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Xylose-containing carbohydrate chains derived from *N*-glycoproteins

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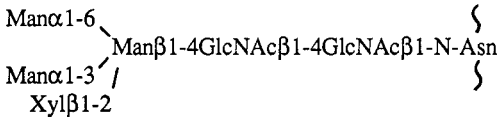
Abstract - Xylose-containing N-linked carbohydrate chains are integral parts of certain plant and animal glycoproteins. In all of the known structures, β-D-xylose is 1-2-linked to β-D-mannose of the trimannosyl-*N*,*N*'-diacetylchitobiose unit. If α-L-fucose is present at the asparagine-linked *N*-acetyl-D-glucosamine, then there are differences in the sites of its attachment, namely, α1-3- or α1-6-linked, depending on the biological origin. The α-D-mannose residues can be substituted with additional monosaccharides or 3-*O*-methylated. The state of the art with respect to structural analysis, organic synthesis, conformational analysis, biosynthesis, lectin binding, and immunological aspects is reviewed.

INTRODUCTION

Glycoproteins are biopolymers consisting of a polypeptide backbone with covalently attached carbohydrate side chains (ref.1). One major class of chains is characterized by the occurrence of oligosaccharides linked to the amide nitrogen of L-asparagine. In general, these so-called N-linked carbohydrate chains share a common pentasaccharide core element $Man_3GlcNAc_2$ (Man, D-mannose; GlcNAc, *N*-acetyl-D-glucosamine) connected via GlcNAc to Asn. Based on typical extensions of this core element, three major types of N-linked glycans have been distinguished, namely, (i) the oligomannose type; (ii) the *N*-acetyllactosamine type; and (iii) the hybrid type, showing characteristics of both the oligomannose and the *N*-acetyllactosamine type (ref. 2).

The existence of β-D-xylose (Xyl), 1-2-linked to β-D-mannose of the core element, has lead to the establishment of an additional type of *N*-glycans, which we have called the xylose type. This type can also include characteristics of the oligomannose or the *N*-acetyllactosamine type, but additional modifications, not comprised by the three major types, do occur too. One of the first examples of the xylose-type chains was found in the proteolytic enzyme bromelain from pineapple stem (ref. 3, 4). Since then, it has become evident that xylose-containing N-linked carbohydrate chains are integral parts of certain plant and animal glycoproteins.



Since the finding of xylose as a constituent of certain molluscan hemocyanins (ref. 5), part of our research program deals with this type of oligosaccharide chains. Over the years, analytical including conformational, synthetic and biosynthetic aspects of especially animal *N*-glycoprotein xylose-containing glycans have our attention, but also plant *N*-glycoprotein glycans of the same type are under current investigation.

This paper will summarize the present knowledge in the field of the xylose-containing carbohydrate chains from *N*-glycoproteins and focus on analytical, synthetic, conformational, biosynthetic, lectin-binding, and immunological aspects of these glycans.

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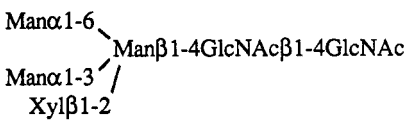
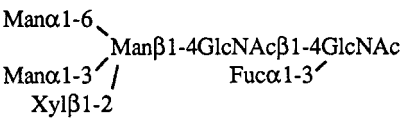
PRIMARY STRUCTURES

Plant glycoproteins

Xylose-containing N-linked oligosaccharides have shown to occur so far in lectins, enzymes, storage proteins and protease inhibitors. Especially, $Man_3XylGlcNAc_2$ seems to be a common structural element of many plant *N*-glycoproteins. It has been found in phaseolin, the major storage protein of the common bean *Phaseolus vulgaris* (ref. 6), together with $Man_9GlcNAc_2$ and $Man_7GlcNAc_2$, and in ascorbic acid oxidase of *Cucurbita pepo medullosa* (ref. 7). Besides $Man_3XylGlcNAc_2$, an extension of this element with L-fucose (Fuc) in α1-3-linkage to the Asn-bound GlcNAc residue has been identified in lectins from five *Erythrina* species (ref. 8), from *Sophora japonica* (ref. 8, 9), from *Lonchocarpus capassa* (ref. 8), and from horse radish peroxidase (ref. 10). In the *Erythrina* species and horse radish peroxidase, the non-fucosylated form occurred as a minor constituent. The protease inhibitor from barbados pride (*Caesalpinia pulcherrima* Sw.) seeds (ref. 11), the *Artocarpus intergrifolia* lectin (ref. 12), and the *Clerodendron trichotomum* agglutinin (ref. 13) contain the fucosylated variant only.

