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Note

Structural analysis of the carbohydrate chain isolated from jacalin lectin*

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The seeds of the jackfruit, *Artocarpus intergrifolia* [1-3], contain a haemagglutinating lectin called jacalin[4] which was found to be a strong mitogen for human T cells and an activator for secretion of immunoglobulin Ig by B cells[5]. In contrast, Saxon *et al.*[6] reported an inhibitor effect on B cell (Ig) production and an activation of T suppressor cells. Jacalin precipitates monomeric and polymeric monoclonal (MC) IgA_1 and polyclonal (PC) milk sIgA, but not MC IgA_2 of both m(1) and m(2) allotypes, MC IgD, IgE, IgM, PC IgG, free secretory component, and J chain[7]. Recently, Hagiwara *et al.*[8] reported the interactions of jacalin with human IgA_1 by use of the latex-agglutination technique. This lectin preferentially bound to nonreducing *α*-D-galactosyl groups. Jacalin recognizes also the terminal *β*-D-Gal*p*-(1→3)-*β*-D-Gal*p*NAc group, as in the IgA_1-hinge, and Gal*p*NAc group, but not the *β*-D-Gal*p*-(1→4)-*β*-D-Glc*p*NAc nor *β*-D-Gal*p*-(1→6)-*β*-D-Glc*p*NAc group and their sialylated extensions[8].

The method described herein allowed us to prepare 250 mg of jacalin. In the profile obtained in sodium dodecyl sulfate-poly(acrylamide)gel electrophoresis (SDS-PAGE) under reducing conditions (Fig. 1), the two peaks correspond to the same peaks given by the lectin obtained by affinity chromatography on various D-galactose-containing columns[8]. The major peak has a mol. wt. of 15 000 and the minor peak of 18 000. The specific activity of the lectin preparation obtained by the procedure described herein was comparable to that of the material obtained by affinity chromatography. The minimum concentration necessary to completely agglutinate a 2% suspension of human red blood cells was 180 ng. mL^{-1}. The carbohydrate composition of the glycopeptide, obtained by Pronase digestion, followed by Bio-Gel P-4 fractionation, was identi-

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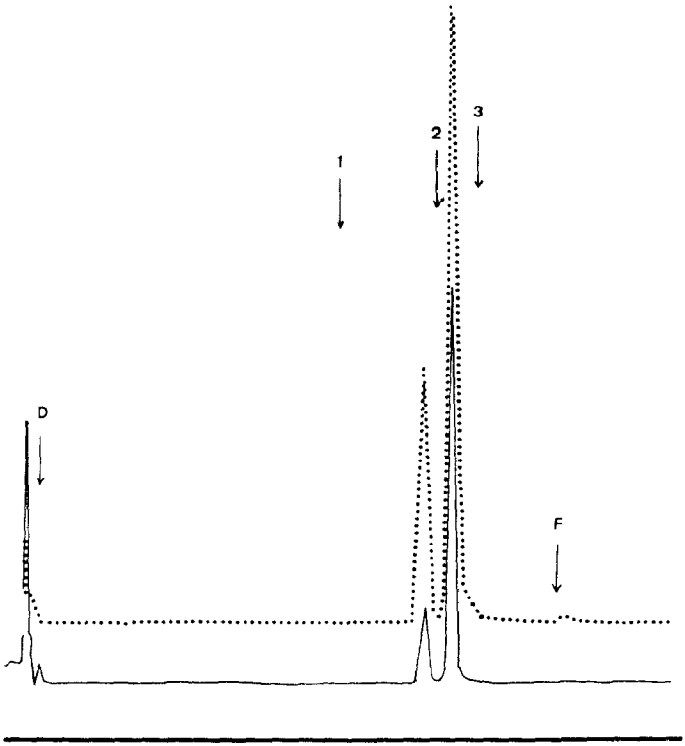

Fig.1. Scannings of 15% SDS-Page analysis under reducing conditions: (...) Lectin purified by affinity chromatography (Vector Lab.); (—) lectin obtained by the procedure described herein. Standards were: (1) Soy-bean trypsine inhibitor (mol. wt. 21 500), (2) myosine light chain (mol. wt. 18 000), and (3) lysosyme (mol. wt. 14 400).

cal to that of the native glycoprotein (Table I), suggesting that the glycopeptide possesses a structure closely related to that of the carbohydrate component of *Sophora japonica* lectin[9] and several plant glycoproteins.

