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Structural analysis of the carbohydrate chains isolated from mistletoe (*Viscum album*)lectin I *

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ABSTRACT

Two glycopeptide fractions prepared from mistletoe (*Viscum album*) lectin I by Pronase digestion were fractioned by affinity chromatography on a concanavalin A–Sepharose column. With 400-MHz ^{1}H NMR spectroscopy, in conjunction with sugar analysis, the following oligosaccharide structures could be determined: two oligomannose-type glycans in the ratio 4:1, one containing six mannose and the other containing five mannose units, both with two 2-acetamido-2-deoxyglucose units. In addition, a mannotriosyl → *N*,*N*'-diacetylchitobiose glycan containing a xylosyl group and an α-fucosyl group (1 → 3)-linked to the 2-acetamido-2-deoxyglycosyl-*1* residue, a common core element of many plant glycoproteins, was also observed.

INTRODUCTION

Three different lectins have been isolated from mistletoe (*Viscum album*) grown on the locust tree (*Robinia pseudoacacia*) by affinity chromatography[1]. The D-galactose-specific mistletoe lectin I (MLI) is a naturally occuring dimer (mol wt 115 000) of two different chains connected by disulfide bonds. Chain A (34 000 daltons) displays mitogenic activity and cytotoxicity, and chain B (29 000 daltons) is a D-galactose-specific lectin[2]. Both chains are glycoproteins and the sugar content of MLI was found to be between 10.1 (ref. 1) and 11% (ref. 3). We describe herein the structural analysis of the carbohydrate chains of *N*-glycosylpeptides isolated from mistletoe (*Viscum album*) lectin I.

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* Dedicated to Professor Jean Montreuil.

EXPERIMENTAL

Materials.—Mistletoe lectin I was isolated from mistletoe (*Viscum album*) grown on the locust tree (*Robinia pseudoacacia*) by affinity chromatography on acid-treated Sepharose as described[1]. Pronase (grade B) was purchased from Calbiochem, (La Jolla, CA, USA); Con A–Sepharose from Pharmacia (Bois d'Arcy, France), and Bio-Gel P-2 (200–400 mesh) from Biorad (Vitry sur Seine, France). [1-C^{14}]Acetic anhydride (0.37–1.1 GBQ/mmol) was purchased from Radiochemical Center (Amersham, UK) and Aqualyte liquid scintillation cocktail from J.T. Baker Chemicals (Deventer, Netherlands).

400 MHz ^1H NMR spectroscopy.—Desalted glycopeptides were repeatedly treated with ^2H_2O (99.95%, Commissariat à l'Énergie Atomique, Saclay, France) at pH 6–7 and room temperature with intermediate lyophilizations. 400-MHz ^1H NMR spectroscopy was performed with a Bruker AM-400 spectrometer (Centre Commun de Mesures, Université des Sciences et Techniques de Lille Flandres–Artois), operating in the pulsed FT mode at a probe temperature of 27°. Chemical shifts (δ) are expressed downfield from the signal of internal sodium 4,4-dimethyl-4-silapentane-1-sulfonate, but were actually measured by reference to internal acetone (δ 2.225 in ^2H_2O at 27°).

Carbohydrate analysis.—Samples of the two glycopeptide fractions (containing 5 μg of total sugars) were methanolyzed (0.5 M methanolic HCl, 24 h, 80°) in the presence of *meso*-inositol as internal standard (1 μg). The per-*O*-(trimethylsilyl) methyl glycosides (after *N*-reacetylation) were analyzed by GLC in a capillary column (0.32 mm × 25 m) of silicone OV 101 (ref. 4).

