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The complete primary structure of abrin-a B chain

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The complete 267 amino acid sequence of abrin-a B chain was determined by analysis of peptides obtained by digestion with trypsin, chymotrypsin, lysyl endopeptidase, *Staphylococcus aureus* V8 protease and thermolysin. The sequence is not identical with that predicted previously by nucleotide sequencing, indicating the presence of isoforms of abrin. Comparison of the amino acid sequence of abrin-a B chain with that of ricin-D B chain reveals a high degree of sequence identity (59%). Abrin-a B chain appears to consist of two domains, each domain with subdomains (α, β, γ) of about 40 amino acid residues.

Abrin-a B chain; Primary structure

1. INTRODUCTION

Abrin-a is one of the potent toxins isolated from the beans of plant *Abrus precatorius* [1,2]. The toxin protein consists of two disulfide-bonded subunits, the A and B chains. The B chain is a galactose-specific lectin that facilitates the binding of abrin to the cell membrane that precedes endocytosis. After entering the cells, the A chain catalytically inactivates 60 S ribosomal subunits by removing adenine from position 4,324 of 28 S r-RNA [3,4], and thereby inhibits protein biosynthesis. Abrin-a A chain itself was virtually non-toxic to intact cells, and the presence of free B chain significantly enhanced the toxicity of A chain to intact cells [5,6].

The A chain of abrin-a has been conjugated to monoclonal antibodies using disulfide-containing cross-linkers. There is considerable interest in the potential use of the conjugate or immunotoxins in cancer chemotherapy [7–10]. The toxicity of immunotoxin was increased in the presence of B chain by enhancing the translocation of A chain into the cytoplasm to exert its cellular cytotoxicity. However, the presence of B chain complicated the complete immunotoxin by its galactose binding activity [11,12]. Recently, recombinant conjugates of cytokine and A chain have been constructed, and the specific killing of cells by the conjugates was demonstrated [13]. The inclusion of B chain with a mutation that alters the galactose binding motif in the recombinant toxin constructs may lead to a modified conjugate with enhanced specificity.

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Recently, a whole genomic DNA of abrin C was cloned and sequenced [14]. The present report presents the complete primary structure of abrin-a B chain, and provides evidence of repetitive genes coding for the B chain.

2. MATERIALS AND METHODS

2.1. *Purification of abrin-a B chain*

Abrin-a was isolated and purified according to the methods described previously [2]. The B chain of abrin-a was obtained by reduction of abrin-a followed by chromatography on a DEAE-cellulose column [15], and then carboxymethylated [16]. The following enzymes were purchased from the sources indicated in parentheses: trypsin, chymotrypsin, and thermolysin (Sigma), *S. aureus* V8 protease (Boehringer-Mannheim), lysyl endopeptidase (Wako Pure Chemicals). The PVDF membranes (Immobilon transfer), 0.45 μm pore size, were obtained from Millipore. All other chemicals were of the highest grade commercially available.

2.2. *Sequence determination*

2.2.1. Enzymatic digestion

Tryptic and chymotryptic digestions were performed in 0.05 M Tris-HCl buffer, pH 8.0, at 37°C for 3 h. Digestion with thermolysin was carried out in 0.1 M NH_4HCO_3 buffer, pH 7.8, at 40°C for 1.5 h. Lysyl endopeptidase digestion was performed in 0.05 M Tris-HCl buffer, pH 8.0, at 37°C for 3 h. The digestions were stopped by the addition of acetic acid. Limited digestion with *S. aureus* V8 protease was performed by incubating the abrin-a B chain in 0.1 M pyridine-acetate-collidine buffer, pH 6.5, at 0°C for 1 h. The products were electroblotted onto PVDF membranes and then stained with Coomassie brilliant blue and the sequence of peptides on the membrane was determined [17].

2.2.2. Fractionation of the peptides

Peptides obtained by digestion with trypsin, chymotrypsin, thermolysin, and lysyl endopeptidase were fractionated by reverse-phase HPLC in μBondapak C18 reverse-phase columns (4.6 × 250 mm) (Waters). The peptides were eluted by a linear gradient of acetonitrile containing 0.07% trifluoroacetic acid from 10 to 70% at a flow rate of 1 ml/min. UV-absorbance of the eluent was monitored at 214 nm.

