Short Communication

Carbohydrate Composition
of Glycopeptides Isolated from
Ricinus communis Lectins

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Castor bean (*Ricinus communis*) seeds contain two types of lectins, ricin and hemagglutinin (CBH). They are homologous proteins and are composed of A- and B-chains which are glycosylated. In a previous paper, Nanno *et al.*¹⁾ reported the carbohydrate composition of glycopeptides from ricin D: one glycopeptide having the composition (GlcNAc₂Man₄) from the A-chain and two glycopeptides having the composition (GlcNAc₂Man₆) and (GlcNAc₂Man₇) from the B-chain. However, Thorpe *et al.*²⁾ reported recently that xylose and fucose were contained in the A-chain of ricin, and we also detected xylose and fucose in the glycopeptide from the A-chain of ricin D.

With regard to the carbohydrate composition of CBH, on the other hand, only the content of mannose and glucosamine in whole CBH has been reported.³⁾

This paper describes the analysis of the carbohydrate composition of glycopeptides from ricin D as well as CBH.

The A- and B-chains of ricin D and CBH were isolated by a modification of the method of Olsnes and Pihl.⁴⁾ The A-chain was further separated into two fractions, which correspond to molecular weights of 32,000 and 31,000, by CM-cellulose column chromatography using a linear gradient of NaCl from 0 to 0.1 M in 5 mM phosphate buffer, pH 6.6. Of these fractions, the major fraction with the smaller molecular weight was employed for this study. Tryptic digestion was done with 2% by weight of trypsin (Worthington Biochemical Corp.) at 37°C and pH 8.0 for

6 hr. Pronase digestion was done at 37°C and pH 7.8, initially with 2% by weight of pronase (Seikagaku Kogyo Co., Ltd.) for 48 hr, and then with an additional 2% by weight of pronase for 24 hr. The glycopeptides were separated by gel filtration on a Sephadex G-50 (Pharmacia Fine Chemical Corp.) column (1.9 × 190 cm) in 0.1 N NH₄OH solution. This was followed by Dowex 1 × 2 column (0.9 × 60 cm) chromatography using a polygradient elution system from 2% pyridine-acetic acid (pH 8.3) to 0.5 N acetic acid as described earlier⁵⁾ except for using pyridine instead of collidine. Further purification of the glycopeptides was done by reverse-phase HPLC (JASCO TWINCLE) with a fine SIL-C₁₈ column (8 × 250 mm) using a linear gradient of 2-propanol from 0 to 70% in 0.1% aqueous TFA solution. The peptides were detected either by the Folin-Lowry method⁶⁾ or by their absorbance at 220 nm, and the glycopeptides by the phenol-H₂SO₄ method.⁷⁾ Neutral sugar was analyzed after methanolysis by a slight modification of the method of Chambers and Clamp⁸⁾ using gas-liquid chromatography apparatus (Shimadzu Co., Ltd. Type GC-8A) with 2% OV-17 on Gas-Chrom Q (80~100 mesh) and nitrogen as the carrier gas. The temperature was raised at a rate of 4°C/min from 110°C to 240°C and the trimethylsilylated sugars were detected by a flame ionization detector. The mannose content was also measured by the method of Dubois *et al.*⁷⁾ with a slight modification. Amino acid analysis was done with an amino acid analyzer (JEOL Type GLC-6A) after hydrolysis with 5.7 N HCl containing 0.05% 2-mercaptoethanol in an evacuated sealed tube at 105°C for 24 hr. The aminosugar was measured by the method of Walworg and Ward⁹⁾ using an amino acid analyzer after hydrolysis with 4 N HCl at 105°C for 4 hr.