Preparation of glycopeptides from mistletoe lectin I.—Mistletoe lectin I (28.5 mg) was exhaustively digested for 72 h with Pronase according to Muramatsu et al.[5]. The resulting glycopeptides were separated from the nonglycosylated peptides by gel filtration on a Bio-Gel P-2 column (3 × 75 cm), equilibrated in water at a flow rate of 9 mL/h. Aliquots (10 μL) of each collected fraction (3 mL) were applied to a silica gel plate (Kieselgel 60, Merck, Darmstadt, Germany). After being dried, the plate was sprayed with a freshly prepared solution of 0.2% orcinol in 20% H_2SO_4. The fractions containing carbohydrates were pooled and lyophilized. These glycopeptides were *N*-[^{14}C]acetylated according to Koide and Muramatsu[6], and desalted on a Bio-Gel P-2 column (2 × 60 cm) equilibrated with water. Fractions (1.5 mL) were collected and the radioactivity of 5-μL aliquots in 4 mL of Aqualyte scintillation cocktail was counted in a Beckman LS-1800 scintillation counter. Desalted *N*-[^{14}C]acetylated glycopeptides were pooled and concentrated with a rotary evaporator under reduced pressure.

Affinity chromatography of N-[^{14}C]acetylated glycopeptides on Con A–Sepharose.—*N*-[^{14}C]Glycopeptides were disolved in 1 mL of NaOAc elution buffer (5 mM NaOAc, pH 5.2, containing 0.1 M NaCl and mM $MnCl_2$, $MgCl_2$ and $CaCl_2$) and applied to a 2.5 × 15 cm column of Con A–Sepharose equilibrated in the acetate buffer. Elution was carried out first with five column-volumes of the

acetate buffer at a flow rate of 9 mL/h and 3-mL fractions were collected. The Con A–Sepharose column was then eluted with 10 mM methyl α-D-glucopyranoside in the acetate buffer, and finally with the same buffer containing 0.5 M methyl α-D-glucopyranoside. After counting the radioactivity of 5-μL aliquots, the retarded fractions and the fractions eluted with 0.5 M methyl α-D-glucopyranoside were pooled separately and desalted on a Bio-Gel P-2 column (2 × 60 cm) equilibrated with water. The two desalted fractions were then lyophilized.

RESULTS

Affinity chromatography on Con A–Sepharose of glycopeptides, prepared from mistletoe lectin I by Pronase digestion, yielded two fractions (Fig. 1). Glycopeptides of Fraction I, weakly interacting with concanavalin A, were eluted from the column by the starting buffer as a retarded fraction. On the contrary, glycopeptides of Fraction II strongly interacted with the lectin and are eluted with 0.5 M methyl α-D-glucopyranoside in the starting buffer.

The relative amounts and carbohydrate compositions of the two glycopeptide fractions are given in Table I. From the yield of the two purified glycopeptide fractions, the carbohydrate content of the mistletoe lectin I was estimated to be 10.5% (w/w) and monosaccharide analysis indicated the glycans to be of the

