Carbohydrate Research, 236 (1992) 181–193
Elsevier Science Publishers B.V., Amsterdam

Structural analysis of the carbohydrate chain of glycopeptides isolated from *Robinia pseudoacacia* seed lectins *

Josiane Wantyghem [a], Nicole Platzer [b], Mireille Giner [a], Christian Derappe [a] and Yves Goussault [c]

[a] *Unité INSERM 180-UAC CNRS 71, 45 Rue des Saints-Pères, F-75270 Paris (France)*
[b] *Laboratoire de Chimie Organique Structurale, Université Pierre et Marie Curie, 4 Place Jussieu, F-75230 Paris (France)*
[c] *Laboratoire de Biochimie, Université Paris V, UFR Biomédicale des Saints-Pères, 45 Rue des Saints-Pères, F-75270 Paris (France)*

(Received July 16th, 1991; accepted in revised form March 5th, 1992)

ABSTRACT

Robinia pseudoacacia seeds contain lectins which are closely related. Pronase digestion of the dimeric and tetrameric lectins, RPA1 and RPA3, gave glycopeptides. The structure of the oligosaccharide was determined by ^{1}H NMR spectroscopy and carbohydrate determination as α-D-Man*p*-(1 → 3)-[β-D-Xyl*p*-(1 → 2)]-[α-D-Man*p*-(1 → 6)]-β-D-Man*p*-(1 → 4)-β-D-Glc*p*NAc-(1 → 4)-[α-L-Fuc*p*-(1 → 3)]-β-D-Glc*p*NAc-(1 → 4)-Asn. It appears that the 34-kDa constitutive polypeptide of RPA1 contains 4–5 carbohydrate chains whereas the 30.5-kDa and 29-kDa subunits of RPA3 contain two and one oligosaccharide chains, respectively.

INTRODUCTION

Robinia pseudoacacia seeds contain several lectins[1,2]. Two of these, RPA1 and RPA3, which exhibit an immunological cross-reactivity, have been particularly studied[1,2], they differ in their biochemical characteristics and in their biological properties. RPA1, which contains more carbohydrate chains than RPA3 is a dimeric molecule of M_r 63 000 constituted of identical subunits, whereas RPA3 is a tetramer of M_r 110 000 that contains two subunits of M_r 30 500 and two subunits of M_r 29 000. Monosaccharide analysis of RPA1 and RPA3 showed an almost identical composition consisting of GlcNAc, Man, Xyl, and Fuc in the molar proportions of ~2:3:1:1. These data suggest that the carbohydrate

Correspondence to: Dr. J. Wantyghem, Unité INSERM 180-UAC CNRS 71, 45 Rue des Saints-Pères, F-75270 Paris, France

* Dedicated to Professor Jean Montreuil.

component occurs as *N*-linked carbohydrate chains, the structure of which would be closely related to that of several plant glycoproteins, including *Erythrina cristagalli*[3], *Sophora japonica*[4], and *Artocarpus integrifolia*[5] lectins and that quantitative differences in total carbohydrate content is due to the number of carbohydrate chains contained in each lectin. In an attempt to further characterize the two lectins, the structure of the carbohydrate chain was established by ^1H NMR spectroscopy of glycopeptides obtained by Pronase digestion of RPA1 and RPA3. Moreover, glycopeptide analysis suggested an amino acid sequence for the glycosylation sites.

EXPERIMENTAL

Preparation of glycopeptides.–The purified lectins were prepared as previously described[1]. A lyophilized lectin sample (59 mg of RPA1, 105 mg of RPA3) was dissolved in 100 mM Tris · HCl buffer (pH 8.0), and 2 mM $CaCl_2$, and boiled for 20 min. Pronase (Calbiochem, Switzerland) was added in a 1:10 ratio (w/w) and the sample was digested for 24 h at 60°. A second digestion of 16 h was performed with the same amount of Pronase. The digest was boiled for 5 min, frozen, and lyophilized. The glycopeptides were separated from peptides and undegraded material by gel filtration in a Bio-Gel P-4 column (1.2 × 46 cm), equilibrated and eluted with 0.7% propanol in water. The elution was monitored by the absorbance at 280 nm and neutral sugars were detected by the orcinol–H_2SO_4 test. The fractions containing glycopeptides were pooled and lyophilized. The column was calibrated with Dextran Blue. ^{14}C-Labeled galactose, and bi- and tri-antennary glycopeptides were prepared by serial lectin-affinity chromatography.

The glycopeptide fraction III obtained from RPA3 was further fractionated on a Sepharose-Con A column (0.7 × 14 cm) (Pharmacia, France) under the conditions described by Merkle and Cummings[6]. The fractions obtained were rechromatographed as described above in the Bio-Gel P-4 column. The carbohydrate composition of the various glycopeptides was determined by the method of Chambers and Clamp[7] by GLC on a Varian 3700 chromatograph. Amino acids were analyzed after hydrolysis (6 M HCl, 0.005% 2-mercaptoethanol, 18 h at 110° under vacuum) with a Bio-tronik autoanalyzer Model LC 6000. Sequence analyses were carried out by automated Edman degradation on 2 nmol of glycopeptides with a gas-phase Applied Biosystem automated sequencer Model 470 A.