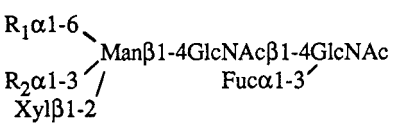


$Man_3XylGlcNAc_2$



$Man_3XylGlcNAc(Fuc\alpha1\text{-}3)GlcNAc$

In ricin D and castor bean hemagglutinin from *Ricinus communis* seeds the oligosaccharides $Man_3XylGlcNAc_2$, $Man_3XylGlcNAc(Fuc\alpha1\text{-}3)GlcNAc$, $Man_4XylGlcNAc_2$ (with the additional Man residue α1-3-linked to Manα1-6) have been detected (ref. 14-16). In addition, ricin contains $Man_{5\text{-}7}GlcNAc_2$ chains (ref. 15), and the agglutinin has $Man_{4\text{-}6}GlcNAc_2$ chains (ref. 16). The agglutinin of *Abrus precatorius* contains $Man_3XylGlcNAc(Fuc\alpha1\text{-}3)$-GlcNAc and $Man_{6\text{-}8}GlcNAc_2$ chains (ref. 17), and the lectin abrin a from this species has $Man_4XylGlcNAc_2$ and $Man_{5\text{-}7}GlcNAc_2$ chains (ref. 18). For S-glycoproteins of the cruciferous genus *Brassica* the major carbohydrate chains turned out to be $Man_3XylGlcNAc(Fuc\alpha1\text{-}3)GlcNAc$ and $GlcNAcMan_3XylGlcNAc(Fuc\alpha1\text{-}3)GlcNAc$ (with the additional GlcNAc residue β1-2-linked to Manα1-6) (ref. 19). For several other legume lectins the presence of fucose and/or xylose has been shown (see ref. 8, 13). In a few cases partial structures have been proposed (ref. 8, 10, 20). In sycamore cell laccase (*Acer pseudoplatanus* L.) (ref. 21) and in the taste-modifying glycoprotein miraculin, isolated from *Richadella dulcifica* berries (ref. 22), the presence of $Man_3XylGlcNAc$-$(Fuc\alpha1\text{-}3)GlcNAc$ together with mono-/di-antennary *N*-acetyllactosamine (Galβ1-4GlcNAc; Gal, D-galactose) type of extensions have been demonstrated, whereby the peripheral GlcNAc residues can be α1-6-fucosylated:



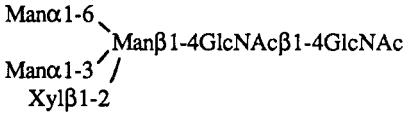
(a) $R_1 = R_2 = Man$ (ref. 21, 22)
(b) $R_1 = Man$; $R_2 = H$ (ref. 22)
(c) $R_1 = Man$; $R_2 = GlcNAc\beta1\text{-}2Man$ (ref. 21)
(d) $R_1 = GlcNAc\beta1\text{-}2Man$; $R_2 = Man$ (ref. 21)
(e) $R_1 = R_2 = GlcNAc\beta1\text{-}2Man$ (ref. 21)
(f) $R_1 = Man$; $R_2 = Gal\beta1\text{-}4(Fuc\alpha1\text{-}6)GlcNAc\beta1\text{-}2Man$ (ref. 22)
(g) $R_1 = Gal\beta1\text{-}4(Fuc\alpha1\text{-}6)GlcNAc\beta1\text{-}2Man$; $R_2 = Man$ (ref. 22)
(h) $R_1 = Gal\beta1\text{-}4(Fuc\alpha1\text{-}6)GlcNAc\beta1\text{-}2Man$; $R_2 = GlcNAc\beta1\text{-}2Man$ (ref. 21)
(i) $R_1 = R_2 = Gal\beta1\text{-}4(Fuc\alpha1\text{-}6)GlcNAc\beta1\text{-}2Man$ (ref. 21, 22)

