.6*	0.4	0.4	0.4	*a*	0.3
Serine	2.9	2.8	2.2	*1.4*	1.3	*1.1*	1.2	1.1	1.1	*0.9*	*a*	0.9
Proline	1.9	2.1	1.9	2.0	1.9	1.9	2.0	2.0	*1.5*	1.5	1.6	*1.4*
Alanine	0.9	1.1	0.9	1.1	*0.2*	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Isoleucine	1.9[b]	*1.1*[b]	*0.1*[b]	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Tyrosine	0.9	0.9	0.4	0.3	0.6	0.7	[a]	0.8	0.9	0.9	*0.7*	0.7

× Not determined.
[a] Not determined due to technical error.
[b] After hydrolysis for 72 hours.

aspartic acid, thus indicating the C-terminal amino acid sequence to be -Asp-Lys. These results permit deduction of the following amino acid sequence for peptide *OT4:* *Ile-Ile-Ser-Ala-Ser-Thr-Cys(O_3H)-Pro-Ser-Tyr-Pro-Asp-Lys.* To check this sequence the peptide was also subjected to partial acid hydrolysis and the hydrolysate chromatographed on a column of SE-Sephadex, the result of which is presented in Fig. 2. Two peaks were obtained. The first peak was

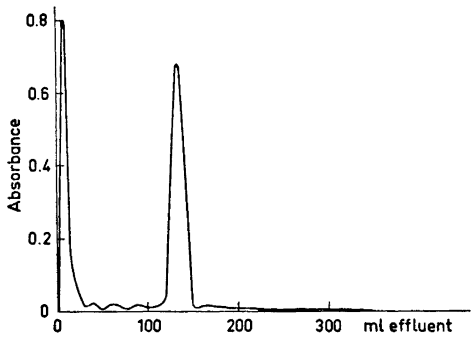

Fig. 2. Separation of a partial acid hydrolysate of peptide OT4 on SE-Sephadex. For details, see text.

shown to be a mixture of free amino acids and peptides. The second peak was a pure dipeptide containing the amino acids tyrosine and proline. Dansylation showed the amino terminal residue to be tyrosine, thus confirming that the sequence Tyr-Pro is present in peptide *OT4.* The sequence of the first 5 amino acid residues of peptide *OT4* is the same as the sequence of residues 5 – 9 of peptide *OC4.* It is therefore concluded that the undetermined sequence of peptide *OC4* is the same as the sequence of residues 6 – 13 of peptide *OT4.*

Peptide OT5. This peptide was obtained in the comparatively low yield of 3 μmol. As seen from Table 6 the amino acid composition of this peptide is the same as that of peptide *OT1* minus one residue of lysine. Dansylation showed the amino terminal residue to be serine. Digestion with carboxypeptidase B liberated arginine which thus is the *C*-terminal amino acid. Carboxypeptidase A + B liberated arginine, glycine, and threonine. It is therefore concluded that peptide *OT5* has been formed from peptide *OT1* by incomplete tryptic hydrolysis.

The complete amino acid sequence of Viscotoxin A2

The sequences of the tryptic peptides *OT2* and *OT3* show that the chymotryptic peptides *OC1, OC2,* and *OC3* must be linked together in the sequence *OC2-OC3-OC1.* Since peptide *OC2* represents the amino terminal sequence of Viscotoxin A2, peptide *OC4* must represent the carboxyl terminal

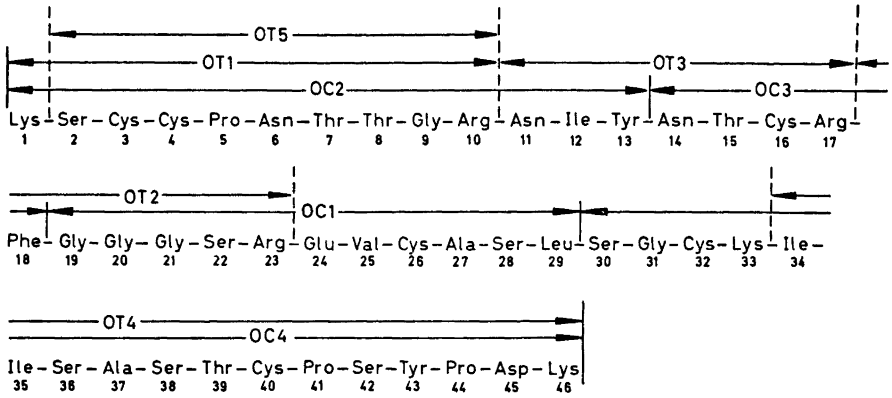

Fig. 3. The amino acid sequence of Viscotoxin A2.

sequence and the four chymotryptic peptides are thus linked together in the intact protein in the order *OC2-OC3-OC1-OC4*, thus establishing the complete amino acid sequence of Viscotoxin A2 as presented in Fig. 3. The sites of attack by trypsin and chymotrypsin are indicated in the figure. It is also obvious that the number of tryptic peptides isolated is not complete. One fragment containing the amino acids 24 – 33 is missing from the series of tryptic peptides. This fragment must have a very low isoelectric point as it contains two residues of cysteic acid and one residue of glutamic acid balanced only by one residue of lysine. It is therefore very probable that this fragment was so strongly adsorbed on the Dowex 1-X2 column that it could not be eluted under the conditions employed.

The amino acid sequence of Viscotoxin A2 is very similar to that of Viscotoxin A3.[5] The 6 half-cystine residues are all in the same position in the two proteins. The amino acid sequence of residues 1 – 14, 16, 17, 20, 23, 26, 27, 29 – 36, and 38 – 46 are the same. The main difference is found in the sequence of residues 18 – 25, where only two residues are the same in both proteins. The presence of glutamic acid at position 24 and the absence of lysine at position 28 are noteworthy.

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