2.2.3. Amino acid analysis

The amino acid analysis was performed with an automatic Beckman 6300 system amino acid autoanalyzer. Samples were hydrolyzed with 6 M HCl containing 0.5% phenol at 150°C for 1.5 h in sealed evacuated tubes.

2.2.4. Sequence analysis

The amino acid sequence of purified peptides was analyzed with an ABI 477A sequencer and an ABI 120 A analyzer using about 200 pmol–1 nmol of peptide dissolved in 0.05% TFA and 50% acetonitrile.

2.2.5. Nomenclature

Peptides derived from the digestion with trypsin, chymotrypsin, thermolysin, lysyl endopeptidase, and *S. aureus* V8 protease are named by letter codes T, C, TH, L and V, respectively, and the numbers are denoting the order in which they appear in the B chain from the N-terminus.

3. RESULTS AND DISCUSSION

The complete amino acid sequence of abrin-a B chain from *A. precatorius* is shown in Fig. 1. Four sets of overlapping peptides (30–50 nmol) were obtained after reverse-phase HPLC of reaction products of four different proteases of 300–500 nmol quantities of abrin-a B chain. The B chain contains 267 amino acid residues. Direct sequencing of abrin-a B chain identified unambiguously the residues 1–23 (Table I). There are four extra amino acid residues at the N-terminus of abrin-a B chain when the sequence was compared and aligned with that of ricin-D B chain (Fig. 2) [18].

Recently, the amino acid sequence predicted by the genomic DNA sequence of abrin C was reported [14,19]. There are 17 variant amino acid residues between the amino acid sequence obtained from the nucleotide sequence and the protein sequence of abrin-a B chain (Fig. 2). These differences could be due to the fact that there are at least four isoforms of abrin present in

Table I

N-terminal sequence of abrin-a B-chain

Residue number	Amino acid	Yield (pmol)
1	Iso	3.71
2	Val	4.87
3	Glu	3.59
4	Lys	4.09
5	Ser	0.55
6	Lys	2.40
7	Iso	2.66
8	Cys	1.22
9	Ser	0.72
10	Ser	0.59
11	Arg	0.32
12	Tyr	2.06
13	Glu	1.58
14	Pro	1.30
15	Thr	1.83
16	Val	3.90
17	Arg	0.98
18	Iso	3.16
19	Gly	2.14
20	Gly	2.36
21	Arg	0.84
22	Asp	1.02
23	Gly	2.46

Numbers stand for position number in the B-chain sequence.

A. precatorius, as reported previously by several laboratories [1,2,20]. Although abrin and ricin are not closely related taxonomically, the B chains of abrin-a and ricin-D have very high amino acid homology with 159 amino acid residues conserved as shown in Fig. 2, which is higher than that of A chains of abrin and ricin, with 102 amino acid residues conserved [21].

