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Short Communication

The Complete Amino Acid Sequence of the A-Chain of Abrin-a, a Toxin Protein from the Seeds of Abrus precatorius†

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Abrin-a is one of the toxic proteins in the seeds of Abrus precatorius (Leguminosae), more toxic than ricin, the toxic protein of Ricinus communis seeds. Abrin-a consists of an A-chain, which inhibits protein synthesis by inactivating the 60 S ribosomal subunits, and a B-chain, which binds to the galactose-containing receptors on the cell surface, and its toxicity is elicited by linking the A- and B-chains with a single disulfide bridge.1) In this paper we describe the complete amino acid sequence of the A-chain of abrin-a and compare it with that of the A-chain of ricin D.

Abrin-a was prepared from the seeds of Abrus precatorius produced in Thailand by the method of Lin et al.2) and its A-chain was isolated by the method of Olsnes et al.3) Tryptic and chymotryptic digestions were done at pH 8.0 and at 37°C for 4 and 2 hr, respectively. Digestions with Staphylococcus aureus V-8 protease, lysylendopeptidase, and pyroglutamate aminopeptidase of the A-chain were done in 50 mM ammonium bicarbonate solution, 50 mM Tris–HCl buffer (pH 9.0) and 50 mM Tris–HCl buffer (pH 8.0), respectively, at 37°C for 24 hr. Peptic digestion of peptide was done in 5% formic acid solution at 37°C for 6 hr. Cyanogen bromide (CNBr) cleavage of the A-chain was done in 70% formic acid at room temperature for 24 hr and dilute acid hydrolysis of peptide was done at 110°C for 20 hr with 0.03 N HCl. Peptides were separated by reverse-phase HPLC (RP-HPLC) with a FINE SIL C₁₈ or a YMC-GEL C₄ column (4.6 x 250 mm) using a 5 mM phosphate buffer (pH 6.0)–acetonitrile system or a 0.1% trifluoroacetic acid–2-propanol system. For separation of the peptides insoluble at pH 4.5 (large peptides), RP-HPLC with an Asahipak ODP-50 column (5.5 x 150 mm) using 5 mM ammonium bicarbonate solution–2-propanol system was used. The fragments obtained by CNBr cleavage or lysylendopeptidase digestion were separated by gel-filtration through a Bio-gel P-30 column (1.9 x 170 cm) in 30% acetic acid solution. Amino acids were analyzed with an amino acid analyzer after hydrolysis in an evacuated sealed tube at 110°C for 24 hr with 6 N HCl containing 0.05% 2-mercaptoethanol. Amino acids of peptides were sequenced by the DABITC/PITC double-coupling method.4)

Twenty-one peptides (T-1 ~ T-21) were isolated from the tryptic digest of the A-chain by RP-HPLC. Out of these peptides, the sequencing of small peptides other than peptide T-8 was completed directly by the DABITC/PITC double-coupling method and the sequence of large peptides (T-15, -16, -17, -19 and -20) was found after fragmentation by chymotryptic or V-8 protease digestion. Dilute acid hydrolysis of the peptide T-8 liberated Asp and Glu, and the resulting peptide was Arg-Pro-Ile-Lys, suggesting the sequence of [Glu-Asx-Arg-Pro-Ile-Lys]. To establish the arrangement of these tryptic peptides, the A-chain was digested with chymotrypsin or V-8 protease and the chymotryptic peptides insoluble at pH 4.5 was further digested with pepsin. The resulting peptides were isolated by RP-HPLC and sequenced. The positions of three lysine residues were

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Fig. 1. Complete Amino Acid Sequence of the A-Chain of Abrin-a. T, peptides from tryptic digestion of the A-chain; C, peptides from chymotryptic digestion of the A-chain; V, peptides from V-8 protease digestion of the A-chain; CB, fragments from CNBr cleavage of the A-chain; CP, peptides from peptic digestion of the chymotryptic peptides insoluble at pH 4.5.

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Fig. 2. Comparison of Amino Acid Sequences between the A-Chains of Abrin-a and Ricin. Identical residues are boxed.
confirmed by sequencing the peptides obtained from lysylendopeptidase-digest of the A-chain. Cleavage of A-chain with CNBr yielded four fragments (CB-I, -II, -III and -IV), of which fragments CB-II, CB-III and CB-IV were sequenced. The amino-terminal residue of the A-chain was released by pyroglutamate aminopeptidase digestion and the resulting A-chain was found to be Asp-Arg-Pro-Ile-.

Thus, the complete amino acid sequence of the A-chain of abrin-a was established as illustrated in Fig. 1. The amino acid composition derived from the sequence of the A-chain of abrin-a is: Asp₁₄Asn₁₄Thr₁₉Ser₂₀Glu₁₅Gln₁₅Pro₁₀Gly₁₄Ala₁₈Val₁₈Cys₁Met₁₃Ile₁₇Leu₂₁Tyr₉Phe₁₁Lys₅His₅Arg₁₈Trp₉ with a corresponding molecular weight of 27,997 daltons. The occurrence of 29 acidic and 21 basic residues is compatible with the isoelectric point of 4.6 of this protein.¹)

Figure 2 demonstrates our alignment of the A-chain of abrin-a and ricin,⁵) in which 106 residues are identical. The identical residues comprise 42% of the sequence of the A-chain of abrin-a. This result suggests that they originate from a common ancestor, although Abrus and Ricinus are not closely related taxonomically.

Details of the sequencing will be described elsewhere.

REFERENCES