500-MHz ^1H NMR spectroscopy.–Glycopeptides were repeatedly treated with ^2H_2O (99.8 atom%, Sigma, France) at p^{2}H 6 with intermediate lyophilizations. ^1H NMR measurements were carried out on a Bruker WM 500 spectrometer operating in the pulse FT mode, at a probe temperature of 27°, and equipped with a Bruker Aspect 3000 computer. Resolution enhancement of the spectra was achieved by Lorentzian-to-Gaussian transformation. Chemical shifts (δ) are expressed downfield from the signal of internal sodium 4,4-dimethyl-4-silapentane-1-sulfonate by indirect reference to internal acetone (δ 2.225).

Poly(acrylamide) gel electrophoresis and affinity blotting.–RPA polypeptides were separated by SDS-PAGE on 12.5% poly(acrylamide) gels according to the procedure of Laemmli[8]. Electrotransfer on PVDF membrane (Millipore, France) was performed as described by Matsudaira[9]. The membrane was then soaked in 3% BSA in phosphate buffered 0.2 M NaCl solution in order to minimize hydrophobic interactions[10]. The blotted paper was sequentially treated with biotin-labeled Con A (Bio-Carb, Sweden) and streptavidin–peroxidase (Amersham, France). Staining was carried out for 5–40 min with 4-chloro-1-naphthol and H_2O_2.

RESULTS

Glycopeptides were obtained by Pronase digestion of RPA1 and RPA3. Fractionation on Bio-Gel P-4 (Fig. 1a) yielded three fractions (I–III) which contained carbohydrates. Similar profiles were observed for the two lectins. Fraction I, which was excluded, was discarded and corresponded to undegraded lectin or large proteolytic fragments. Fraction II was eluted at the position of purified, biantennary *N*-linked glycopeptides. Affinity chromatography on immobilized Con A of Fraction III, obtained from RPA3, showed that no glycopeptide was eluted with methyl α-D-glucopyranoside or -mannopyranoside. The unretained fraction gave a rather extended peak (Fig. 1b), which was divided into a nonretained (NR) and a retained peak (R). Both peaks were further chromatographed as described above on Bio-Gel P-4. The weakly retained Fraction R gave two fractions, R1 (eluted as II) and R2 (eluted as III). The carbohydrate and amino acid compositions of the five fractions (II-RPA1, II-RPA3, NR, R1, and R2) are reported in Tables I and II. The 500-MHz ^1H NMR spectra of these glycopeptides in ^2H_2O were recorded, and the spectra of three of them (II-RPA3, R1, and R2) are shown in Figs. 2–4. Signals of the structural reporter groups were assigned on the basis of their chemical shifts (Table III) and coupling constants (Table IV). The relevant NMR parameters of the various glycopeptides of RPA1 and RPA3 were compared to those of reference compounds[4,5,11]. Signals of the anomeric protons are well defined, except for II-RPA3 and R2 where H-1 of GlcNAc^1, Man^4, and Fuc^5 partially overlapped. Evidence for the presence of Fuc was obtained from the doublet of the methyl group in C-5 at δ 1.28, and from the multiplet of H-5 at δ 4.70. Structural reporter group signals of H-2, -3, and -5 of Xyl are well defined and their chemical shifts were further confirmed by selective irradiation. The small coupling constants of H-1 of Fuc are consistent with an α-L configuration, whereas the values obtained for H-1 of GlcNAc^1 and GlcNAc^2 are indicative of a β-D configuration. Amino acid analysis (Table II) revealed the presence of Asx in every glycopeptide. This is the only amino acid found in the R1 compound (multiplet of β, β′ protons at δ 2.72 and 2.86). Attributions of the signals due to additional amino acids are summarized in Table V. For the R2 compound, signals between δ 7.20 and 7.50 (Fig. 4) were associated with the aromatic core of Trp. Signals corresponding to Pro and Val could be observed in the δ 2.6–3.4 region. These