It has been proposed previously that the B chain of

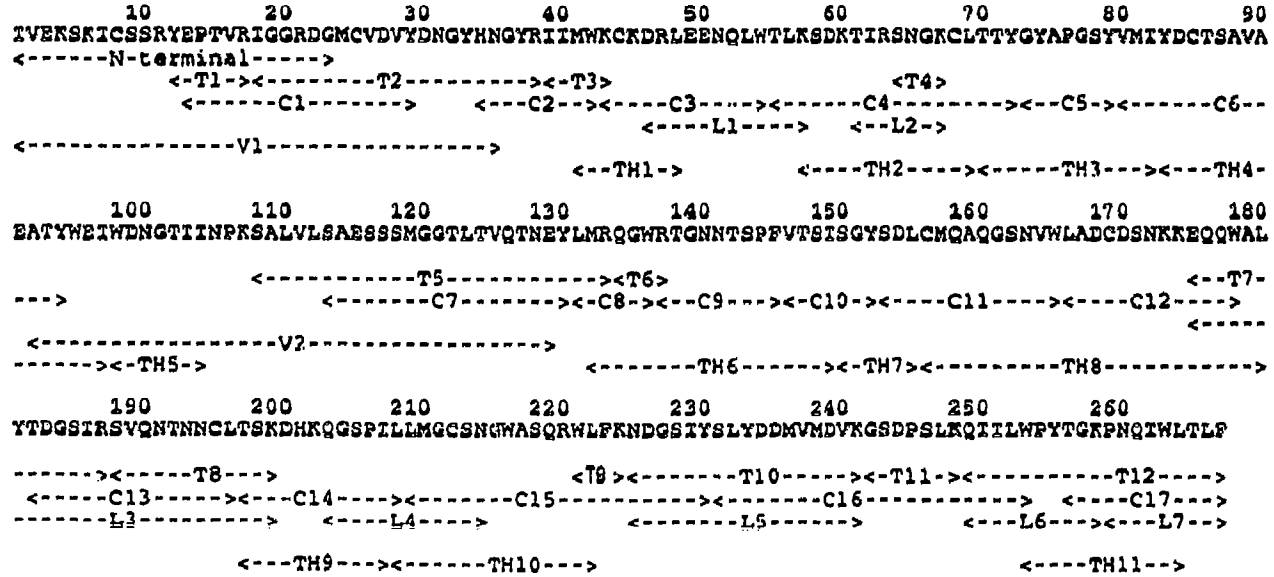

Fig. 1. Summary of the results of sequence analysis of the abrin-a B chain. T, trypsin peptides; C, chymotrypsin peptides; L, lysyl endopeptidase peptides; V, *Staphylococcus aureus* V8 protease peptides; T, thermolysin peptides. Sequences of all peptides were completely determined.