Non-fucosylated (a), (c), (d), and (e) were detected in rice α-amylase from germinated seedlings, together with $Glc_1Man_9GlcNAc_2$ and $Man_{5\text{-}9}GlcNAc_2$ (ref. 23). In pineapple stem bromelain, compound (b) has been detected (ref. 3, 4). Recently, free $Man_3XylGlcNAc(Fuc\alpha1\text{-}3)GlcNAc$, together with $Man_5GlcNAc$, have been isolated from the extracellular medium of a white campion (*Silene alba*) suspension culture (ref. 24).

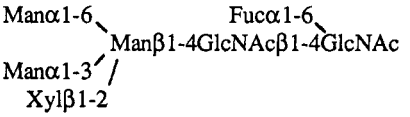
Animal glycoproteins

Up to now only two examples of animal *N*-glycoproteins containing the $Man_3XylGlcNAc_2$ structural element have been reported. Both glycoproteins are hemocyanins, high-molecular-mass copper-containing oxygen carriers, isolated from the hemolymph of the molluscan species *Helix pomatia* (terrestrial snail) and *Lymnaea stagnalis* (fresh-water snail). The glycans of both species can be divided into low- and high-molecular-mass compounds, for which highly unusual structures have been established, including the presence of methylated monosaccharides like 3-*O*-methyl-D-galactose (3MeGal) and 3-*O*-methyl-D-mannose (3MeMan).

The low-molecular-mass structures of *H. pomatia* hemocyanin comprise $Man_3XylGlcNAc_2$ (minor) and its α1-6-fucosylated extension (major) (ref. 25) :



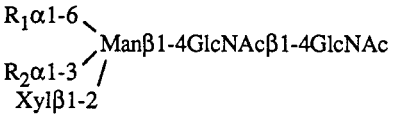
$Man_3XylGlcNAc_2$



$Man_3XylGlcNAc(Fuc\alpha1\text{-}6)GlcNAc$

Extension of these elements, leading to high-molecular-mass mono- and/or di-antennary compounds, consist of the incorporation of 3MeGal, Gal, GlcNAc, *N*-acetyl-D-galactosamine (GalNAc) and Fuc. Although definite structures are not yet available, clear evidence exists for the occurrence of the GalNAcβ1-4GlcNAcβ1-2Man sequence.

The structures of the low- and high-molecular-mass carbohydrate chains of *L. stagnalis* hemocyanin have been completely analyzed (ref. 26, 27), giving rise to the following series of compounds :



(a) $R_1 = R_2 = 3MeMan$
(b) $R_1 = 3MeGal\beta1\text{-}3GalNAc\beta1\text{-}4GlcNAc\beta1\text{-}2Man$; $R_2 = 3MeMan$
(c) $R_1 = Man$; $R_2 = 3MeGal\beta1\text{-}3GalNAc\beta1\text{-}4GlcNAc\beta1\text{-}2Man$
(d) $R_1 = R_2 = 3MeGal\beta1\text{-}3GalNAc\beta1\text{-}4GlcNAc\beta1\text{-}2Man$
(e) $R_1 = Fuc\alpha1\text{-}2Gal\beta1\text{-}3GalNAc\beta1\text{-}4GlcNAc\beta1\text{-}2Man$; $R_2 = 3MeMan$
(f) $R_1 = Man$; $R_2 = Fuc\alpha1\text{-}2Gal\beta1\text{-}3GalNAc\beta1\text{-}4GlcNAc\beta1\text{-}2Man$
(g) $R_1 = R_2 = Fuc\alpha1\text{-}2Gal\beta1\text{-}3GalNAc\beta1\text{-}4GlcNAc\beta1\text{-}2Man$
(h) $R_1 = 3MeGal\beta1\text{-}3GalNAc\beta1\text{-}4GlcNAc\beta1\text{-}2Man$;
$R_2 = Fuc\alpha1\text{-}2Gal\beta1\text{-}3GalNAc\beta1\text{-}4GlcNAc\beta1\text{-}2Man$