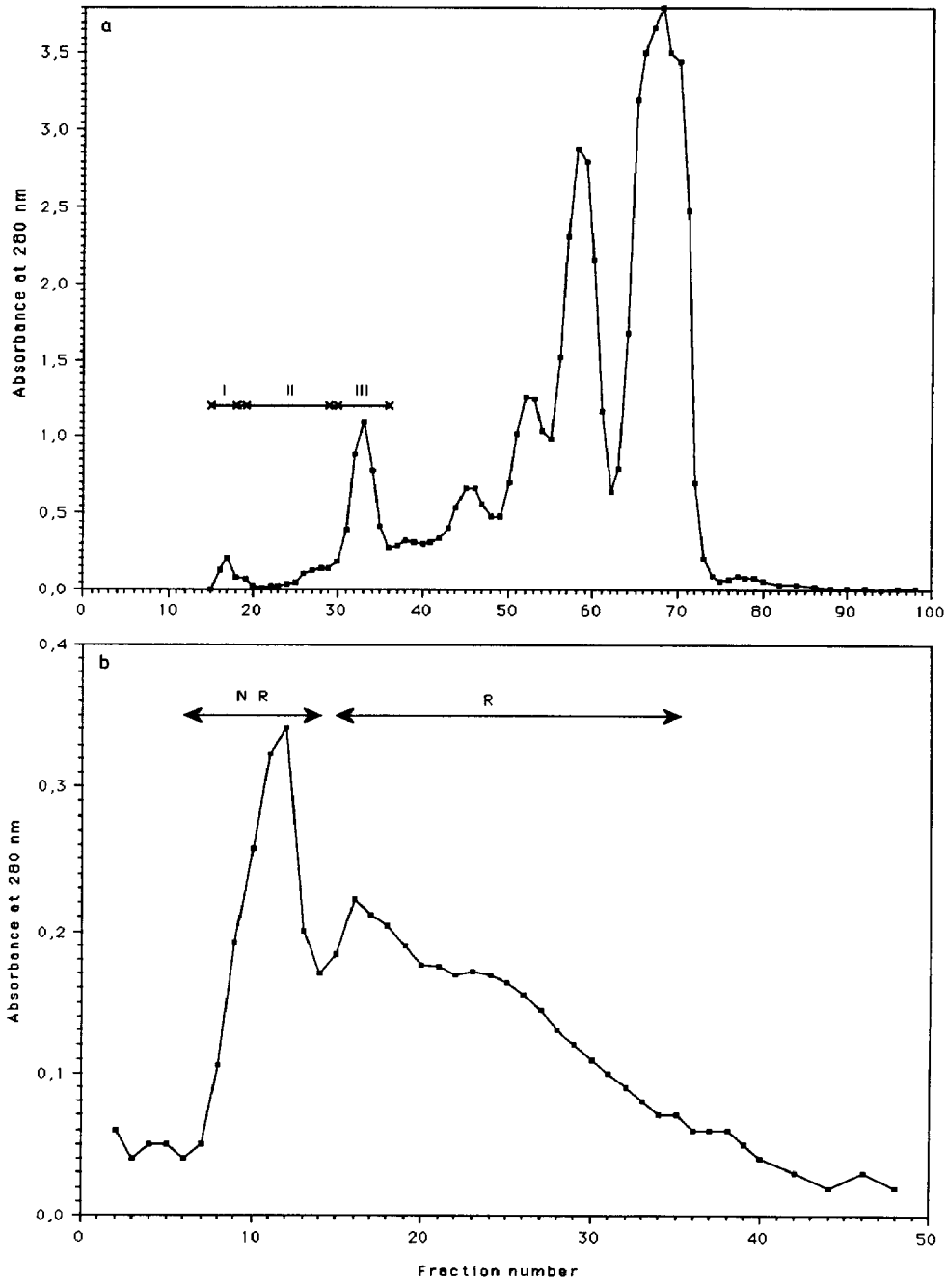


Fig. 1. Glycopeptide fractionation: (a) Gel filtration on Bio-Gel P-4 of the Pronase digest of RPA3. (b) Con A-affinity chromatography of Fraction III isolated by gel filtration. The volume of the column fractions was 1 mL in each case.

TABLE I

Carbohydrate composition of the various fractions [a]

Fraction	Xyl	Fuc	Man	GlcNAc
II-RPA1	1.14	0.95	3.0	1.46
II-RPA3	1.08	0.83	3.0	1.33
III-RPA3				
NR	0.98	0.62	3.0	1.31
R1	0.84	0.88	3.0	1.36
R2	0.66	1.03	3.0	1.63

[a] Molar composition (Man content of each glycopeptide is taken as 3).

TABLE II

Amino acid composition of the various fractions [a]

Fraction	Asx	Glx	Pro	Gly	Ala	Thr	Ser	Leu	Lys	Val
II-RPA1	1.0	0.72	0.65	0.51	0.25	[b]	[b]	[b]		
II-RPA3	1.0								0.79	
III-RPA3										
NR	1.0	[b]	[b]	[b]						[b]
R1	1.0									
R2	1.0		0.97							0.86

[a] Molar compositions (Asx content of each glycopeptide is taken as 1). [b] Traces.

two amino acids were detected in a molar ratio close to that of Asx (0.97 and 0.86, respectively, Table II). The low amount of glycopeptide NR prevented any accurate determination of the peptide sequence. The amino acid composition of the glycopeptide released from RPA1 is complex and includes amino acids present in RPA3 glycopeptides. This fraction appeared as a mixture of peptides or glycopeptides (or both). The amino acid sequence of glycopeptides II-RPA3 and R2 was determined by sequential Edman degradation and is reported in Table V.

Affinity labeling with biotin-labeled Con A showed that the polypeptide chains of RPA1 (34 kDa) and RPA3 (30.5 and 29 kDa) are glycosylated (Fig. 5) and that the staining intensity is correlated with the molecular mass (34 > 30.5 > 29 kDa). No reaction was obtained in the presence of methyl α-D-mannopyranoside.