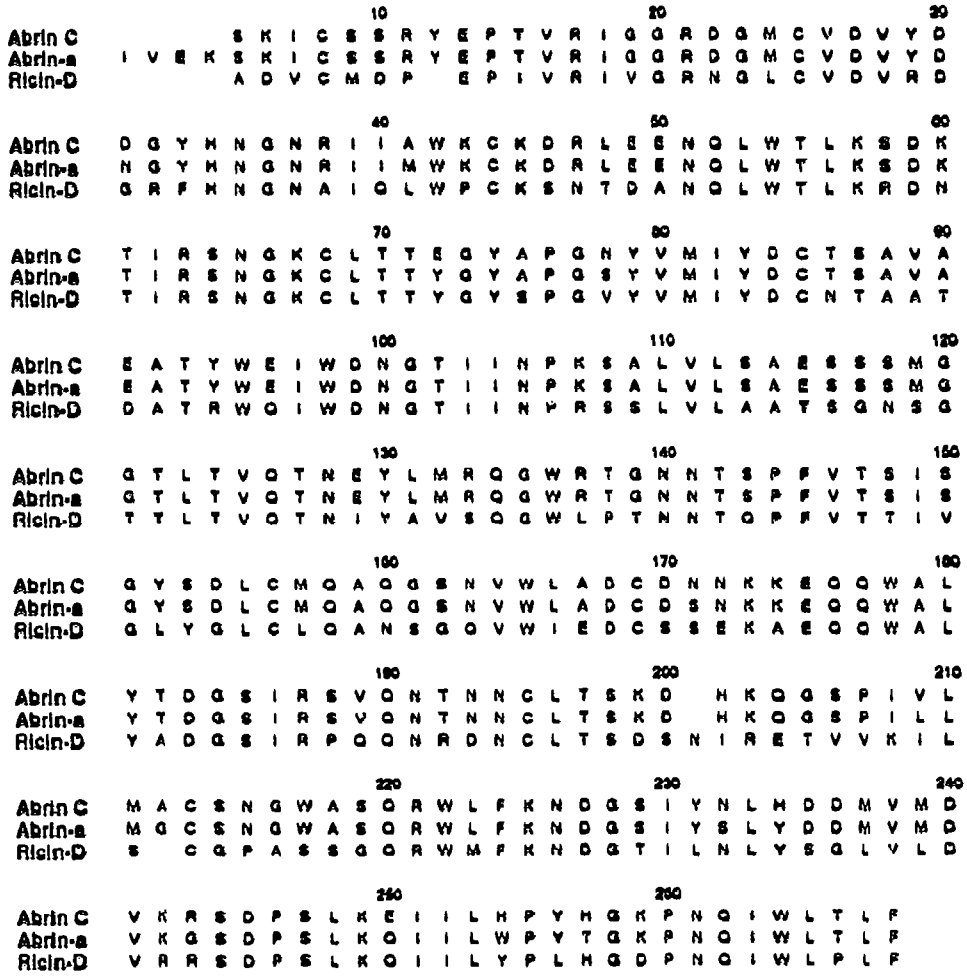

Fig. 2. Comparison of amino acid sequences of B-chain of abrin C, abrin-a and ricin-D.

ricin-D may be a product of gene duplication [22,23]. By comparing the amino acid sequence of ricin-D B chain with that of abrin-a, it is revealed that the B chain of abrin-a was also shown to be the product of a series of gene duplications. Abrin-a B chain appears to be composed of two domains, and each domain consists of three subdomains (α, β, γ) with about 40 residues, which are homologous with each other. There are many strongly conserved key residues in the domains of abrin-a B chain, such as 1/2 Cys, Trp and Ile residues. When these residues are aligned, several areas of amino acid homology exist between the N- and C-terminal parts of

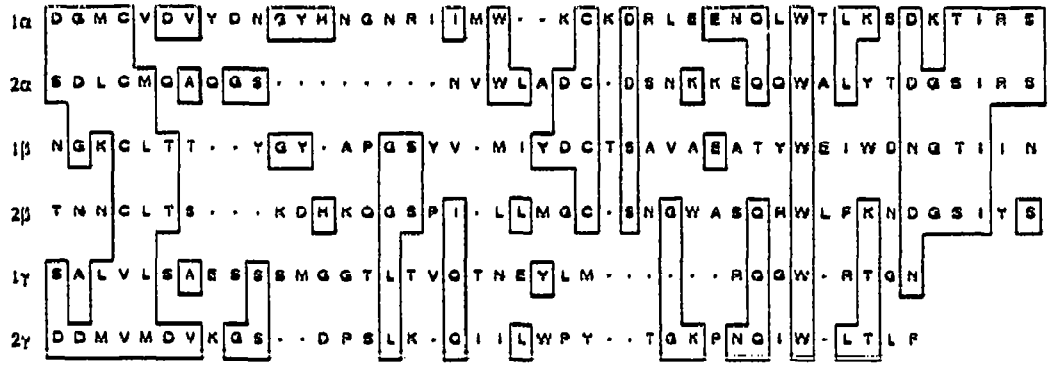

Fig. 3. Amino acid sequence alignment of the subdomain units of abrin-a B chain. The six sequences from abrin-a B chain are aligned from amino- to carboxy-terminus and are arranged to maximize their similarities. The subdomain units correspond to residues as follows: 1α, 22–64; 2α, 153–188; 1β, 65–105; 2β, 192–231; 1γ, 108–140; 2γ, 234–267. Boxes enclose identical residues between two subdomain units.

the B chain (Fig. 3). Abrin-a B chain shows two galactose binding sites, Asn^{51} and Asn^{200}, which are located in units 1 and 2, and are stabilized by hydrogen bonds of the conserved acidic amino acids, Asp^{27} and Asp^{230} [24]. The sequence Gln-X-Trp is highly conserved and forms in five of the subdomains, where X is any residue, and Gln and Trp are invariant. Our findings strengthen the proposals that the B chain of abrin-a, as well as that of ricin-D, may have come from the same ancestor of galactose-binding peptide of about 40 residues [24,25]. The duplication of the gene produced the $(\alpha, \beta, \gamma)_2$ motif, and the fusion of multiple galactose-binding peptides would make the galactose-binding protein more effective.

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