The interpretation, in terms of primary structural assignments, of the 400-MHz, ^{1}H-n.m.r. spectrum of the glycopeptide obtained by Pronase digestion (Fig. 2) was

TABLE I

Carbohydrate composition of jacalin lectin and glycopeptide derived from lectin

Compound	*Monosaccharides*[a]			
	Xylose	*Fucose*	*Mannose*	*2-Acetamido-2-deoxyglucose*
Jacalin lectin	1.0	0.9	3.0	1.3
Glycopeptide	1.0	0.9	3.1	1.3

[a]Molar composition, xylose content being taken as 1.0.

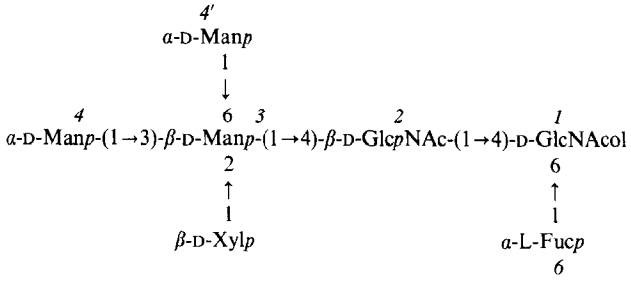

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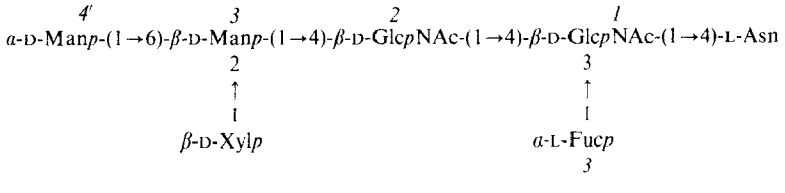
TABLE II

Relevant ^{1}H-n.m.r. parameters characteristic of constituent monosaccharides for the glycopeptides derived from jacalin lectin, and for reference coupounds **1**, **2** and **3**[a]

Residue or group	*Reporter group*	*Chemical shifts in structures*			
		1[b]	**2**[c]	**3**[d]	**3**[d]
GlcNAc^1	H-1		5.121	5.082	5.042
	H-2	4.219			
	NAc	2.058	2.000	1.993	1.988
GlcNAc^2	H-1	4.718	4.579	4.568	4.561
	NAc	2.081	2.066	2.053	2.050
Man^3	H-1	4.884	4.839	4.849	4.846
	H-2	4.270	4.268	4.265	4.263
Man^4	H-1	5.124		5.122	5.120
	H-2	4.040		4.037	4.038
Man$^{4'}$	H-1	4.914	4.913	4.910	4.909
	H-2	3.982	3.988	3.980	3.974
Fuc^6	H-1	4.898			
	H-5	4.077			
	CH_3	1.225			
Fuc^3	H-1		5.136	5.134	5.128
	H-5		4.722	[f]	4.706[g]
	CH_3		1.285	1.277	1.274
Xyl	H-1	4.449	4.474	4.464	4.462
	H-2	3.379	3.385	3.372	3.374
	H-3	3.453	3.456	3.451	3.447
	H-5*a*	3.253	3.273	3.258	3.255

[a] Chemical shifst (δ) are given relative to the methyl signal of internal acetone (δ 2.225 for a solution in ^2H_2O at 27°). [b] From *H. pomatia* α-hemocyanin[10]. [c] From bromelain[11]. [d]From glycopeptide Fraction II from *S. japonica* lectin[9]. [e] From jacalin (this work). [f] Not determined. [g] Value obtained by homodecoupling of CH_3 of Fuc^3.



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based on the spectral data for oligosaccharide alditols obtained by hydrazinolysis of *H.pomatia*, α-hemocyanin[10] (**1**), glycopeptide from bromelain[11] (**2**), and glycopeptide Fraction III from *S. japonica* lectin[9] (**3**) (see Table II). The lectin glycopeptide having a D-xylosyl group on the tri-D-mannoside core gave for 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-5^{a} of Xyl values comparable to those observed for glycopeptide fraction III from *S. japonica* lectin[9], glycopeptide from *Erythrina cristagalli* lectin[12], and compound **1** corresponding to the oligosaccharide alditol obtained by hydrazinolysis of *H. pomatia* α-hemocyanin[10]. On the basis of these data, it was concluded that, in jacalin, the D-xylosyl group is linked to Man^{3} by a β-(1→2) linkage, and that Man ^{4} and Man $^{4'}$ are in the nonreducing terminal position. The same n.m.r. values were also observed for the chemical shifts of Fuc for jacalin and the *S. japonica* lectin, indicating that the L-fucosyl group is linked to GlcNAc^{1} by an α-(1→3) linkage. The evidence for this linkage was shown by the chemical shifts of the