DISCUSSION

The chemical shift values for the carbohydrate units observed for the various glycopeptides obtained from RPA1 and RPA3 are comparable to those observed for other plant glycoproteins by ^{1}H NMR techniques. It could be assumed that the main structure 1 of the oligosaccharide component of these two lectins consists of a heptasaccharide where the common $Man_3GlcNAc_2$ pentasaccharide core of *N*-glycoproteins is substituted with an α-L-fucosyl group (1 → 3)-linked to the (potentially) reducing 2-acetamido-2-deoxy-D-glucose unit and a β-D-xylosyl group

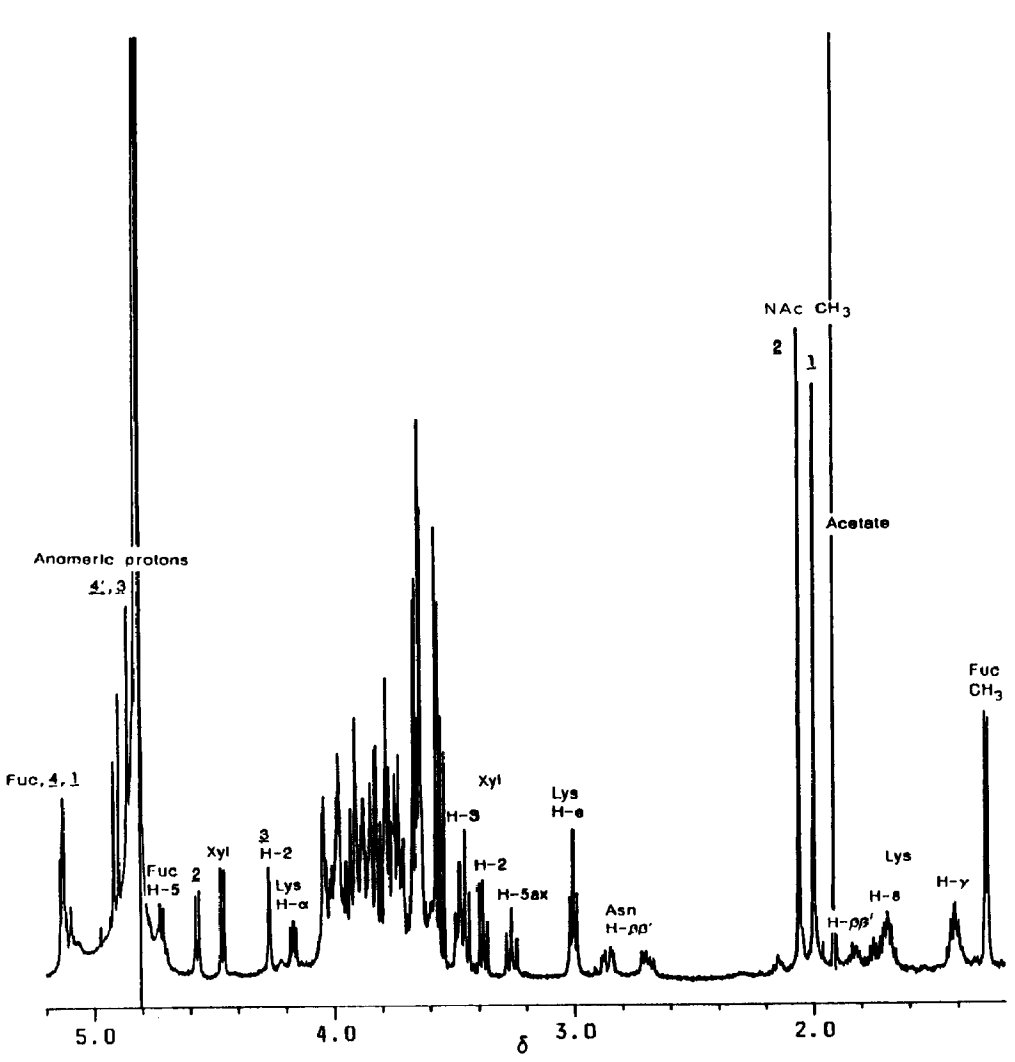
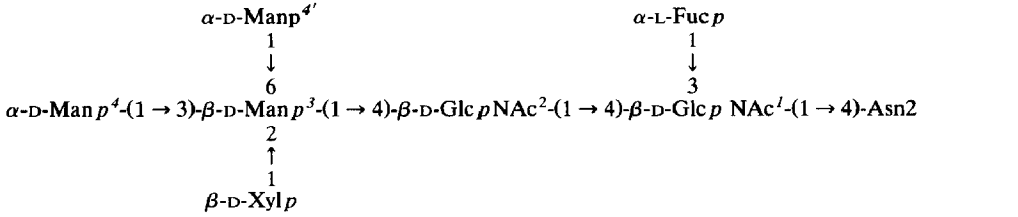


Fig. 2. Structural reporter groups of the resolution-enhanced, 500-MHz ^{1}H NMR spectrum of RPA3 glycopeptide II (II-RPA3) (900 μg of neutral hexoses).

(1 → 2)-linked to the β-D-mannosyl residue; the α-L-fucosyl group exhibits the characteristic linkage observed in plant glycoproteins. Amongst the plant oligosaccharides that have been examined so far, two types of carbohydrate component have been reported: high-mannose glycans found for instance in soybean ag-



