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The abundance of additional *N*-acetyllactosamine units in N-linked tetraantennary oligosaccharides of human Tamm-Horsfall glycoprotein is a donor-specific feature

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Previously, treatment of Tamm-Horsfall glycoprotein (THp) from different donors with endo-β-galactosidase has been shown to liberate a tetra- and a Sd^a-active pentasaccharide, concluding the presence of N-linked carbohydrate chains containing additional *N*-acetyllactosamine units. These type of oligosaccharides were not found in a detailed structure elucidation of the carbohydrate moiety of THp of one male donor, suggesting a donor-specific feature for these type of structures. Therefore, THp was isolated from four healthy male donors and each subjected to endo-β-galactosidase treatment in order to release these tetra- and Sd^a-active pentasaccharide. Differences were observed in the total amount of released tetra- and Sd^a-active pentasaccharide of the used donors (42, 470, 478, 718 μg/100 mg THp), indicating that the presence of repeating *N*-acetyllactosamine units incorporated into the N-glycan moiety of THp is donor specific. Furthermore, a higher expression of the Sd^a determinant on antennae which display *N*-acetyllactosamine elongation was observed, suggesting a better accessibility for the β-*N*-acetylgalactosaminyltransferase. In order to characterize the N-glycans containing repeating *N*-acetyllactosamine units, carbohydrate chains were enzymatically released from THp and isolated. The tetraantennary fraction, which accounts for more than 33% of the total carbohydrate moiety of THp, was used to isolate oligosaccharides containing additional *N*-acetyllactosamine units. Five N-linked tetraantennary oligosaccharides containing a repeating *N*-acetyllactosamine unit were identified, varying from structures bearing four Sd^a determinants to structures containing no Sd^a determinant (see below).

One compound was used in order to specify the branch location of the additional *N*-acetyllactosamine unit, and it appeared that only the Gal-6′ and Gal-8′ residues were occupied by a repeating *N*-acetyllactosamine unit.

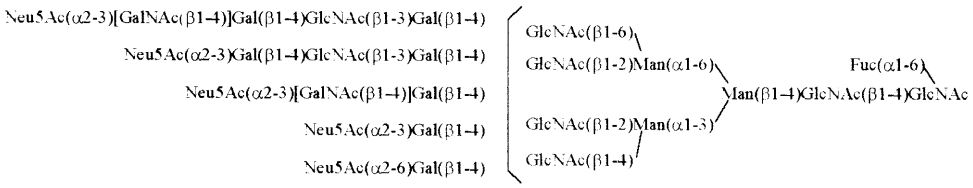
Key words: Tamm-Horsfall glycoprotein/carbohydrate/NMR/*N*-acetyllactosamine repeat/donor specificity

Introduction

Human Tamm-Horsfall glycoprotein (THp) is the most abundant protein in normal human urine, being excreted in quantities up to about 100 mg/day (Tamm and Horsfall, 1950). THp is produced by the kidney where it is expressed via a phosphatidylinositol anchor on the endothelium of the thick ascending limb of the loop of Henle (Sikri *et al.*, 1979; Rindler *et al.*, 1990). The physiological function of THp is still subject of study. Several studies have indicated that THp acts as a natural inhibitor of microbial infection of the urinary tract and bladder (Duncan, 1988; Parkkinen *et al.*, 1988). Furthermore, THp contains immunomodulating properties consisting of inhibiting T-cell proliferation, triggering the inflammatory response of neutrophils and stimulating the proliferation of human mononuclear cells (Muchmore and Decker, 1985; Horton *et al.*, 1990; Sathyamoorthy *et al.*, 1991; Yu *et al.*, 1992, 1993; Thomas *et al.*, 1993a,b; Toma *et al.*, 1994). These immunomodulating properties are probably carbohydrate-regulated (Muchmore and Decker, 1987; Muchmore *et al.*, 1987, 1990a,b; Sherblom *et al.*, 1988; Dall' Olio *et al.*, 1991; Sathyamoorthy *et al.*, 1991), inducing the necessity to elucidate the carbohydrate structures displayed by THp.

