Revised amino acid sequence of the B-chain of ricin D due to loss of tryptophan in the cyanogen bromide cleavage

Tomohiro Araki and Gunki Funatsu

Laboratory of Biochemistry, Faculty of Agriculture, Kyushu University, Fukuoka 812, Japan

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The complete amino acid sequence of the ricin D B-chain was redetermined using the DABITC/PITC double-coupling method. By comparing the presently established amino acid sequence with the sequence reported earlier which was determined by the manual Edman degradation technique, it was found that the identification of 26 amino acid residues had to be revised. Furthermore, the ricin D B-chain was shown to consist of 262 amino acid residues rather than 260 as previously described. In addition, based on the new amino acid sequence, the sequence homology between the N- and C-terminal domain of this chain was found to be 31%.

Ricin D B-chain *Amino acid sequence* Ricinus communis *lectin* *Castor bean toxic protein*

1. INTRODUCTION

Ricin D, one of the toxic lectins of castor beans (*Ricinus communis*), has been shown to consist of 2 polypeptide chains (A- and B-chains) which are linked together by a single disulfide bond [1,2]. The biological function of the A-chain is known to be associated with the cytotoxicity of ricin, whereas the B-chain induces internalization of the A-chain by binding to the galactose-containing receptors on the cell surface [3]. In [4] we reported the amino acid sequence of the B-chain of ricin D, isolated from the above-mentioned seeds. In recent studies we noted that some of the Trp residues were missing and other amino acid residues, which were determined by the manual Edman degradation procedure [5], were earlier misinterpreted.

This paper describes briefly the redetermination of the complete amino acid sequence of the B-chain of ricin D.

2. MATERIALS AND METHODS

The B-chain of ricin D was isolated and purified according to [6]. The resulting B-chain was carboxymethylated [7] and digested with 1/50 (w/w) trypsin (type XII, Sigma), pH 8.0 for 4 h, at 37°C. This digest was then adjusted to pH 4.0 with 5% acetic acid and fractionated by centrifugation into a supernatant (Ts) and precipitate (Tp). After its concentration, the Ts fraction was fractionated further by centrifugation yielding a supernatant (Ts-A) and a precipitate (Ts-B). The Tp fraction was digested with 1/25 (w/w) chymotrypsin (Worthington), pH 8.0 for 2 h, at 37°C.

Each peptide fraction was applied to a C_{18} column which was developed with a gradient system consisting of 5 mM phosphate buffer, pH 6.0, and 60% acetonitrile in the same buffer. The peptide of each peak was purified and simultaneously desalted employing the same column but a different gradient system, namely 0.1% trifluoroacetic acid (TFA) and 60% acetonitrile in 0.1% TFA. To establish their amino acid sequences, the tryptic and chymotryptic peptides were then sequenced with the aid of the DABITC/PITC double-coupling method [8]. The C-terminal sequence of the B-chain was determined with the aid of carboxypeptidase Y [9].

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3. RESULTS AND DISCUSSION

The HPLC elution patterns of the tryptic peptides derived from the Ts-A and Ts-B fractions and the chymotryptic peptides derived from the Tp fraction are shown in fig.1. The tryptic peptides were designated T1–T20 and the chymotryptic peptides C1–C6. Nineteen tryptic peptides, excepting T12, were purified from the Ts-A and Ts-B fractions. The Tp fraction yielded 6 chymotryptic peptides. The total sequence of the latter peptides equals that of T12 so that it was not necessary to sequence this peptide.

It should be noted that the C-terminal Lys residues of peptides T5, T6 and C6 were not detected by the DABITC/PITC double-coupling method because of the hydrophobicity of the ϵ-DABITC/PITC derivative of this amino acid. With regard to peptides T11 and C4, the Asn residues which are linked to the carbohydrate unit were identified as aspartic acid by amino acid analysis of the hydrolysate of the aqueous phase remaining after all Edman steps had been carried out.