1

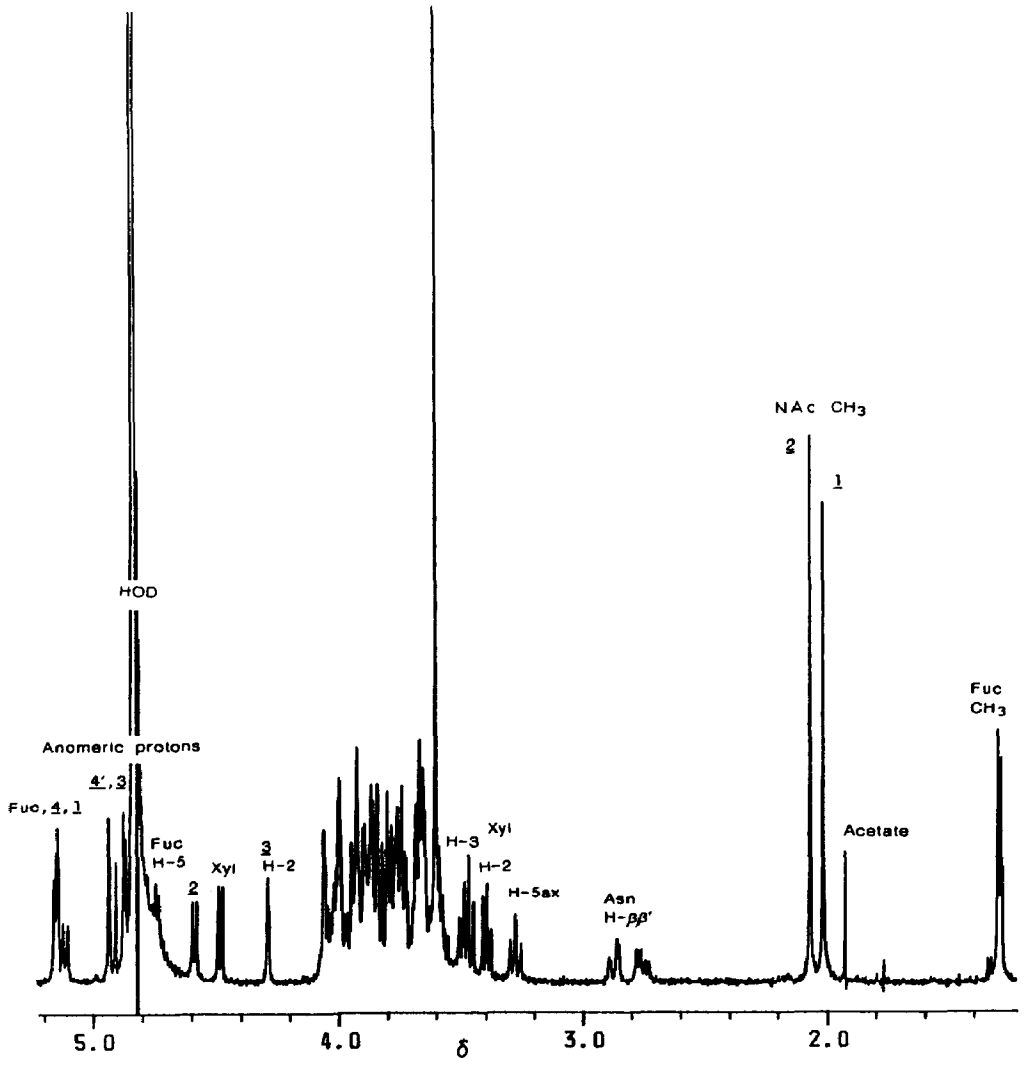


Fig. 3. Structural reporter groups of the resolution-enhanced, 500-MHz ^{1}H NMR spectrum of RPA3 glycopeptide III (R1) (490 μg of neutral hexoses).

glutinin[12] or biantennary, complex glycans which were identified in bromelain[13], sycamore laccase[14], and the protease inhibitor of *Caesalpinia pulcherrima*[15], and in various lectins[3–5]. The biosynthesis and posttranslational maturation of *N*-linked glycans in plants[16] follow the same initial steps as those of animal glycoproteins[17], and complex-type glycans result from the processing of the Man_9 structures in the Golgi apparatus but, plant glycoproteins differ from animal glycoproteins by the addition of a β-D-xylosyl group or a (1 → 3)-linked α-L-fucosyl group (or both) to the $Man_3GlcNAc_2$ core[18]. Studies of the seed storage proteins of the common bean, phytohemagglutinin[19], and phaseolin[20], which have one high-mannose and