Treatment of THp from a urinary pool of different donors with endo-β-galactosidase revealed the tetrasaccharide Neu5Ac(α2–3)-Gal(β1–4)GlcNAc(β1–3)Gal and the pentasaccharide Neu5Ac-(α2–3)[GalNAc(β1–4)]Gal(β1–4)GlcNAc(β1–3)Gal (Donald *et al.*, 1983). These data suggest that *N*-acetyllactosamine repeats must be present on the antennae of the oligosaccharides of THp. A detailed structure elucidation of the N-glycan moiety of THp of one male donor yielded di-, tri-, and, most of all, tetraantennary structures, possibly fucosylated, sialylated, and/or sulfated (Hård *et al.*, 1992). The Sd^a determinant was present as the trisaccharide Neu5Ac(α2–3)[GalNAc(β1–4)]Gal incorporated in tri- and tetraantennary N-glycans. No N-glycans containing repeating *N*-acetyllactosamine units were found. Therefore, we hypothesized that the occurrence of poly-*N*-acetyllactosamine-containing N-glycans in THp is donor specific.

In this report, THp samples of four male donors, including the one used by Hård *et al.* (1992), were investigated for the presence of *N*-acetyllactosamine repeats, by a combination of enzymatic and chromatographic methods. Furthermore, the N-glycans



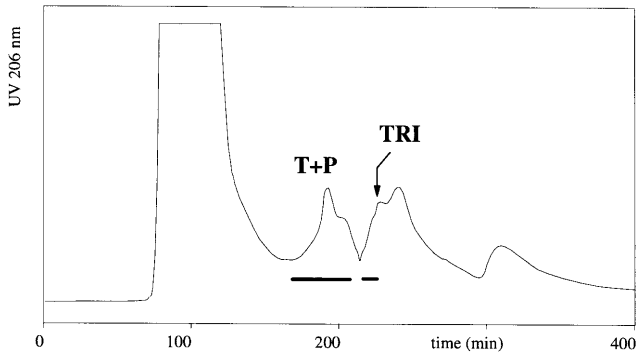


Fig. 1. Separation pattern at 206 nm on Bio-Gel P-4 of the endo-β-galactosidase-digest of Tamm-Horsfall glycoprotein. Elutions were performed on a Bio-Gel P-4 column (50 × 1.0 cm, Bio-Rad) with 10 mM NH_4HCO_3, pH 7.0, at a flow rate of 15 ml/h. Fractions **T+P** and **TRI**, containing the released carbohydrates (orcinol/H_2SO_4), were isolated.

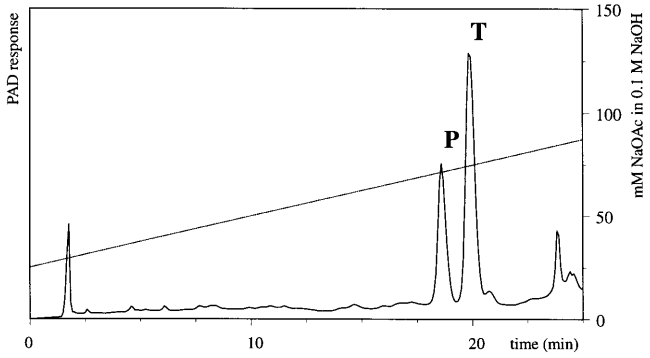


Fig. 2. Fractionation pattern of fraction **T+P** on CarboPac PA-1 with pulsed amperometric detection. Elutions were carried out with a concentration gradient of NaOAc in 0.1 M NaOH as indicated in the figure, at a flow rate of 4 ml/min. **P** and **T** are the enzymatically released Sd^a-active pentasaccharide and tetrasaccharide, respectively.

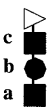
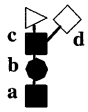
containing repeating *N*-acetyllactosamine units were isolated from the tetraantennary oligosaccharide fraction of the carbohydrate moiety of THp of a single male donor, and characterized by 600 MHz ^{1}H-NMR spectroscopy.

Results

The repeating *N*-acetyllactosamine units present in the carbohydrate moiety of THp were generated by digesting THp (100 mg, donor **HR**) with endo-β-galactosidase (Scudder *et al.*, 1983). The digest was fractionated by gel permeation chromatography on Bio-Gel P-4 (Figure 1) yielding one main oligosaccharide-containing fraction (**T+P**), and a highly contaminated minor one (**TRI**). Fraction **T+P** was subfractionated by HPAEC on CarboPac PA-1 affording two main fractions **T** and **P** (Figure 2), which were characterized by 1D and 2D 600 MHz ^{1}H-NMR spectroscopy. Relevant ^{1}H-NMR parameters in terms of structural-reporter-group signals are listed in Table I.

