Short Communication

Primary Structure of Ala Chain
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Ricin D is one of toxic lectins isolated from large grain of castor bean seeds (*Ricinus communis*) produced in Thailand.¹⁾ Ricin D consists of two types of polypeptide chains—Ile (or A)²⁾ chain and Ala (or B) chain—which are linked with a single disulfide bridge.³⁾ The Ile chain inhibits protein synthesis, while the Ala chain induces not only cytoagglutination, but also internalization of the Ile chain into the cytoplasm by binding to the cell surface receptors carrying galactose.²⁾

In previous paper,⁴⁾ we reported complete amino acid sequence of the Ile chain of ricin D. With regard to the primary structure of the Ala chain of ricin D, on the other hand, isolations of ten tryptic peptides from the performic acid oxidized Ala chain⁵⁾ and of cyanogen bromide fragments from the reduced Ala chain have been reported.⁶⁾

This paper summarizes the determination of complete amino acid sequence of the Ala chain of ricin D.

The Ala chain of ricin D was isolated from performic acid oxidized, reduced-carboxymethylated or reduced ricin D as previously described,^{3,7)} and these were referred to as

O-Ala chain, R₀CM-Ala chain or R-Ala chain, respectively. Tryptic or chymotryptic digestion was performed at pH 8.0 at 37°C and peptic digestion was in 5% formic acid at 37°C. CNBr cleavage of R-Ala chain was

carried out in 70% formic acid at room temperature for 24 hr and the resulted product was reduced with 2-mercaptoethanol and carboxymethylated. Partial acid hydrolysis of peptide was performed with 0.03 N HCl in 40% acetic acid at 107°C for 18 hr.

Separation of the peptides was performed by gel filtration on Sephadex G-25 or Biogel P-30 either in 5% or 30% acetic acid, Dowex 1×2 column chromatography, paper chromatography using nBuOH: H₂O: AcOH: pyridine (15: 12: 3: 10) and paper electrophoresis. Amino acid analyses were performed with a JEOL amino acid analyzer. The amino acid sequences of peptides were determined either by manual Edman degradation,⁸⁾ dansyl-Edman degradation⁹⁾ or subtractive Edman degradation.¹⁰⁾ Resulted PTH-amino acid and DNS-amino acid were identified by thin-layer chromatographies on silica gel plates and on polyamide layer, respectively.

Ten small tryptic peptides as well as free arginine (T1~T11) were obtained from the O-Ala chain by gel filtration on Sephadex G-25 and Dowex 1×2 column chromatography followed by paper chromatography, and nine peptides out of them were sequenced by manual Edman degradation.⁵⁾ CNBr treatment of R-Ala chain cleaved two methionine bonds out of three in the Ala chain and yielded three fragments (CB-I, -II and -III). Amino acid compositions and N-terminal sequences of these fragments revealed the alignment of fragments (CB-II)-(CB-I)-(CB-III).⁶⁾

The fragment CB-I, a largest fragment consisted of 140 amino acid residues and oligosaccharides, was digested with trypsin and separated into soluble and insoluble fractions in 30% acetic acid. Seven peptides (CB-I-T1 ~ CB-I-T7) were isolated from the soluble fraction and the insoluble fraction was purified by washing with 70% formic acid (CB-I-T8). Though the sequences of six peptides out of these eight peptides were determined directly by manual Edman degradation, the sequences of peptides CB-I-T3 (19 residues) and CB-I-T8 (66 residues) were determined by sequencing the subfragments after peptic digestion of

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fragment CB-I and chymotryptic digestion of peptide CB-I-T8. From the chymotryptic digest of peptide CB-I-T8, six peptides (CB-I-T8-C1~CB-I-T8-C6) were isolated and sequenced either by manual- or dansyl-Edman degradation. On the other hand, twelve peptides (CB-I-P1~CB-I-P12) were obtained from peptic digest of fragment CB-I and some of them were sequenced at need. Partial acid hydrolysis of peptide CB-I-T8 yielded peptide CB-I-T8-A, and N-terminal sequence of peptide CB-I-T8-A revealed that peptide CB-I-P9 was followed by peptide CB-I-P2. From these results, the sequences of peptides CB-I-T3 and CB-I-T8 were established, and moreover the alignment of the tryptic peptides in fragment CB-I was established to be (CB-I-T4)-(-T1)-

(-T8)-(-T3)-(-T5)-(-T6)-(-T2)-(-T7).