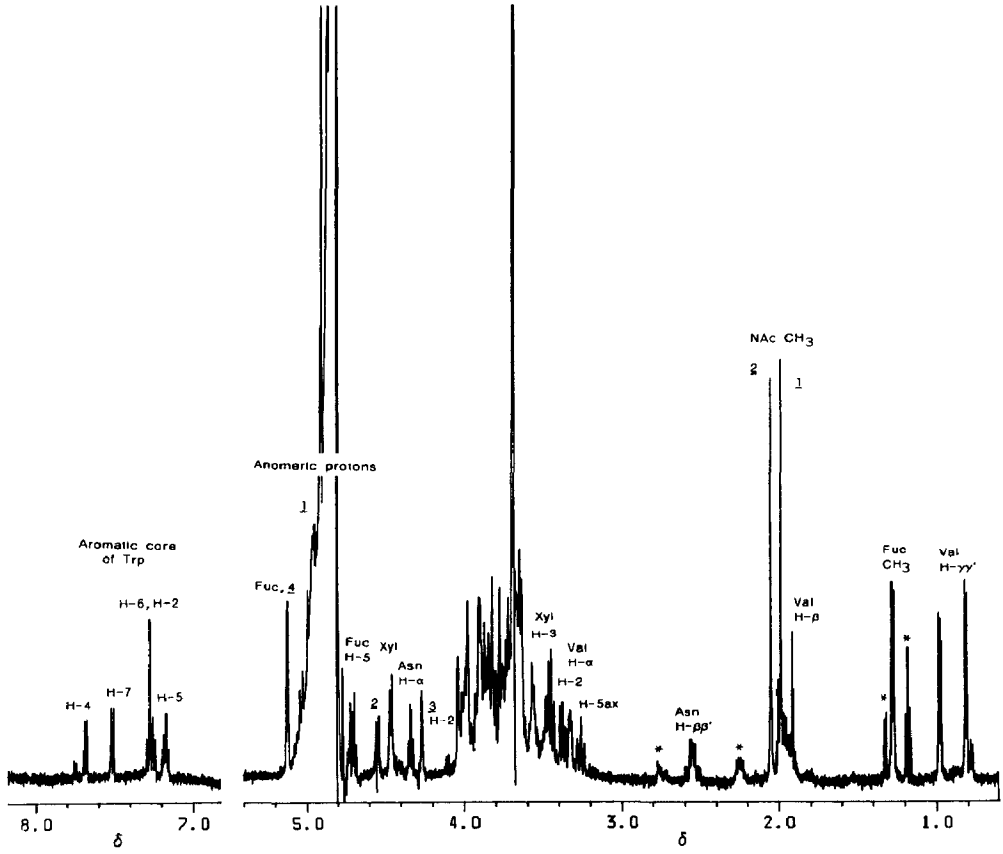


Fig. 4. Structural reporter groups of the resolution-enhanced, 500-MHz ^{1}H NMR spectrum of RPA3 glycopeptide III (R2) (440 μg of neutral hexoses); * not attributed

one modified chain, have shown that the high-mannose chain is almost completely protected from glycosidase digestion in vitro. It was inferred that the accessibility of this structure would be the determinant in its processing and conversion towards the complex form. However the mechanism which determines this process has still to be resolved. The high-mannose oligosaccharide of phaseolin is linked to an Asn residue located in a hydrophobic protein domain, whereas the processed glycan, either $Man_7GlcNAc^2$ or complex glycan, is found in a hydrophilic domain. Thus, extensive processing, i.e., formation of the complex glycan, is observed only when the high-mannose site is unoccupied[19]. In contrast, the high-mannose and complex glycans of phytohemagglutinin are in a hydrophilic environment[20], and the deletion of one of the two glycosylation sites by site-directed mutagenesis does not modify the structure of the residual glycan[21]. This would indicate that protein folding rather than the hydrophobic or hydrophilic character of the glycosylation site determines the conversion of high-mannose structures into complex ones. In this study, glycans of *Robinia pseudoacacia* lectins seem to be solely of the complex

TABLE III

Chemical shifts (δ) of the carbohydrate component of RPA glycopeptides

Residue or group, and reporter group	Reporter compound [a]			RPA glycopeptide				
	A	B	C	II (RPA1)	II (RPA3)	III-RPA3		
						NR	R1	R2
β-D-Glcp^1NAc								
H-1	5.05	5.08	5.04	5.11	5.09	5.09	5.09	5.03
NAc	2.01	1.99	1.99	1.99	1.99	1.99	1.99	1.98
β-D-Glcp^2NAc								
H-1	4.61	4.57	4.56	4.57	4.57	4.57	4.57	4.53
NAc	2.07	2.05	2.05	2.05	2.05	2.06	2.05	2.04
β-D-Manp^3-(1 → 4)								
H-1	4.87	4.89	4.85	4.84	4.85	4.85	4.84	4.85
H-2	4.26	4.26	4.26	4.26	4.27	4.27	4.27	4.26
α-D-Manp^4-(1 → 3)								
H-1	5.12	5.12	5.12	5.12	5.12	5.12	5.12	5.12
H-2	4.04	4.04	4.04	4.04	4.04	4.04	4.04	4.03
α-D-Man$p^{4'}$-(1 → 6)								
H-1	4.91	4.91	4.91	4.91	4.91	4.92	4.91	4.90
H-2	3.98	3.98	3.97	3.98	3.98	3.99	3.97	3.97
β-D-Xyl$p$-(1 → 2)								
H-1	4.45	4.46	4.46	4.46	4.46	4.47	4.46	4.46
H-2	3.37	3.37	3.37	3.37	3.37	3.38	3.37	3.37
H-3	3.44	3.45	3.45	3.45	3.45	3.45	3.45	3.45
H-5*a*	3.25	3.26	3.25	3.26	3.26	3.27	3.26	3.26
α-L-Fuc$p$-(1 → 3)								
H-1		5.13	5.13	5.13	5.13	5.13	5.13	5.12
H-5			4.71	4.71	4.72	4.72	4.71	4.71
CH_3		1.28	1.27	1.27	1.28	1.28	1.28	1.26

[a] A, Glycopeptide from ascorbic acid oxidase of *Cucurbita pepo medullosa*[10]. B, Glycopeptide from *Sophora japonica* lectin[4]. C, Glycopeptide from *Artocarpus integrifolia* lectin (jacalin)[5].

type. Thus, it appears that the glycosylation sites of RPA1 and RPA3 are easily accessible to the glycosidases and glycosyltransferases involved in the processing of high-mannose structures.