As to the new amino acid sequence (fig.2) the following points deserve further comment. It was found that this sequence contains 3 additional Trp residues when compared with the earlier sequence. In this regard it is of interest to note that Trp-90 which is located at the N-terminus of T11 could not be recovered when the Trp-containing tryptic peptide was purified from the corresponding cyanogen bromide fragment. This new finding indicates that, under the conventional conditions employed for the cyanogen bromide cleavage, Trp residues are lost if these residues occur at the N-terminal position of the peptides. As to His, 2 of these residues (His-29, His-251) were earlier misinterpreted because the manual Edman degradation method was used which is known to cleave the His-X peptide bond during the coupling step [10,11] so that the PTH derivatives of His and the subsequent amino acid are formed simultaneously. As the PTH derivative of the latter amino acid is usually extracted into the organic phase and PTH-His remains in the aqueous phase, PTH-His may be overlooked. In contrast, under the conditions employed in the DABITC/PITC

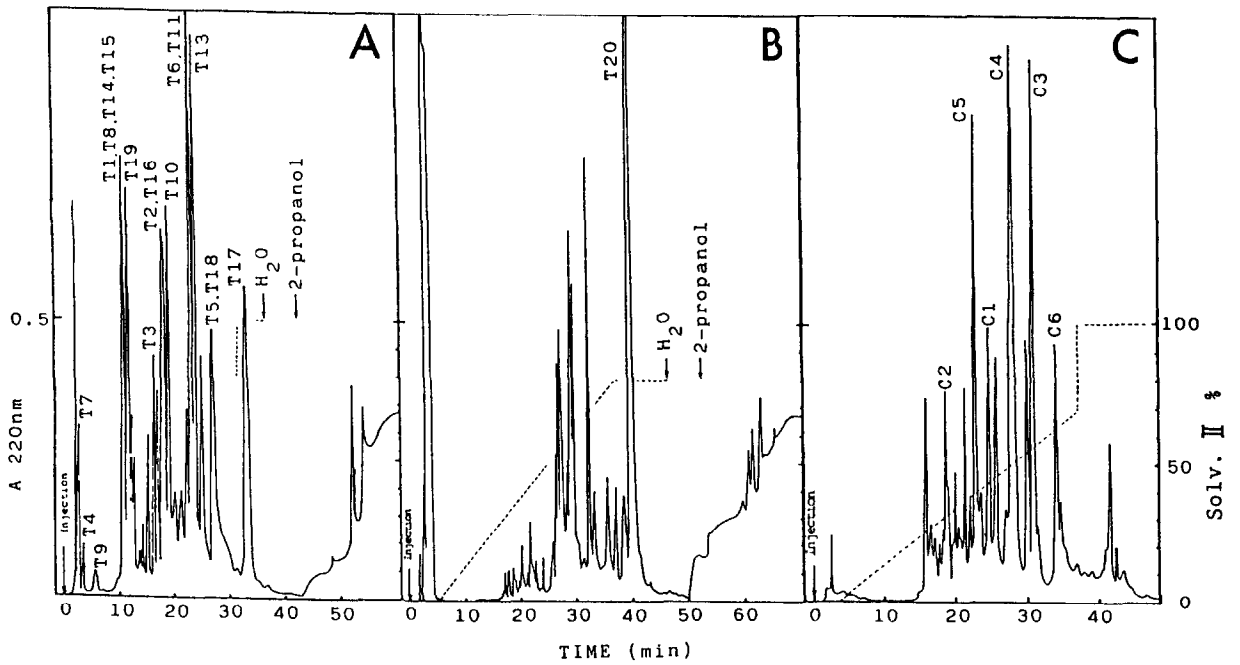

Fig.1. HPLC of tryptic and chymotryptic peptides of Ts-A (A), Ts-B (B) and Tp (C) fractions. Peptides were eluted with a gradient system consisting of 5 mM phosphate buffer, pH 6.0 (solvent I), and 60% acetonitrile in the same buffer (solvent II).