Fraction **T** contained the tetrasaccharide Neu5Ac(α2–3)-Gal(β1–4)GlcNAc(β1–3)Gal earlier reported as the corresponding alditol in case of THp (Donald and Feeney, 1986). The structural-reporter-group signals fit those reported by Hokke *et al.* (1991), and in a 2D TOCSY experiment most of the ring protons could be assigned (Figure 3a). Fraction **P** contained the Sd^a-antigen

Table I. ^{1}H-Chemical shifts of structural-reporter-group protons of the constitutent monosaccharides of the liberated tetra- **(T)** and Sd ^{a}-active pentasaccharide **(P)**, derived from human Tamm-Horsfall glycoprotein

Residue	Reporter group	Chemical shift (δ, ppm) in	
		T^a 	**P**^{a} 
Gal-**a**	H-1α	5.225	5.229
	H-1β	4.562	4.568
	H-2α	3.856	3.860
	H-2β	3.536	3.535
	H-3α	3.892	3.908
	H-3β	3.701	3.705
	H-4α	4.202	4.202
	H-4β	4.147	4.148
GlcNAc-**b**	H-1	4.721	4.721
	H-2	3.72[b]	3.72
	H-3	3.72	3.72
	H-4	3.72	3.72
	H-5	3.580	3.585
	NAc	2.040	2.040
Gal-**c**	H-1	4.556	4.556
	H-2	3.572	3.366
	H-3	4.116	4.155
	H-4	3.961	4.116
Neu5Ac	H-3e	2.757	2.668
	H-3a	1.798	1.926
	H-6	nd[c]	3.487
	NAc	2.031	2.031
GalNAc-**d**	H-1	-	4.746
	H-2	-	3.92
	H-3	-	3.683
	H-4	-	3.92
	NAc	-	2.015

Chemical shifts are given at 300 K and were measured in ^2H_2O relative to internal acetone (δ 2.225). Compounds are represented by short-hand symbolic notation: open triangles, Neu5Ac(α2-3); solid circles, GlcNAc; solid squares, Gal; open diamonds, GalNAc.
[a]**T**, Neu5Ac(α2–3)Gal(β1–4)GlcNAc(β1–3)Gal; **P**, Neu5Ac(α2–3)[GalNAc-(β1–4)]Gal(β1–4)GlcNAc(β1–3)Gal.
[b]Some values are given with only two decimals because of spectral overlap.
[c]nd, Not determined.

active pentasaccharide Neu5Ac(α2–3)[GalNAc(β1–4)]Gal(β1–4)-GlcNAc(β1–3)Gal. Attachment of GalNAc to Gal produced a set of characteristic ^{1}H chemical shifts for Neu5Ac, GalNAc and Gal, previously described for the alditol analog (Donald and Feeney, 1986) and Sd^a-antigen containing N-glycans (Hård *et al.*, 1992). In a 2D TOCSY experiment, most of the ring protons could be assigned (Figure 3b). ^{1}H-NMR analysis of fraction **TRI** revealed the presence of the nonsialylated trisaccharide Gal(β1–4)GlcNAc(β1–3)Gal (data not shown; cf. compound **D_O7.4** in Hokke *et al.*, 1994). The disaccharide GlcNAc(β1–3)Gal was not found, indicating the absence of poly-*N*-acetyllactosamine units as an element of the carbohydrate moiety of THp.

In order to investigate if the presence of the di-(*N*-acetyl-lactosamine) element is donor specific, THp (100 mg) was isolated from four healthy male donors (**HR**, **CF**, **YB**, and **PM**) including the donor used by Hård *et al.* (1992) (**PM**). All four