Although the biantennary, complex-type oligosaccharide of RPA3 has the minimal structure recognized by Con A[22], it failed to interact with the immobilized lectin, and the presence of xylose or fucose could impair its retention by the lectin. The presence of a core (1 → 6)-linked α-L-fucosyl group in complex, biantennary *N*-linked oligosaccharides does not interfere with the binding to Con A, whereas certain oligosaccharides possessing a (1 → 4)-linked ("bisecting") 2-acetamido-2-deoxy-β-D-glucopyranosyl group are slightly retarded. The D-xylosyl group of plant glycoproteins has been considered analogous to the bisecting 2-acetamido-2-deoxy-D-glycosyl group of complex glycans of animal glycoproteins[3] and would influence

TABLE IV

Coupling constants (Hz) of the carbohydrate component of RPA glycopeptides

Redidue or group	II-RPA1	II-RPA3	III-RPA3		
			NR	R1	R2
β-D-Glcp^1NAc					
$J_{1,2}$		9.5	9.0	9.5	8.5
β-D-Glcp^2NAc					
$J_{1,2}$	7.5	8.2	9.5	8.2	9.5
β-D-Manp^3-(1 → 4)					
$J_{1,2}$					
$J_{2,3}$	2.3	2.8	2.0	3.2	
α-D-Manp^4-(1 → 3)					
$J_{1,2}$	1.5	1.7	1.5	1.7	
$J_{2,3}$	3.0	3.2	3.5	3.3	
α-D-Man$p^{4'}$-(1 → 6)					
$J_{1,2}$		1.7	1.5	2.0	1.5
$J_{2,3}$		3.5	3.5	3.0	3.0
β-D-Xyl$p$-(1 → 2)					
$J_{1,2}$	8.0	7.6	8.0	7.6	8.0
$J_{2,3}$	9.2	9.2	9.0	9.2	9.0
$J_{3,4}$	9.2	9.2	9.0	9.2	9.0
$J_{4,5a/5a,5e}$	10.9	11.1	11.5	11.1	11.0
α-L-Fuc$p$-(1 → 3)					
$J_{1,2}$		3.9	4.0	3.8	
$J_{5,6}$	6.5	7.0	6.5	6.5	6.5

the interaction with Con A. Faye et al.[19] reported that the modified glycopeptide of phytohemagglutinin is not retained on Sepharose–Con A, and Jaikaran et al.[23] described the preparation of germin, a wheat germ glycoprotein which contains complex *N*-glycans, by affinity chromatography on Con A. Our results are in agreement with both observations and suggest that the exposure of the carbohydrate chain would be different in glycoproteins and glycopeptides. The ability of discriminating between different glycopeptides through their peptide component further emphasizes the importance of the peptide part in the interaction with Con A.

From the proportions of neutral sugars detected after Pronase treatment of RPA1 and RPA3, the respective carbohydrate content of the two lectins was estimated to be 17 and 6%. Based on the molecular mass of each lectin, it could be estimated that RPA1 and RPA3 have, respectively, 8–10 and 6 glycan chains per molecule. Since it appeared that the 30.5-kDa constitutive polypeptide of RPA3 is more intensively stained than the 29-kDa chain and that the molecular-mass difference could be accomodated by one glycan chain, it could be assumed that (a) the 29-kDa chain and the 30.5-kDa chain bear, respectively, one and two glycan

TABLE V

Chemical shifts observed for the peptide component of RPA glycopeptides

Fraction	Sequence [a]	Atom	Chemical shift (δ)				
			Asn	Lys	Val	Pro	Trp
II-RPA1	[b]						
II-RPA3	Asn → Lys	H-α		4.16			
		H-$\beta\beta'$	2.69, 2.86	1.82, 1.75			
		H-γ		1.41			
		H-δ		1.69			
		H-ϵ		3.00			
III-RPA3	Asn → X						
NR		H-$\beta\beta'$	2.75, 2.86				
R1		H-$\beta\beta'$	2.72, 2.86				
R2	Val → Pro						
	→ Trp → Asn	H-α	4.32		3.31	(4.46)	(4.46)
		H-$\beta\beta'$	2.57		1.91		
			2.53			~ 2.0	
		H-$\gamma\gamma'$			0.80, 0.96		
		H-δ				~ 3.45	
		H-2					7.26
		H-4					7.67
		H-5					7.16
		H-6					7.24
		H-7					7.49

[a] By Edman degradation. [b] Not determined.

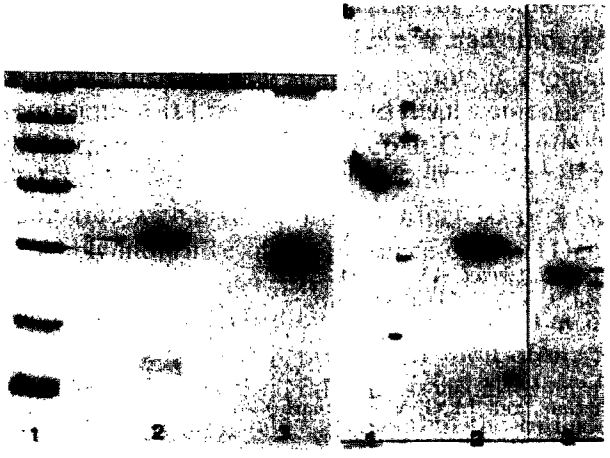


Fig. 5. SDS-PAGE analysis of RPA1 and RPA3: (a) Coomassie Blue staining. (b) Biotin-labeled Con A staining after electrotransfer. (1) Standard proteins (phosphorylase b, bovine serum albumin, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor, and lysozyme); (2) RPA1; and (3) RPA3.