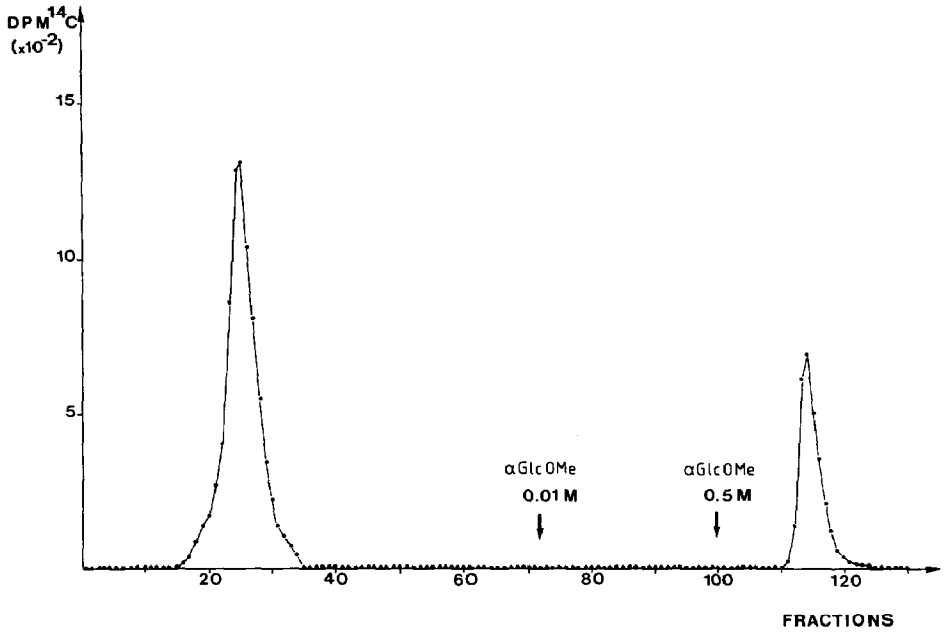


Fig. 1. Fractionation pattern of the glycopeptides from mistletoe lectin I by affinity chromatography on Con A–Sepharose; flow rate 9 mL/h; fraction volume, 3 mL. Abbreviation: αGlcOMe, methyl α-D-glucopyranoside.

TABLE I

Relative amounts and molar carbohydrate composition of glycopeptides isolated from mistletoe lectin I

Monosaccharides	Fraction I	Fraction II
Fuc	0.7	0
Xyl	1	0
Man [a]	3	3
GlcNAc [b]	1.3	0.7
Relative amounts (%) of Fractions	53	47

[a] The number of mannose residues was arbitrarily estimated to be 3. For Fraction II, this value should be ~5.8 for 2 GlcNAc residues, according to the ^{1}H NMR data, which indicate $Man_6GlcNAc_2$ and $Man_5GlcNAc_2$ oligomers in a ratio of 4:1. [b] These values are lower than expected because of the high stability of the GlcNAc-Asn linkage towards methanolysis.

N-glycosidic type (Table I). The sugar components of Con A-weakly bound glycopeptides (Fraction I; yield, 1.6 mg) are fucose, xylose, mannose, and *N*-acetylglucosamine in the molar ratio 0.7:1.0:3.0:1.3 (relative to mannose, 3) (Table I). This result suggested that the Con A-weakly bound glycopeptides contain sugar chains closely related to the xylose-containing glycans found in several plant glycoproteins, such as pineapple stem bromelain[7], as well as in a number of legume lectins as reported by Ashford et al.[8].

The interpretation of the 400 MHz ^{1}H NMR spectrum of the Con A-weakly bound Fraction I (Fig. 2a) was based on the spectral data obtained for the glycopeptide from bromelain[9] (**1**) and for the major glycopeptide III from *Sophora japonica* lectin[10] (**2**). The chemical shifts of H-1 and H-2 of Man-*3*, Man-*4*, and Man-*4'*, and H-1, H-2, H-3, and H-5a of Xyl (Table II) are identical to those observed for the glycopeptide from *S. japonica* lectin[10]. These data demonstrate the presence of a β-D-xylosyl group linked to Man-*3* by a (1 → 2) linkage, and Man-*4* as well as Man-*4'* groups in a nonreducing, terminal position. The presence of an α-L-fucosyl group linked to GlcNAc-*1* by a (1 → 3) linkage is shown by the chemical shifts of the *N*-acetyl proton of GlcNAc-*1* at δ 1.99, previously observed for the bromelain glycopeptide[9] and for the glycopeptide III from *S. japonica* lectin[10]. The compositional data and ^{1}H NMR spectroscopy data indicated that the structure of the carbohydrate moiety of the Con A-weakly bound glycopeptides from MLI is a xylosylmannotriosyl-*N N'*-diacetylchitobiose having an α-L-fucosyl group linked to GlcNAc-*1* by an (1 → 3) linkage (**2**).