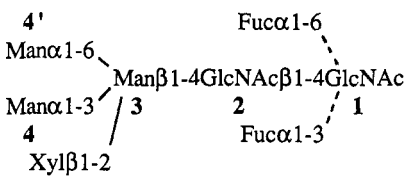
The fucosylation patterns of the xylose-containing N-linked carbohydrate chains in the discussed plant and animal glycoproteins need further comment. The α1-6 fucosylation of the Asn-bound GlcNAc in *H. pomatia* hemocyanin fits the usual finding in higher-animal glycoproteins (ref. 1, 2). In contrast, in plant glycoproteins the fucosylation of the Asn-bound GlcNAc occurs in a α1-3 linkage. The α1-6 fucosylation of the peripheral GlcNAc in laccase and miraculin deviates from the α1-3 fucosylation of this residue in higher-animal glycoproteins (SSEA-1 immunodeterminant; ref. 2). The $Fuc\alpha1\text{-}2Gal\beta1\text{-}3GalNAc\beta$ element, as observed in *L. stagnalis* hemocyanin, occurs also in higher-animal glycoconjugates (human blood group H type 4) (ref. 28).

The presence of the $GalNAc\beta1\text{-}4GlcNAc$ in stead of the $Gal\beta1\text{-}4GlcNAc$ sequence is not unique for *N*-glycoproteins from lower-animal species. For the higher-animal species, typical examples are the glycohormones lutropin and thyrotropin (ref. 29). However, in the latter glycoproteins the GalNAc residue is always terminated by a 4-linked sulfate group, in stead of $Fuc\alpha1\text{-}2Gal\beta1\text{-}3$ or $3MeGal\beta1\text{-}3$ elements.

Structural studies have been carried out on glycopeptides (ref. 4, 6, 9, 12, 30), on oligosaccharides, obtained via hydrazine treatment and *N*-acetylation, in their reduced form (ref. 4, 8, 25-27) or derivatized with 2-aminopyridine (ref. 11, 14-18), and on enzymatically (peptide-N^4-(*N*-acetyl-β-glucosaminyl)asparagine amidase; PNGase-A or F) released oligosaccharides in their native form (ref. 7, 30) or derivatized with 2-aminopyridine (ref. 13, 21, 22). It has to be noted that α1-3-linked Fuc at the Asn-bound GlcNAc can be partly removed during the hydrazinolysis procedure (β-elimination). Furthermore, there are indications that in contrast to PNGase-A, PNGase-F does not release carbohydrate chains having α1-3-linked Fuc at the Asn-bound GlcNAc. In general, monosaccharide analysis, methylation analysis (ref. 31), and ^1H-NMR spectroscopy (ref. 32) played major roles in all studies. In Table 1 a survey is presented of the ^1H-NMR chemical shifts of structural-reporter groups of the constituent monosaccharides for the non-fucosylated, the α1-3-fucosylated, and the α1-6-fucosylated $Man_3XylGlcNAc_2$ structural elements, as glycopeptides, oligosaccharide-alditols, free oligosaccharides, and oligosaccharide pyridylamino derivatives.

TABLE 1. ^{1}H-Chemical shifts of structural-reporter groups of the constituent monosaccharides for the non-fucosylated (M_3XGn_2), the α1-3-fucosylated ($M_3XGn(F\alpha1\text{-}3)Gn$), and the α1-6-fucosylated ($M_3XGn(F\alpha1\text{-}6)Gn$) Manα1-6(Manα1-3)(Xylβ1-2)GlcNAcβ1-4GlcNAc structural elements, as glycopeptides, oligosaccharide-alditols, reducing oligosaccharides, and oligosaccharide pyridylamino derivatives from xylose-containing carbohydrate chains of *N*-glycoproteins. Chemical shifts (δ) are given at 25-27°C, in ppm downfield from internal sodium 4,4-dimethyl-4-silapentane-1-sulfonate in ^2H_2O (internal standard, acetone δ 2.225). For numbering of the monosaccharide residues, see structure below.