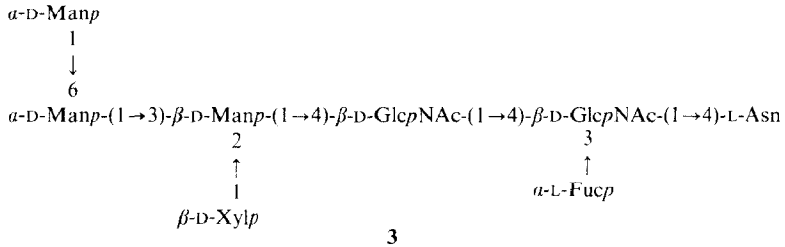

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TABLE III

Methylation analysis of glycopeptide from Pronase digestion of jacalin lectin

Methyl ethers of methyl glycosides	*Molar ratio*
2,3,4-Me_3-Fuc	0.84[a]
2,3,4-Me_3-Xyl	0.4[a]
2,3,4,6-Me_4-Man	1.7
4-Me-Man	1.0[b]
3,6-Me_2-GlcNAc	1.2
6-Me-GlcNAc	0.7[c]

[a] This value is lower than expected because of the relatively high volatility of this compound. [b] 4-Me-Man taken as 1.0. [c] This value is lower than expected because of the high stability of the GlcNAc-Asn linkage towards methanolysis.

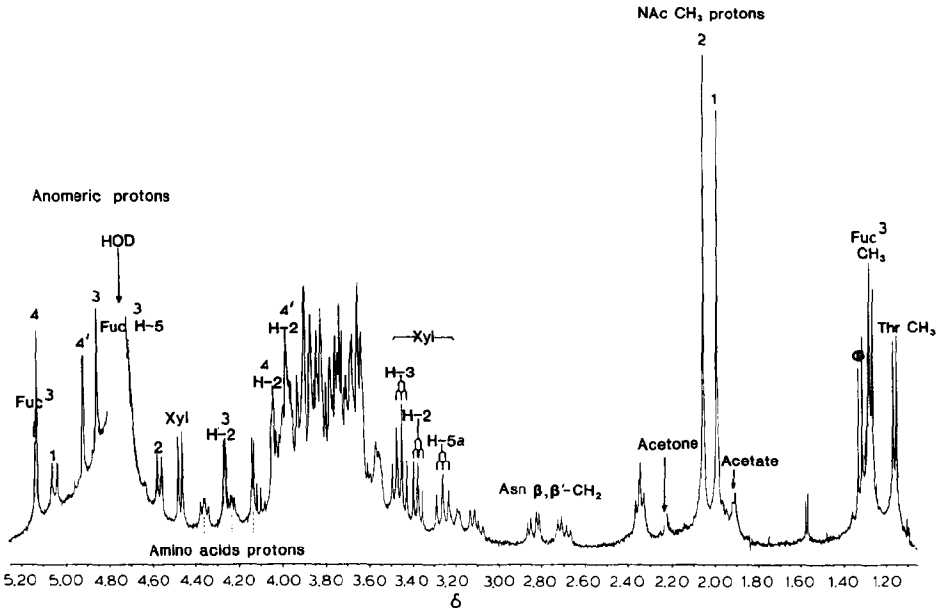

Fig. 2. 400-MHz, ^{1}H-n.m.r. spectrum of jacalin glycopeptide. (Φ) Signal produced by a frequently occuring, nonprotein, noncarbohydrate material.

N-acetyl protons of GlcNAx^1 and GlcNAc^2 (δ 1.988 and 2.049, respectively), previously observed for compound[11] **2** and for *S. japonica* lectin glycopeptide[9].