The sugar composition of the Con A-strongly bound glycopeptides (Fraction II) (yield, 1.4 mg) indicated that the oligosaccharide chains consist only of mannose and *N*-acetylglucosamine in a molar ratio of 3:1 (Table I) and are of the oligomannosidic type. The interpretation of the 400-MHz ^{1}H NMR spectrum of the Con A-strongly bound Fraction II (Fig. 2b) was based on the spectral data obtained with Compound **63**, $Man_6GlcNAc_2$-Asn[11], and with pyridylaminoglycans prepared from Ricin D[12], and from *Ricinus communis* agglutinin[13], except for the chemical shifts of H-1 of GlcNAc-*2* and those of H-2 of GlcNAc-*1* which result

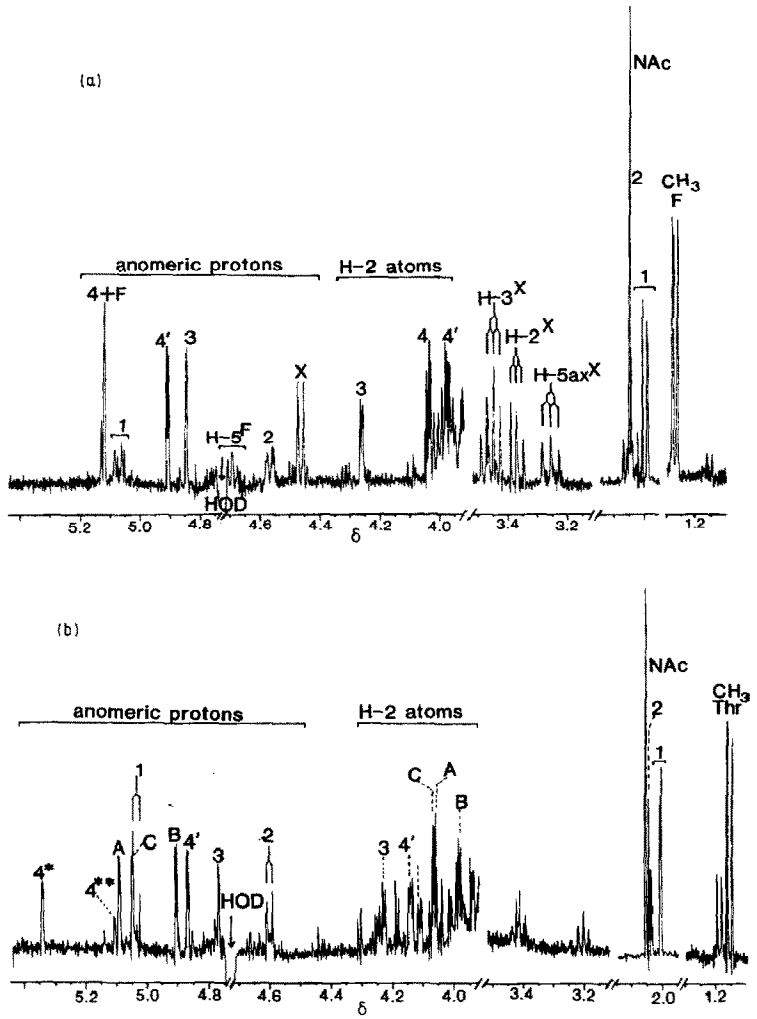
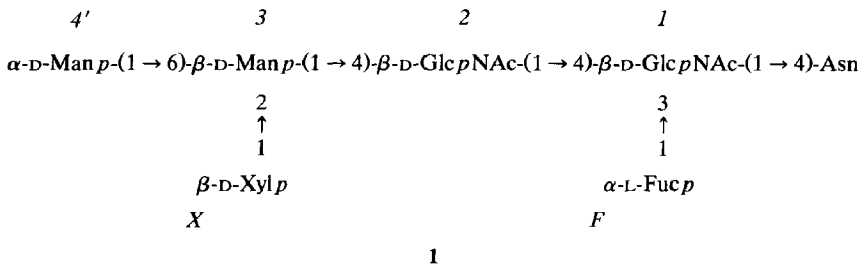


Fig. 2. 400-MHz ^{1}H NMR spectra of the glycopeptides isolated from mistletoe lectin I: (a) Con A-weakly bound D-xylose-containing glycopeptide Fraction I; (b) Con A-strongly bound glycopeptide Fraction II.

from the pyridylamino group of GlcNAc-*1*[14]. The chemical shifts of Con A-strongly bound Fraction II (Table III) are in good agreement with those of Compound **63**[11]. From these results, the structure of the carbohydrate moiety of the Con A-strongly bound glycopeptides from MLI is the oligomannoside glycan **3**.