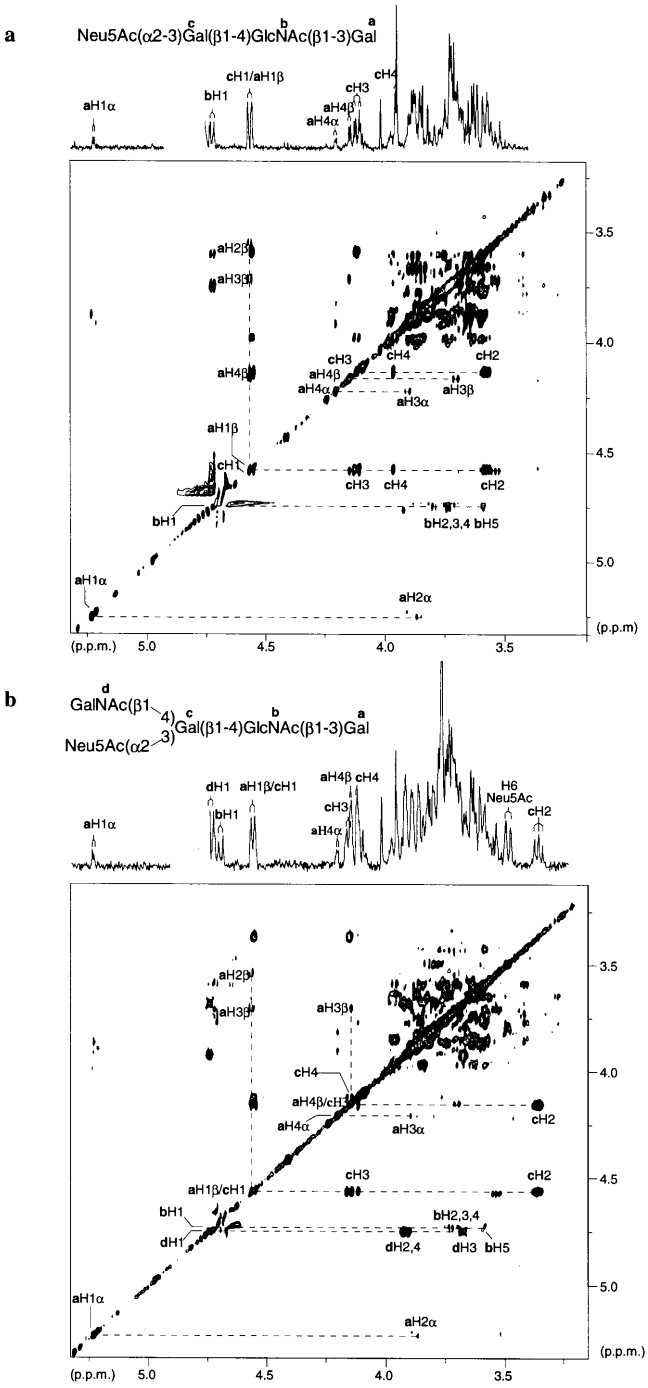


Fig. 3. Resolution-enhanced 500-MHz 1D ^{1}H-NMR spectra and 600-MHz 2D clean TOCSY spectra (τ_m 100 ms) at 300 K of the tetrasaccharide (a) and the Sd^a-active pentasaccharide (b) obtained from endo-β-galactosidase-treated human Tamm-Horsfall glycoprotein. Dotted lines are drawn for Gal-**a**, GlcNAc-**b**, Gal-**c** and GalNAc-**d** to show the scalar-coupled network of their anomeric protons.

samples were separately digested with endo-β-galactosidase and in each case the released pool of tetrasaccharide and Sd^a-antigen active pentasaccharide was isolated by gel permeation chromatography on Bio-Gel P-4. Quantification of the pool and determination of the molar ratio of the tetra- and pentasaccharide in the pool was performed by a phenol/sulfuric acid assay (Dubois *et al.*, 1956) and HPAEC on CarboPac PA-1, respectively. The results are compiled in Table II.

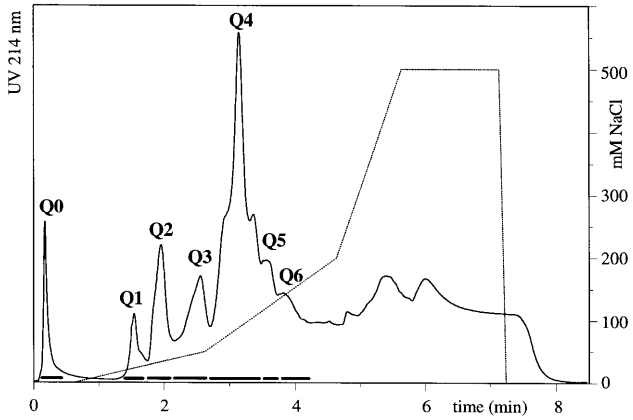


Fig. 4. Fractionation pattern at 214 nm on a FPLC Resource Q column of the carbohydrate-containing Superdex 75 fraction, derived from PNGase-F-treated human Tamm-Horsfall glycoprotein. Elutions were performed with linear concentration gradients of NaCl in H_2O as indicated in the figure, at a flow rate of 4 ml/min.