		M_3XGn_2				$M_3XGn(F\alpha1\text{-}3)Gn$				$M_3XGn(F\alpha1\text{-}6)Gn$		
Residue	Reporter group	ref. 6 ~Asn	ref. 25 ~ol	ref. 7 free	ref. 15 ~PA	ref. 12 ~Asn	ref. 8 ~ol	ref. 24 free	ref. 11 ~PA	ref. 30 ~Asn	ref. 25 ~ol	ref. 30 free
GlcNAc-**1**	H-1	5.048	n.d.	5.189[a] 4.703[b]	n.d	5.042	n.d.	5.162[a] 4.685[b]	n.d.	5.088	n.d.	5.181[a] 4.694[b]
	H-2	n.d.	4.239	n.d.	4.35	n.d.	4.31	n.d.	4.483	n.d.	4.219	n.d.
	NAc	2.010	2.057	2.039	n.d.	1.988	n.d.	2.030[a] 2.025[b]	1.934[c]	2.010	2.058	2.040
GlcNAc-**2**	H-1	4.608	4.634	4.615[a] 4.607[b]	4.649	4.561	n.d.	4.561[a] 4.545[b]	4.613	4.686	4.718	4.666[a] 4.673[b]
	NAc	2.070	2.073	2.074	n.d.	2.050	n.d.	2.048[a] 2.051[b]	2.056	2.086	2.081	2.088[a] 2.085[b]
Man-**3**	H-1	4.869	4.883	4.875	n.d.	4.846	4.87	4.849	4.868	4.872	4.884	4.873
	H-2	4.264	4.270	4.265	4.257	4.263	4.27	4.265	4.270	4.267	4.270	4.266
Man-**4**	H-1	5.121	5.122	5.122	5.128	5.120	5.12	5.120	5.135	5.122	5.124	5.123
	H-2	4.037	4.039	4.041	4.040	4.038	4.03	4.038	4.050	4.037	4.040	4.038
Man-**4'**	H-1	4.911	4.913	4.912	4.921	4.909	4.91	4.910	4.925	4.914	4.914	4.913
	H-2	3.981	3.983	3.982	3.995	3.974	3.98	3.975	4.000	3.979	3.982	3.981
Xyl	H-1	4.447	4.449	4.453	4.438	4.462	4.45	4.462	4.452	4.449	4.449	4.450
	H-2	3.373	3.377	3.375	n.d.	3.374	3.38	3.375	n.d.	3.377	3.379	3.375
	H-3	3.438	3.437	3.444	n.d.	3.447	3.44	3.450	n.d.	n.d.	3.453	3.456
	H-5ax	3.248	3.250	3.253	n.d.	3.255	3.25	3.257	n.d.	3.252	3.253	3.253
α1-3Fuc	H-1	-	-	-	-	5.128	5.02	5.080	5.060	-	-	-
	H-5	-	-	-	-	4.706	4.23	4.721	4.254	-	-	-
	CH_3	-	-	-	-	1.274	~1.2	1.273	1.206	-	-	-
α1-6Fuc	H-1	-	-	-	-	-	-	-	-	4.878	4.898	4.890[a] 4.898[b]
	H-5	-	-	-	-	-	-	-	-	n.d.	4.077	4.100
	CH_3	-	-	-	-	-	-	-	-	1.206	1.225	1.210[a] 1.222[b]



Codes: ~Asn, glycopeptides; ~ol, oligosaccharide-alditols; free, reducing oligosaccharides; ~PA, oligosaccharide pyridylamino derivatives. [a] Due to α-anomeric form; [b] Due to β-anomeric form; [c] In ref. 13: δ 1.974.

ORGANIC SYNTHESIS

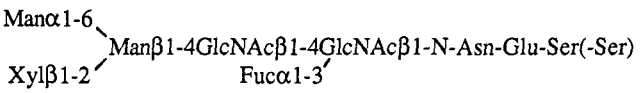
In the framework of conformational analysis and biosynthesis also attention has been paid to the organic synthesis of structural elements of xylose-containing carbohydrate chains from *N*-glycoproteins.