However, the presence of a signal of low intensity at δ 5.108 is characteristic of an unsubstituted Man-*4* residue. This indicated the presence of a second oligomannoside-type glycan containing five D-mannose units (**4**). According to the ratio of Man 4* to Man 4** (Fig. 2b), this oligomannoside compound **4** represents ~ 20% of Con-A strongly bound Fraction II. All the other ^{1}H NMR parameters



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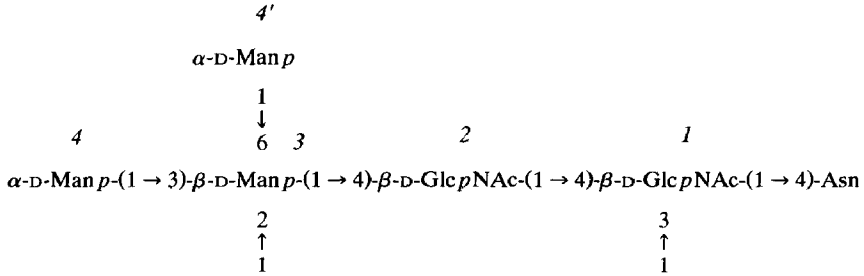

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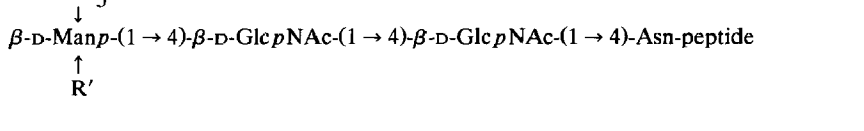
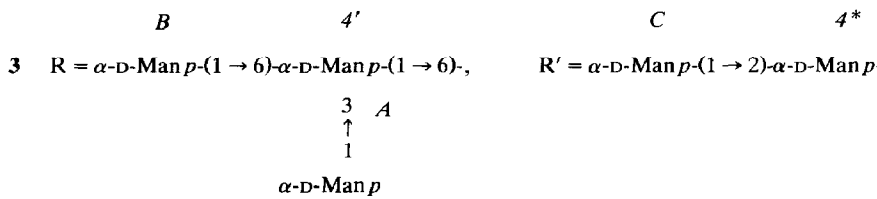
related to the mannose units common to the two oligomannoside-type glycans are completely superimposable.

DISCUSSION

In this study, mistletoe lectin I was shown to contain two different *N*-glycosyl-linked glycans. The first, compound **2**, is a common core element found in plant





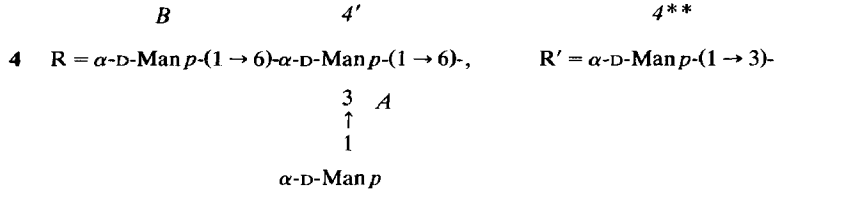


TABLE II

^{1}H-Chemical shifts of structural reporter groups of the constituent monosaccharides for the glycopeptide fraction I derived from mistletoe lectin I and for reference compounds derived from bromelain (**1**) and from lectin (**2**) [a]

