PRIMARY STRUCTURE OF THE ALANINE SUBUNIT OF RICIN T FROM SEEDS OF THE CENTRAL ASIAN CASTOR OIL PLANT. V. PEPTIDES OBTAINED BY CYANOGEN BROMIDE CLEAVAGE

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The cleavage of the carboxymethylated Ala subunit of ricin T by cyanogen bromide has been performed. Four fragments have been isolated in the individual state by gel filtration and high-voltage paper electrophoresis. The sequence of the cyanogen bromide peptides in the protein chain has been established. Partial structures of three cyanogen bromide fragments have been determined.

Continuing an investigation to establish the primary structure of the Ala-subunit of ricin T, we have carried out its cleavage with cyanogen bromide in order to determine the locations of the tryptic peptides.

Three methionine residues have been detected previously in the amino acid composition of the Ala-subunit of the protein [1]. In a study of the tryptic peptides, three methionine-containing peptides were isolated: T-2, T-3, and T-8 [2]. Consequently, cleavage with cyanogen bromide may be expected to form four fragments.

The reduced and carboxymethylated Ala-subunit was incubated with cyanogen bromide in 70% formic acid solution at room temperature for 24 h, as described in [3]. Then the product of cyanogen bromide cleavage was diluted with distilled water and lyophilized.

TABLE 1. Amino Acid Compositions of the Cyanogen Bromide Peptides from the Ala-Subunit of Ricin T

Amino acid	B-1	B-2	B-3	B-4	Ala-subunit
CmCys	5	3		1	9
Asp	17,8	12,27	7,34	0,61	38
Thr	15,9	3,65	1,00		21
Ser	11,6	3,75	4,40		20
Glu	16,0	3,24	1,78		21
Pro	2,54	3,63	4,28		11
Gly	11,31	5,69	2,98		20
Ala	8,42	1,99	0,99	0,54	12
1/2 Cys	—	—	—	—	—
Val	7,85	6,40	2,60	0,75	18
Met	—	—	—	—	3
Ile	8,65	3,91	2,91		16
Leu	12,65	4,91	7,67		26
Tyr	3,75	2,63	1,63		9
Phe	0,76	1,20	2,20		4
Lys	2,95	1,98	1,62		7
His	—	0,74	0,99		2
Arg	5,64	5,54	0,62		13
Trp*	+	+	+	—	6
Hse	1	1	—	1	—
Total number of residues	134	67	44	5	256
N-terminal amino acid	Ile	Asn	Phe	Ala	Ala
C-terminal amino acid	Hse	Hse	Ser	Hse	Ser
Yield, %	66,7	13,3	12	3	

*Determined qualitatively with the aid of the Ehrlich reagent, as described previously [2].

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The mixture of peptides was separated by gel filtration on a column of Sephadex G-50 in 10% acetic acid. This gave four fractions, designated B-1, B-2, B-3, and B-4. The fractions were collected, diluted with water, and lyophilized. The purity of the fractions was estimated by chromatography on a cellulose-coated plate in the butanol—pyridine—acetic acid—water (15:10:3:12) system, and their N-terminal amino acids were determined. Fractions B-1, B-2, and B-3 contained impurities, while fraction B-4 proved to be homogeneous. Fractions B-1, B-2, and B-3 were purified further by preparative high-voltage paper electrophoresis. The homogeneity of the peptides was estimated by determining N-terminal amino acids. Table 1 gives the amino acid compositions of the four cyanogen bromide peptides obtained.

Partial N-terminal sequences were determined by Edman's method with the determination of the amino acids split out in the form of the PTH derivative in combination with dansylation [2]. The results of the investigation are given below:

B-1 Ile-Tyr-Asn-CmCys-Asn-Thr-Ala-...
B-2 Asn-Pro-Ile-Pro-Glu-Arg-...
B-3 Phe-Lys-Asn-Ser-Gly-Thr-...
B-4 Ala-Asp-Val-CmCys-Hse

The N-terminal sequence of the cyanogen bromide peptide B-4 agreed completely with the N-terminal sequence of the initial protein.

A comparison with the results on the determination of the N- and C-terminal amino acids of the initial protein and of the cyanogen bromide peptides showed that the N-terminal fragment was peptide B-4 and the C-terminal fragment B-3, which differed from the C-terminal sequence of cyanogen bromide fragment βCB-III of the Ala-subunit of ricin D [3]. Peptide B-1 was a continuation of the N-terminal sequence of the initial protein, following after peptide B-4. Peptide B-2 followed peptide B-1.