Four xylose-containing oligosaccharides have been synthesized, namely, Xylβ1-2Manβ1-OMe, Manα1-3(Xylβ1-2)Manβ1-OMe, Manα1-6(Xylβ1-2)Manβ1-OMe, and Manα1-6(Manα1-3)(Xylβ1-2)Manβ1-OMe (ref. 33). The trisaccharide Manα1-6(Xylβ1-2)Manβ1-OMe represents the terminal sequence of the bromelain glycan, whereas the tetrasaccharide Manα1-6(Manα1-3)(Xylβ1-2)Manβ1-OMe is the frequently occurring terminal sequence in plant glycoproteins, and has also been found in the *H. pomatia* hemocyanin (see above). Preliminary data concerning additional studies aimed at the preparation of larger structures with Xyl incorporated have been reported (ref. 34, 35).

Besides these xylose-containing oligosaccharides, also other structural elements of the antennae of the hemocyanin carbohydrate chains have been synthesized, namely, GalNAcβ1-4GlcNAcβ1-2Manα1-O$(CH_2)_8$COOMe (ref. 36) and GalNAcβ1-4GlcNAcβ1-OMe (ref. 37). Although the trisaccharide was in principle prepared as a substrate for the 4-O-sulfation of terminal GalNAc in the glycohormone glycan studies, this compound, together with the disaccharide methyl glycoside, have also shown to be useful substrates in biosynthetic studies of snail hemocyanin glycans (see below).

CONFORMATIONAL ANALYSIS

Many studies have appeared on the conformational analysis of *N*-glycoprotein glycans (ref. 38-42). In this context also attention has been paid to the conformational analysis of xylose-containing N-linked carbohydrate chains and of partial structures.



^{1}H- and ^{13}C-NMR assignments have been obtained for the carbohydrate part of a bromelain derived glycopeptide, using homo- and heteronuclear correlation spectroscopy, two-dimensional homonuclear Hartmann-Hahn and nuclear Overhauser enhancement (NOE) experiments. The assignments of the ^{1}H-NMR spectra have made possible a conformational analysis of all glycosidic linkages (ref. 43). In Table 2 a summary is presented of torsional angles (φ,ψ) for the various glycosidic linkages and the rotamer population about the C5-C6 bond of the β-D-Man residue, as determined by ^{1}H-NMR measurements in combination with HSEA (ref. 38) calculations. According to the measured coupling constants ($J_{5,6}$ = 1 Hz; $J_{5,6'}$ = 2.1 Hz) the rotamer population about the C5-C6 linkage of β-Man is dominated by the presence of the $P_{\omega=180}$ (gg) rotamer. Based on these data a time-averaged conformational model is presented in Fig. 1, and it can be proposed that this structure gives a considerable contribution to the ensemble of actual conformers.

TABLE 2. Conformational data of bromelain glycopeptide

linkage	φ/ψ	$P_{\omega=180}$
Xylβ1-2Man	50/0	
Manα1-6Man	-40/170	>98%
Manβ1-4GlcNAc	60/0	
GlcNAcβ1-4GlcNAc	50/10	
Fucα1-3GlcNAc	45/30	

φ : H-1',C-1',O,C-x with x = 2, 3, 4 or 6
ψ : C-1',O,C-x,H-x with x = 2, 3 or 4;
C-1',O,C-6,C-5 for 1-6 linkage
ω : O-6,C-6,C-5,H-5 ($P_{\omega=180}$ corresponds with gg)

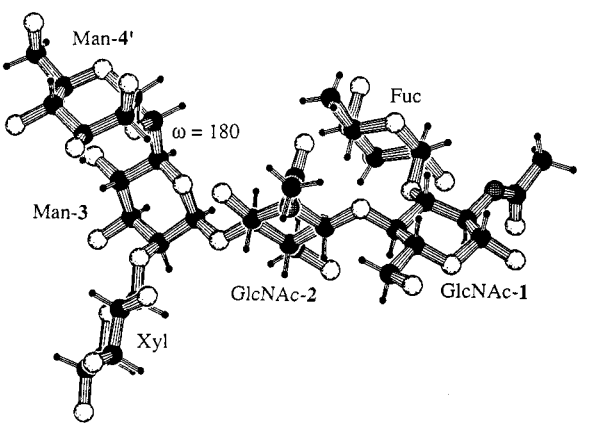


Fig. 1. Molecular model of the bromelain glycopeptide for the $P_{\omega=180}$ (gg) rotamer.