Residue or group	Reporter group	*N*-Glycosylasparagine from		
		Bromelain[9] (**1**)	*Sophora japonica* lectin[10] (III) (**2**)	Glycopeptide Fraction I from MLI
GlcNAc-*1*	H-1	5.121	5.082	5.076 [b] / 5.067
	NAc	2.000	1.993	1.991 [b] / 2.008
GlcNAc-*2*	H-1	4.579	4.568	4.566 [b] / 4.570
	NAc	2.066	2.053	2.051
Man-*3*	H-1	4.839	4.849	4.848
	H-2	4.268	4.265	4.265
Man-*4*	H-1		5.122	5.122
	H-2		4.037	4.039
Man-*4'*	H-1	4.913	4.910	4.910
	H-2	3.988	3.980	3.976
Fuc-*3*	H-1	5.136	5.134	5.127
	H-5	4.722	[c]	4.703
	CH_3	1.285	1.290	1.274
	H-1	4.474	4.464	4.466
	H-2	3.385	3.372	3.372
Xyl	H-3	3.456	3.451	3.450
	H-5a	3.273	3.258	3.256

[a] Chemical shifts (δ) are given downfield from the signal of sodium 4,4-dimethyl-4-silapentane-1-sulfonate in ^2H_2O at 27°. [b] Two signals due to the heterogeneity of the peptide part. [c] Not detected.

N-glycosylproteins as in the protease inhibitor from barbados pride seeds (*Caesalpinia pulcherrina*)[15] or in the taste-modifying protein, miraculin, from berries of *Richadella dulcifica*[16]. The same glycan was also characterized in lectins from *Clerodendron trichotomum*[17], from five *Erythrina* species[8], from *Sophora japonica*[8,10], from *Lonchocarpus capassa*[8], from *Ricinus communis* Ricin D[12] and agglutinin[13], and from *Artocarpus integrifolia*[18].

The other glycans characterized in the mistletoe lectin I are two oligomannoside-type glycans (**3** and **4**) containing, respectively, six and five mannose and two *N*-acetylglucosamine units in a 4:1 ratio according to the ^{1}H NMR data (Fig. 2b). The same glycans were also characterized together with another oligomannoside-type glycan containing seven mannose residues in Ricin D[12] or with an oligomannoside-type glycan with four mannose units in *Ricinus communis* agglutinin[13]. In *Ricinus communis* Ricin D[12] and agglutinin[13], as well as in mistletoe lectin I, both a

TABLE III

^{1}H-Chemical shifts of structural reporter groups of the constituent monosaccharides for the glycopeptide Fraction II derived from mistletoe lectin I and for reference compounds, Compound **63** (ref. 11) and pyridylaminoglycan derived from Ricin D (ref. 12) [a]

Residue or group	Reporter group	Compound 63[11]	Pyridylaminoglycan from Ricin D[12]	Glycopeptide Fraction II from MLI
GlcNAc-*1*	H-1			5.039
	NAc			2.008 [b]
				2.010
GlcNAc-*2*	H-1	4.621	4.646	4.604
	NAc			2.060
Man-*3*	H-1	~ 4.78		4.771
	H-2	4.232	4.225	4.232
Man-*4*	H-1	5.345	5.357	5.344
	H-2	4.100	4.120	4.114
Man-*4'*	H-1	4.869	4.878	4.871
	H-2	4.145	4.155	4.144
Man-*A*	H-1	5.090	5.098	5.095
	H-2	4.070	4.073	4.065
Man-*B*	H-1	4.908	4.914	4.908
	H-2	3.980	3.995	3.984
Man-*C*	H-1	5.052	5.059	5.052
	H-2	4.070	4.073	4.068

[a] Chemical shifts (δ) are given downfield from the signal of sodium 4,4-dimethyl-4-silapentane-1-sulfonate in ^2H_2O at 27°. [b] Two signals due to the heterogeneity of the peptide part.

fucosyl- and xylosyl-containing glycan and oligomannoside-type glycans containing 4–7 mannose residues were found simultaneously.

Ricinus communis Ricin D[12] and agglutinin[13], as well as the mistletoe lectin I[2], are glycoproteins consisting of A and B chains linked by disulfide bonds; A chains are powerful toxins and B chains possess the sugar-binding sites. In *Ricinus communis* Ricin D[12] and agglutinin[13], the A-toxic chains possess only fucosyl- and xylosyl-containing glycans. To further establish analogies between those *Ricinus communis* glycoproteins and the mistletoe lectin I, the glycan structures present on isolated A and B chains of MLI remain to be studied.

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