chains, and (b), their polypeptide chains would have similar size. Two glycopeptides were isolated from RPA3, an Asn-Lys dipeptide (II-RPA3) and a tetrapeptide having a Val-Pro-Trp-Asn sequence (R2) which is rather hydrophobic. These glycopeptides would represent either the same or two different glycosylation sites. Besides the valine and proline residues, NR also contains trace amounts of Glx and Gly and could result from a different cleavage of the "R2" site, or could correspond to a third glycosylation site. Whether the different polypeptides of RPA1 and RPA3 represent different glycosylated forms of the same apoprotein remains to be determined. Evidence for two (or three) glycosylation sites could argue for differences in the sequence of the 29- and 30.5-kDa chains of RPA3, but it could also account for a partial occupancy of these sites. Indeed although the sequence of phytohemagglutinin-E has three glycosylation sites[24], only two of them are generally occupied[19]. These observations raise the question of the influence that glycosylation might have on the oligomerization of RPA1 as the presence of four to five glycan chains per RPA1 subunit could prevent the association in tetramer.

ACKNOWLEDGMENTS

This work was supported by INSERM (U 180), the UFR Biomédicale des Saints-Pères, the Fondation pour la Recherche Médicale, and the Ligue contre le Cancer. The authors are indebted to Dr. A.-M. Fiat (CNRS UA 1188), to M.-M. Bierge, C. Bauvy, and C. Goulut-Chassaing for their assistance in the sequence and the chemical analyses.

REFERENCES

1 J. Wantyghem, C. Goulut, J.-P. Frénoy, E. Turpin, and Y. Goussault, *Biochem. J.*, 237 (1986) 483–489.
2 G. Fleischmann and H. Rüdiger, *Biol. Chem. Hoppe-Seyler*, 367 (1986) 27–32.
3 D. Ashford, R.A. Dwek, J.K. Welply, S. Amatayakul, S.W. Homans, H. Lis, G.N. Taylor, N. Sharon, and T.W. Rademacher, *Eur. J. Biochem.*, 166 (1987) 311–320.
4 B. Fournet, Y. Leroy, J.-M. Wieruszeski, J. Montreuil, R.D. Poretz, and R. Goldberg, *Eur. J. Biochem.*, 166 (1987) 321–324.
5 C. Capon, F. Piller, J.-M. Wieruszeski, Y. Leroy, and B. Fournet, *Carbohydr. Res.*, 199 (1990) 121–127.
6 R.K. Merkle and R.D. Cummings, *Methods Enzymol.*, 138 (1987) 232–259.
7 R.E. Chambers and J.R. Clamp. *Biochem. J.*, 125 (1971) 1009–1018.
8 U.K. Laemmli, *Nature*, 227 (1970) 680–685.
9 P. Matsudaira, *J. Biol. Chem.*, 262 (1987) 10035–10038.
10 R. Hawkes, *Anal. Biochem.*, 123 (1982) 143–146.
11 G. D'Andrea, J.B. Bouwstra, J.P. Kamerling, and J.F.G. Vliegenthart, *Glycoconjugate J.*, 5 (1988) 151–157.
12 L. Dorland, H. Van Halbeek, and J.F.G. Vliegenthart, *J. Biol. Chem.*, 256 (1981) 7708–7711.
13 H. Ishihara, N. Takahashi, S. Oguri, and S. Tejima, *J. Biol. Chem.*, 254 (1979) 10715–10719.
14 N. Takahashi, T. Hotta, H. Ishihara, M. Mori, S. Tejima, R. Bligny, T. Akazawa, S. Endo and Y. Arata, *Biochemistry*, 25 (1986) 388–395.

15 S. Hase, S. Koyama, H. Daiyasu, H. Takemoto, S. Hara, Y. Kobayashi, Y. Kyogoku, and T. Ikenaka, *J. Biochem. (Tokyo)*, 100 (1986) 1–10.
16 H. Hori, D.W. James, and A.D. Elbein, *Arch. Biochem. Biophys.*, 215 (1982) 12–21.
17 R. Kornfeld and S. Kornfeld, *Annu. Rev. Biochem.*, 54 (1985) 631–664.
18 K.D. Johnson and M.J. Chrispeels, *Plant Physiol.*, 84 (1987) 1301–1308.
19 L. Faye, A. Sturm, R. Bollini, A. Vitale, and M.J. Chrispeels, *Eur. J. Biochem.*, 158 (1986) 655–661.
20 A. Sturm, J.A. Van Kuik, J.F.G. Vliegenthart, and M.J. Chrispeels, *J. Biol. Chem.*, 262 (1987) 13392–13403.
21 T.A. Voelker, E.M. Herman, and M.J. Chrispeels, *Plant Cell*, 1 (1989) 95–104.
22 L. Bhattacharyya, M. Haraldsson, and C.F. Brewer, *J. Biol. Chem.*, 262 (1987) 1294–1299.
23 A.S.I. Jaikaran, T.D. Kennedy, E. Dratewka-Kos, and B.G. Lane, *J. Biol. Chem.*, 265 (1990) 12503–12512.
24 L.M. Hoffman and D.D. Donaldson, *EMBO J.*, 4 (1985) 883–889.