Additional conformational studies have been carried out on the synthetic oligosaccharides Manα1-3(Xylβ1-2)Manβ1-OMe, Manα1-6(Xylβ1-2)Manβ1-OMe, and Manα1-6(Manα1-3)(Xylβ1-2)Manβ1-OMe, using rotating-frame NOE (ROESY) experiments in combination with HSEA calculations (ref. 44, 45).

Comparison of the torsional angles in Manα1-6(Xylβ1-2)Manβ1-OMe (Manα1-6Man ϕ/ψ, -50/180; Xylβ1-2Man ϕ/ψ, 50/0) with those of the terminal glycan element in the bromelain glycopeptide (Table 2), shows similar values for ϕ and ψ. However, the coupling constants ($J_{5,6}$ = 2.3 Hz; $J_{5,6'}$ = 5.2 Hz) of the hydroxymethyl group of β-Man indicate a time-averaged rotamer population about the C5-C6 linkage of $P_{\omega=180}$ (gg) : $P_{\omega=-60}$ (gt) = 40 : 60, indicating the conformational influence of the α1-3-linked Fuc in the bromelain glycopeptide.

Analysis of the tetrasaccharide Manα1-6(Manα1-3)(Xylβ1-2)Manβ1-OMe shows similar torsional angles as observed for the two trisaccharides (Manα1-6Man ϕ/ψ, -60/170; Manα1-3Man ϕ/ψ, -40/-20; Xylβ1-2Man ϕ/ψ, 40/5) and a similar rotamer population for the hydroxymethyl group of β-Man as found for Manα1-6(Xylβ1-2)Manβ1-OMe ($P_{\omega=180}$ (gg) : $P_{\omega=-60}$ (gt) = 40 : 60). The ϕ/ψ data for the 1-6- and 1-3-linkage agree with those of conventional diantennary structures (ref. 41). The tetrasaccharide element forms the terminal part of e.g. the major low-molecular-mass carbohydrate chain of *H. pomatia* hemocyanin (Man_3XylGlcNAc(Fucα1-6)GlcNAc). Detailed NMR studies of this carbohydrate chain yielded for the hydroxymethyl group of β-Man $J_{5,6}$ and $J_{5,6'}$ values of 2.7 and 4.7 Hz, respectively, indicating the presence of both gg and gt rotamers. This illustrates that in the case of an α1-6-linked Fuc at the Asn-bound GlcNAc, the situation is similar to that found for non-xylosylated α1-6-fucosylated N-linked glycans (ref. 30, 41). Molecular modelling of xylose-containing oligosaccharides with either α1-3- or α1-6-linked Fuc clearly shows the great difference in spatial structure for these two types of compounds.

BIOSYNTHESIS

Studies directed at the biosynthesis of Xyl/Fuc-containing carbohydrate chains in plant glycoproteins show different results. Based on the structural determination of $Glc_1Man_9GlcNAc_2$ and $Man_{5-9}GlcNAc_2$ in the mature form of rice α-amylase (ref. 23), a pathway has been proposed similar to that in animal systems (ref. 2). No comment is given to the processing pathway occurring after $Man_5GlcNAc_2$. In case of *P. vulgaris* cotyledons (ref. 6, 46, 47) it was found that $Man_5GlcNAc_2$-Asn is the acceptor for the first GlcNAc residue added. A further trimming and elongation yields then $GlcNAc_2Man_3GlcNAc_2$-Asn (see ref. 2). The substrates for the incorporation of Fuc turned out to be $GlcNAcMan_5GlcNAc_2$-Asn, $GlcNAcMan_3GlcNAc_2$-Asn, and $GlcNAc_2Man_3GlcNAc_2$-Asn. Only the latter two compounds were substrates for xylosyltransferase. The formation of the structures missing the terminal GlcNAc residues is not documented sofar (ref. 46). Based on a series of identified structures, including $Man_4XylGlcNAc_2$, for ricin D and castor bean hemagglutinin from *R. communis* seeds, another route has been proposed (ref. 14). No information is available with respect to the biosynthesis of the antennary structures in laccase (ref. 21) and miraculin (ref. 22).