Since both oligosaccharide moieties in the A-chains of ricin D and CBH are linked to Asn-10,^{10,11)} the glycopeptides were isolated from the pronase digests of the A-chains by Sephadex G-50 gel filtration followed by Dowex 1 × 2 column chromatography and

TABLE I. CARBOHYDRATE AND AMINO ACID COMPOSITIONS OF GLYCOPEPTIDES FROM RICIN D AND CBH

Sugars and Amino acids	Glycopeptides from ricin D			Glycopeptides from CBH			
	A-Chain	B-Chain		A-Chain	B-Chain		
	RA-1	RB-1	RB-2	HA-1	HB-1	HB-2	HB-3
Fuc	0.86 (1)			0.75 (1)	0.81 (1)		
Xyl	1.12 (1)			0.94 (1)	1.03 (1)		
Man	2.91 (3)	5.68 (6)	6.27 (6)	2.80 (3)	2.87 (3)	5.94 (6)	6.23 (6)
GlcNAc	1.87 (2)	1.67 (2)	1.64 (2)	1.77 (2)	1.78 (2)	1.84 (2)	1.96 (2)
CM-Cys					0.89 (1)		
Asp	1.00 (1)	2.00 (2)	2.00 (2)	1.00 (1)	1.00 (1)	2.00 (2)	2.00 (2)
Thr	0.94 (1)	0.92 (1)	0.91 (1)	0.92 (1)			0.82 (1)
Glu			0.99 (1)				1.00 (1)
Pro			0.93 (1)				0.88 (1)
Gly		1.06 (1)					
Ile	1.01 (1)			1.03 (1)			
Phe	1.07 (1)			0.96 (1)			
Arg						0.93 (1)	
Trp ^a			+				+
Corresponding sequence	10 I-N-F-T	95 D-N-G-T	135 N-N-T- Q-P-W	10 I-N-F-T	79 N-C	95 D-N-R	135 N-N-T- Q-P-W

^a Tryptophan was spectrophotometrically detected without hydrolysis.

reverse-phase HPLC. Only one glycopeptide was isolated from the A-chains of ricin D and CBH, and these are referred to as glycopeptide RA-1 and HA-1, respectively. Since the oligosaccharide moieties in the B-chain are linked to Asn-95 and Asn-135 in ricin D⁽¹²⁾ and to Asn-79, Asn-95, and Asn-135 in CBH,⁽¹³⁾ the glycopeptides from the B-chains were first fractionated as tryptic glycopeptides and then isolated as pronase glycopeptides. Gel filtration of the tryptic digests of the B-chains on a Sephadex G-50 column yielded two glycopeptide fractions (RBT-1 and RBT-2) from ricin D and three fractions (HBT-1, HBT-2 and HBT-3) from CBH. Fractions RBT-1 and HBT-1 were purified by Sephadex G-50 gel filtration followed by Dowex 1×2 column chromatography. Fractions RBT-2, HBT-2 and HBT-3 were digested with pronase after purification by Dowex 1×2 column chromatography, and the resulting glycopeptides were purified by Sephadex G-50 gel filtration. One glycopeptide each was isolated from fractions RBT-1, HBT-1, and HBT-2 and these are

FIG 1. Gas Chromatograms of Methanolysates of Glycopeptides from the A- and B-Chains of Ricin D (A) and CBH (B).

Samples were methanolized with inositol as an internal standard and then analyzed. (1) fucose, (2) xylose, (3) mannose, (4) *N*-acetylglucosamine, and (ST) inositol.

referred to as glycopeptides RB-1, HB-1, and HB-2, respectively. Fractions RBT-2 and HBT-3 were separated into two glycopeptide

fractions of which the major fraction was a pure glycopeptide. These are referred to as glycopeptides RB-2 and HB-3, respectively.

The amino acid and carbohydrate compositions of these glycopeptides were analyzed. As shown in Fig. 1, mannose, xylose, and fucose were found as neutral sugars in glycopeptides RA-1, HA-1, and HB-1, while only mannose was found in glycopeptides RB-1, RB-2, HB-2, and HB-3. The amino acid and carbohydrate compositions of these glycopeptides are shown in Table I. The oligosaccharide chain linked to Asn-10 in both A-chains and Asn-79 in the B-chain of CBH are of the complex type; it consists of xylose, fucose, mannose, and *N*-acetylglucosamine in a molar ratio of 1:1:3:2. The oligosaccharide chains linked to Asn-95 and Asn-135 in both B-chains are of the high mannose type; they consist of mannose and *N*-acetylglucosamine in a molar ratio of 6:2.

Details of this study and the complete structure of these glycopeptides will be published in

a following paper.

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