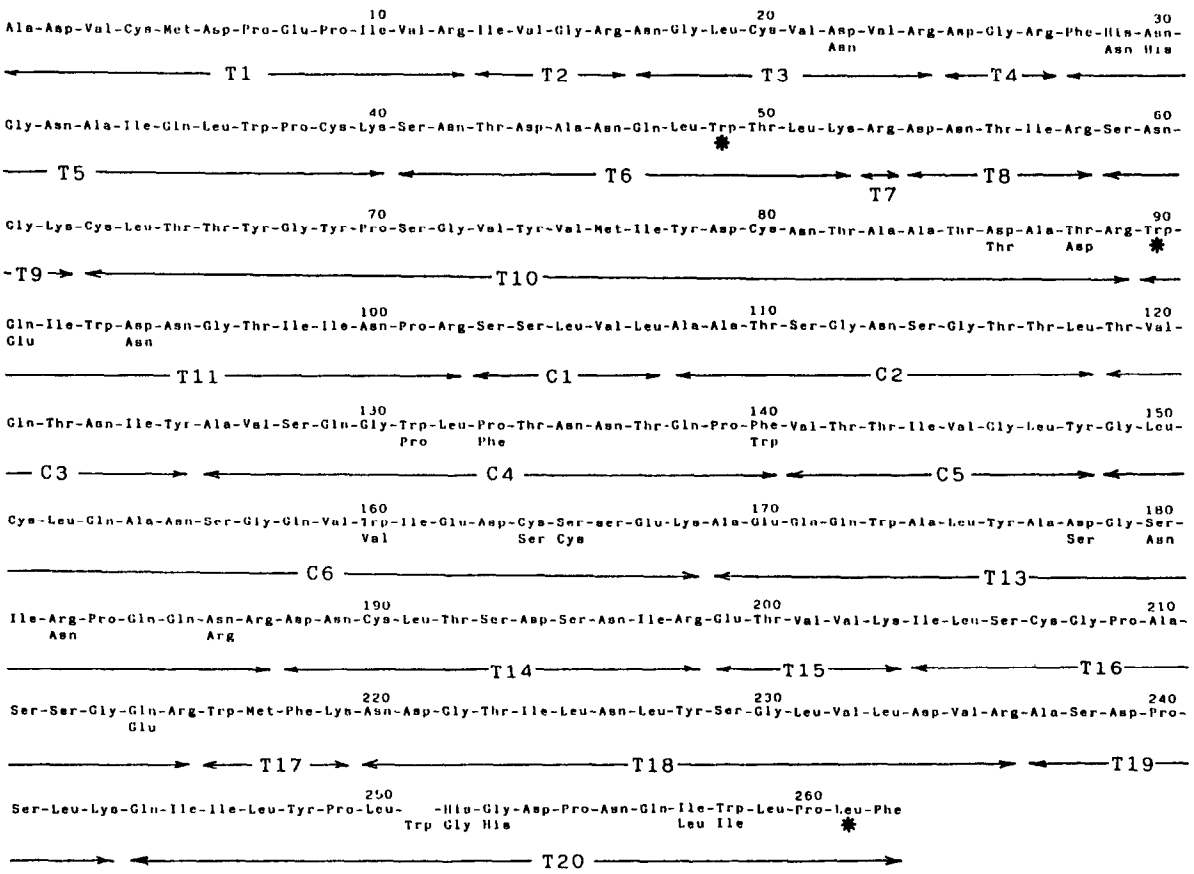

Fig.2. The complete amino acid sequence of the ricin D B-chain. The peptides obtained by tryptic and chymotryptic digestion are indicated by T and C, respectively. Amino acids under the sequence are those reported in the earlier sequence. The amino acids missing in the earlier sequence are marked with an asterisk.

double-coupling procedure, the His-X peptide bond is stable. Moreover, the reactions involved with the latter method protect completely the amide group of Asn and Gln. In addition, the detection of the derivatives of Thr and Ser by the latter method is also unambiguous. Further ambiguity of certain other amino acid residues in the previously reported sequence was now found to be due to the considerably lower sensitivity of the PTH-amino acid on thin-layer chromatography than that of the DABTH-amino acid.

The new amino acid sequence of the B-chain proved to consist of 262 amino acid residues (fig.2). From the amino acid and the sugar compositions [12], a molecular mass of 31557 Da was calculated. Further support for the correctness of the revised amino acid sequence is given by the corresponding DNA sequence [13]. The positions of residues 69 and 70 were reported to be Ser and Pro from the DNA sequence as compared with Pro and Ser in this work. Also, Arg-237 deduced from the DNA sequence was in the position of Ala-237. Hence, it is possible that these differences could be explained by the use of a ricin D variant since Lamb et al. [13] utilized different castor bean seeds.

The N-terminal (residues 1–135) and C-terminal half (residues 136–262) of the polypeptide chain has been shown earlier to possess intramolecular homology [14]. The new sequence homology between these 2 domains was found to be 31% as compared with the earlier value of 19%. With regard to the most conserved amino acid residues it should be noted that 8 of the 9 Cys residues

which are involved in the intramolecular disulfide bonds are highly conserved in the 2 domains, i.e. S-S bond 20–39 corresponds to S-S bond 151–164 and S-S bond 63–80 corresponds to S-S bond 190–207. In addition, this study shows that the Trp residues were also conserved: residues 37, 49, 90 and 131 correspond to residues 160, 173, 216 and 258. As to Trp-93, this residue occurred only once, namely in the N-terminal domain. Thus, it can be assumed that these amino acid residues (Trp and S-S) are involved in the conservation of the structure and function of ricin D.

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