Recently, data have been obtained regarding the biosynthesis of the antennae of *L. stagnalis* hemocyanin glycans (ref. 48). Using the substrates GalNAcβ1-4GlcNAcβ1-2Manα1-O$(CH_2)_8$COOMe and GalNAcβ1-4GlcNAcβ1-OMe, it has been shown that connective tissue of the snail *L. stagnalis* contains a β1-3-galactosyltransferase which is capable of transferring Gal from UDP-Gal to GalNAcβ1-4GlcNAc-R. It has been established that this UDP-Gal:GalNAcβ1-4GlcNAc-R β1-3-galactosyltransferase differs clearly in substrate specificity from the UDP-Gal:GalNAcα-R β1-3-galactosyltransferase occurring in porcine submaxillary gland microsomes and from the UDP-Gal:Galβ-R β1-3-galactosyltransferase in *L. stagnalis* albumen glands. On the basis of data obtained for incubations in the presence of albumen gland, it appears that this gland contains both the UDP-Gal:GalNAcβ1-4GlcNAc-R and UDP-Gal:Galβ-R β1-3-galactosyltransferase activities.

In connection with the hemocyanin glycans of *L. stagnalis*, also attention has been paid to the biosynthesis of 3-*O*-methylated monosaccharides (ref. 49). It was found that only very small amounts of injected 3-*O*-[^{3}H]-methyl-D-mannose were incorporated into the hemocyanin carbohydrate chains. However, injection of L-[methyl-^{14}C]methionine led to the incorporation of the labeled methyl group into 3MeMan and 3MeGal of the hemocyanin oligosaccharides.

LECTIN BINDING

Investigation of the reactivity of the glycan moiety of fruit *C. trichotomum* agglutinin (Man_3XylGlcNAc(Fucα1-3)GlcNAc) towards various lectins gave the following results (ref. 13) : i. The presence of Xylβ1-2 in the N-linked oligosaccharide does not affect the binding with mannose-specific lectins as Concanavalin A, *Lens culinaris* agglutinin, *Pisum sativum* agglutinin, and *Vicia faba* agglutinin; ii. Lentil, pea and *Vicia fabia* lectins can bind to the α1-3Fuc-containing N-linked oligosaccharide; iii. *Ulex europeus* agglutinin I can bind to the α1-3-linked Fuc residue; iv. Wheat germ agglutinin does not bind to the discussed oligosaccharide.

IMMUNOLOGY

A number of studies have appeared dealing with antibodies that recognize Xyl/Fuc-containing oligosaccharides from plant *N*-glycoproteins (ref. 10, 50-53), and can be illustrated as follows.

One of the monoclonal antibodies raised against extracts of the rachis abscission zone of *Sambucus nigra* L., which preferentially recognizes abscission-zone proteins, cross-reacts with several enzymes from higher plants and with lectins from *P. vulgaris* and *E. cristagalli* (ref. 10). $Man_3XylGlcNAc(Fuc\alpha 1\text{-}3)GlcNAc$, isolated from horse radish peroxidase, turned out to be a potent inhibitor of the interaction process. The production of a rat monoclonal antibody to the Xyl/Fuc-containing oligosaccharides demonstrates that these structural elements are highly immunogenic in animals.

In case of polyclonal antibodies raised against carrot (*Daucus carota*) cell wall β-fructosidase (ref. 51), it was shown that the binding activity was associated with $Man_3XylGlcNAc(Fuc\alpha 1\text{-}3)GlcNAc$, $Man_3XylGlcNAc_2$, and $ManXylGlcNAc_2$, but not with $Man_3GlcNAc_2$, suggesting that the primary binding is to the $Xyl\beta 1\text{-}2Man$ element.

CONCLUDING REMARKS

A survey has been presented with respect to the state of the art in the field of the *N*-glycoprotein xylose-containing glycans. Especially since 1985 many details have become available about this interesting group of carbohydrate chains. The reports on primary structures have highly activated additional research focused on conformational analysis, organic synthesis, biosynthesis, lectin studies and immunological studies. It is anticipated that in the near future the biosynthetic pathways will be unravelled for the xylose/fucose-containing carbohydrate chains of both plant and animal origin. Also detailed studies aimed at the understanding of the biological significance of this specific class of oligosaccharides will be the subject of future research programs.

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