These results were confirmed by the analysis of the methyl ethers produced by methanolysis of the permethylated glycopeptide (Table III). The molar ratio of the methyl ethers is in agreement with the structure of the carbohydrate component of the glycopeptides from *S. japonica*[9] and *E. cristagalli*[12], *i.e.*, 2,3,4,6-tetra-*O*- and 4-*O*-methylmannoside derived from the trimannoside core substituted by a xylosyl-(1→2) group. The presence of per-*O*-methyl fucose and 2-amino-2-deoxy-6-*O*-methylglucose in the same ratio allowed us to conclude that the fucosyl group is linked to GlcNAc^1 by a (1→3) linkage. On the basis of the carbohydrate composition, methylation analysis, and ^{1}H-n.m.r. data structure **3** is proposed for the glycopeptide from jacalin. It is a biantennary, oligomannoside type with a β-D-xylosyl group linked to the β-D-mannosyl residue by a (1→2) linkage and an α-L-fucosyl group linked α-(1→3) to a 2-acetamido-2-deoxyglucose residue. Thus, this structure is a new example of a D-xylose-containing plant glycoprotein, it had been demonstrated previously γ for bromelain[11,13], *Vicia graminea* lectin[14], Sycamore cell laccase[15], lectins from *Erythrina cristagalli*[12] and *S. japonica*[9], more recently ricin[16], and ascorbic acid-oxidase of *Cucurbita pepo medullosa*[17].

EXPERIMENTAL

400-MHz, ^{1}H-n.m.r. spectroscopy. — The glycopeptide (0.3 mg) was repeatidly

treated with ^2H_2O (99,95% atom ^2H, C.E.A. France) at pD 7 with intermediate lyophilizations. Finally, the spectrum of the glycopeptide was recorded with a Bruker AM-400 WB spectrometer (Centre Commun de Mesure, Université des Sciences et Techniques de Lille Flandres-Artois) operating in the pulsed F.t. mode, at a probe temperature of 27°, and equipped with a Bruker Aspect 3000 computer.

Preparation of lectin from jackfruit (Artocarpus intergrifolia). — Fresh jackfruits were obtained from a local oriental market. The seeds (112 g) were collected and homogeneized in phosphate-buffered saline solution-5mM EDTA (600 mL) to give a crude extract. The isoluble material was removed by centrifugation and the lectin was precipitated by addition of 60% $(NH_4)_2SO_4$. The precipitate was collected, dissolved in 20 mM sodium cacodylate (pH 6.2) and dialyzed against the same buffer. The resulting material was passed through a column (2.5 × 30 cm) of SP-Sephadex (Pharmacia). The lectin was eluted by 100mM NaCl in the same buffer. The fractions active in the haemagglutination assay were pooled and dialyzed against 20 mM Tris · HCl (pH 8). The retentate was applied onto a column (2 × 20 cm) of DEAE-cellulose (Whatman, DE-52). All the active fraction was in the void volume. The purity was monitored by sodium dodecyl sulfate–poly(acrylamide) gel electrophoresis under reducing conditions[18].

Pronase digestion. — Purified lectin (200 mg) was dissolved in 150 mM Tris·acetate (50mL), pH 8.0, and 15mM $CaCl_2$ and predigested (30 min at 37° in the same buffer). Pronase (0.5 mL, 8 mg·mL^{-1}, Serva, Heidelberg, GFR) was added. After 16 h at 45–47°, the pH was measured (at 25°) and found to be 7.7. M Tris was added to increase the pH (at 25°) to 8.0, and another aliquot of Pronase (0.5 mL, 8 mg·mL^{-1}) was added. After 8 h the procedure was repeated. The digest was cooled to room temperature and cold (−20°) ethanol (450 mL) was added. The mixture was kept for 4 h at −20°. The precipitate was collected by centrifugation and dissolved in 50mM Tris·HCl (pH 8.0) containing 100 mM NaCl (4 mL). Only part of the precipitate could be redissolved; the remaining peptides were removed by centrifugation. Glycopeptides were separated from the nonglycosylated peptides by gel filtration in a Sephadex G-50 column (1.5 × 150 cm), equilibrated and developed in water. Sugars were detected by the orcinol–H_2SO_4 test. The fractions containing glycopeptides were concentrated and further purified by gel filtration in a Bio-Gel P-4 column (1.5 × 120 cm) equilibrated in water

Carbohydrate analysis. — A sample of glycopeptide (containing 5 μg of total sugars) was methanolyzed (0.5 M methanolic HCl, 24 h, 80°) in the presence of *meso*-inositol as internal standard (1 μg), and the per-*O*-trimethylsilylated methyl glycosides (after *N*-reacetylation) were analyzed[19] by g.l.c. in a capillary column (25 m × 0.32 mm) of Silicone OV 101.

Methylation analysis. — Micromethylation analysis of glycopeptide (60 μg) was performed according to the method of Ciucanu and Kerek[20]. The methyl ethers were identified after methanolysis (0.5M methanolic HCl, 24 h, 80°) of per-*O*-methylglycopeptide and *O*-acetylation (1:1, v/v acetic anhydride–pyridine) by g.l.c.-m.s.[21].

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