Thus, four fragments have been isolated in homogeneous form from a cyanogen bromide hydrolysate of the Ala-subunit of ricin T. Their sequence in the polypeptide chain of the protein has been established.

EXPERIMENTAL

Carboxypeptides A from Worthington, Sephadex G-50 (fine), and FN-18 paper were used.

Cleavage of the Ala-Subunit of the Protein with Cyanogen Bromide. A solution of 600 mg of the reduced and carboxymethylated protein in 20 ml of 70% HCOOH was added to a solution of 127.2 mg of cyanogen bromide. The mixture was left at 20°C for 24 h and was then diluted tenfold with distilled water and was lyophilized.

The mixture of cyanogen bromide peptides was fractionated on Sephadex G-50 in a column (2 × 150 cm) previously equilibrated with 10% CH_3COOH at a rate of elution of 20 ml/h. The emergence of the peptides was monitored with the aid of a SF-16 at 280 and 232 nm. The fractions were combined in accordance with the spectrophotometric results and were lyophilized.

The preparative separation of the mixtures of peptides on paper was carried out by high-voltage electrophoresis at pH 6.4 and a voltage of 2000 V in the pyridine—acetic acid—water (25:1:224) system. The peptides were revealed with the aid of ninhydrin and were eluted from the paper with 10% acetic acid.

The amino acid compositions of the peptides were determined on an amino acid analyzer [1].

The determination of the N-terminal amino acids and amino acid sequences was carried out as described in [2].

The C-terminal amino acid sequences of the peptides were determined by enzymatic hydrolysis with carboxypeptidase A followed by analysis of the hydrolysate on a LKB-4107 amino acid analyzer as described previously [1].

CONCLUSIONS

1. Four individual peptides including 256 amino acid residues have been obtained by the cleavage of the CM-Ala-subunit of ricin T with cyanogen bromide.

2. The sequence of the cyanogen peptides in the polypeptide chain of the initial protein has been established.

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MECHANISM OF POLYMERIZATION OF COLCHICIDYL-L-LYSINE N-CARBOXYANHYDRIDE

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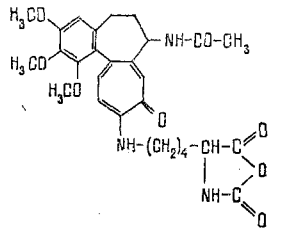
It has been shown that the polymerization of colchicidyl-L-lysine N-carboxyhydride under the action of amines obeys the general laws of the occurrence of anionic polymerization and is described, when primary amines are used, by a mechanism of "normal" amine addition and, when tertiary amines are used by the "activated monomer" mechanism.

Colchicine and its derivatives are attracting the attentions of research workers by their extremely interesting properties and, in the first place, their very strong cytostatic effect [1]. Many investigations have been devoted to the replacement of the tropolone methoxy group by various amino and alkylamine groups, including amino acid residues [2-4]. The introduction of amino acid residues into the molecule provides the possibility of obtaining polypeptides biodegradable in the organism under the action of proteases. The possibility of the gradual disruption of the polymeric chain ensures a prolongation of the action of drugs [5]. The initial monomers are α-amino acid N-carboxyanhydrides (CAs), since synthesis based on them permits a wide variation of the molecular mass of the polymers and the complete exclusion of racemization [6].

In order to elucidate the influence of the structure of the alkaloid on the process of anionic polymerization of CAs, we investigated the mechanism of initiation and chain growth in the polymerization of colchicidyl-L-lysine N-carboxyanhydride, which had been obtained previously [7].



Since the polymerization of CAs is a direct method of obtaining polypeptides, and the polymerization reaction may be brought about by various initiators (amines, metal alkoxides, aprotic bases [8]), the mechanism of initiation and chain growth largely depends on the nature of the initiating agent. As a rule, in the polymerization of CAs in the presence of primary amines the reaction takes place in the manner of a "normal" amine addition [9], and the polymer obtained contains a fragment of the initiator as the terminal group.

A different pattern is observed in the case where tertiary amines are used. The molecule of an aprotic base initiates the reaction by splitting out a proton from a CA molecule, and the particle formed – an "activated" monomer – interacts with the following CA molecule

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