The sequence of fragment CB-II, N-terminal fragment consisted of 75 amino acid residues, was determined by sequencing the tryptic and chymotryptic peptides from fragment CB-II. Nine peptides as well as free arginine (CB-II-T1~CB-II-T10) were isolated from tryptic digest of fragment CB-II by gel filtration on Sephadex G-25 followed by Dowex 1×2 column chromatography and sequenced completely by manual Edman degradation. From chymotryptic digest, on the other hand, eight peptides (CB-II-C1~CB-II-C8) were isolated, and their amino acid compositions and the sequence of peptide CB-II-C1, which contained two lysine and two arginine, were determined. These results established the alignment of the

FIG. 1. Primary Structure of Ala Chain of Ricin D.

T, C, P, CB and A numbers represent the peptides resulting from tryptic, chymotryptic, peptic digestions, CNBr cleavage and partial acid hydrolysis, respectively.

tryptic peptides in fragment CB-II to be (CB-II-T9)-(-T2)-(-T8)-(-T5)-(-T7)-(-T6)-(-T1)-(-T4)-(-T3)-(-T10).

Direct manual Edman degradation of fragment CB-III, smallest fragment derived from C-terminus of the Ala chain, revealed the twenty amino acids of N-terminal sequence of fragment CB-III. Three tryptic peptides (CB-III-T1~CB-III-T3) and six peptic peptides (CB-III-P1~CB-III-P6) were isolated by paper chromatography and Dowex 1×2 column chromatography, respectively, and sequenced by manual Edman degradation. From these results, the sequence of fragment CB-III was established.

Since the alignment of fragments CB-I, CB-II and CB-III in the Ala chain is (CB-II)-(CB-I)-(CB-III) as described above, the complete amino acid sequence of the Ala chain of ricin D was established as illustrated in Fig. 1.

Oligosaccharides consisted of mannose and glucosamine (probably N-acetylglucosamine) were detected in peptides CB-I-T1 and CB-I-T8-C1. Since the sequences of two glycopeptides isolated from the proteolytic digest of O-Ala chain of ricin D were

(glucosamine₂mannose₆)

Asx-Asx-Gly-Thr

and (glucosamine₂mannose₇)

Asx-Asx-Thr-Glx-Pro,¹¹⁾

these two oligosaccharide moieties were located at 93rd and 133rd positions in the Ala chain, respectively.

Locations of the cysteine residue linked with that of the Ile chain and four disulfide bridges in the Ala chain were performed by isolating S-carboxymethylcysteine-containing peptide and cystine-containing peptides from the tryptic digest of R₀CM-Ala chain, respectively. Tryptic peptides from R₀CM-Ala chain were separated into soluble and insoluble fractions by adjusting the digest to pH 4.5 and each fraction was fractionated by gel filtration on Biogel P-30 in 5% acetic acid. S-carboxymethylcysteine- or cystine-containing peptides were purified by Dowex 1×2 column chromatography and paper chromatography. Cys-

tine-containing peptides were oxidized with performic acid, and the resulted peptides were separated by paper chromatography and identified. Thus, the cysteine residue participated in the linkage to the Ile chain was identified to be cysteine at 4th position, and four disulfide bridges were located between Cys20 and Cys39, Cys62 and Cys79, Cys149 and Cys163, and Cys188 and Cys205.

The amino acid composition derived from the sequence of the Ala chain of ricin D is: Asp₁₄Asn₂₄Thr₂₁Ser₂₀Glu₇Gln₁₃Pro₁₃Gly₂₀Ala₁₅Val₁₈Met₃Ile₁₈Leu₂₄Tyr₉Phe₄Lys₇His₂Arg₁₃Cys₉Trp₆, which is in fairly good agreement with that from the total hydrolysis of the intact or O-Ala chain. Molecular weight calculated from the sequential analysis is 31,432 (including glucosamine₄ and mannose₁₃). The 22 basic and 21 acidic amino acid residues distributes in both N- and C-terminal regions and there is no charged residues in the central region between Ser101 and Ile159. Many continuous sequences of hydrophobic amino acids occur over the chain; especially C-terminal region is a typical region including the leucine and isoleucine-rich hydrophobic amino acids.

The secondary structure predicted from the amino acid sequence by the method of Chou and Fasman¹²⁾ indicated that the Ala chain possesses four α -helical regions (27 amino acid residues, 10.4% of total residues), twelve β -sheet regions (82 residues, 32.3%) and thirty-one β -turn. Details of the sequence determination and the prediction of secondary structure will be described elsewhere.¹³⁾

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