Table II. Quantification and molar ratio analysis of the total amount of tetra- and Sd^a-antigen-active pentasaccharide

Donor code	µg Tetra- and pentasaccharide/ 100 mg THp	Molar % pentasaccharide[a]	Molar % tetrasaccharide
HR	478	50	50
CF	470	35	65
PM	42	41	59
YB	718	28	72

[a]The molar ratio was calculated from the peak areas of the HPAEC-profiles, assuming the PAD response to be equal for both oligosaccharides.

For the isolation and characterization of intact N-glycans containing additional *N*-acetyllactosamine units, a batch of THp (700 mg, **HR**) was isolated from 20 l pooled urine. Monosaccharide analysis revealed the presence of Fuc, Man, Gal, GalNAc, GlcNAc, and Neu5Ac in a molar ratio of 0.9:3.0:3.7:1.1:5.5:2.9, in accordance with literature data (Hård *et al.*, 1992). The glycoprotein was treated with PNGase-F and the liberated N-glycans were isolated by gel permeation chromatography on Superdex 75. The carbohydrate-containing fraction was desalted and further fractionated by FPLC on Resource Q yielding seven fractions, denoted **Q0**–**Q6** (Figure 4). Fraction **Q4** consisted of tetra-charged oligosaccharides accounting for more than 33% of the total carbohydrate content of THp (Hård *et al.*, 1992). After desalting, this fraction was subfractionated by HPLC on Lichrosorb-NH_2 (Figure 5) yielding 13 fractions, denoted **Q4.1**–**Q4.13**. Only fractions **Q4.9**, **Q4.12**, and **Q4.13** contained oligosaccharides with repeating *N*-acetyllactosamine units, as revealed by specific structural-reporter-group signals for additional *N*-acetyllactosamine units by ^{1}H-NMR spectroscopy (Hokke *et al.*, 1991). These fractions were subjected to HPAEC on CarboPac PA-1 (Figure 6) affording fractions **Q4.9.1**–**Q4.9.8**, **Q4.12.1**–**Q4.12.7**, and **Q4.13.1**–**Q4.13.4**. ^{1}H-NMR spectroscopy was used to elucidate the oligosaccharide structures of these HPAEC fractions. Relevant ^{1}H chemical shifts of the specific structural-reporter-group signals of oligosaccharides containing additional *N*-acetyllactosamine units are compiled in Table III. The numbering of the monosaccharide residues is exemplified in the structure of compound **Q4.13.3**.

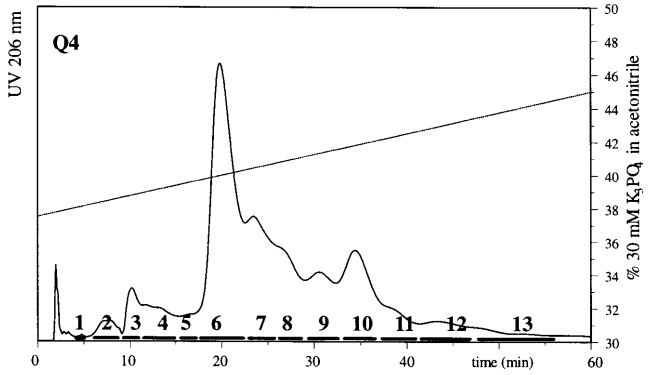


Fig. 5. Separation pattern at 206 nm of FPLC fraction **Q4** obtained from human Tamm-Horsfall glycoprotein on a HPLC Lichrosorb-NH_2 column. Elution was performed with a linear concentration gradient of 30 mM K_2HPO_4/KH_2PO_4, pH 6.8, and acetonitrile as indicated in the figure, at a flow rate of 1.5 ml/min.

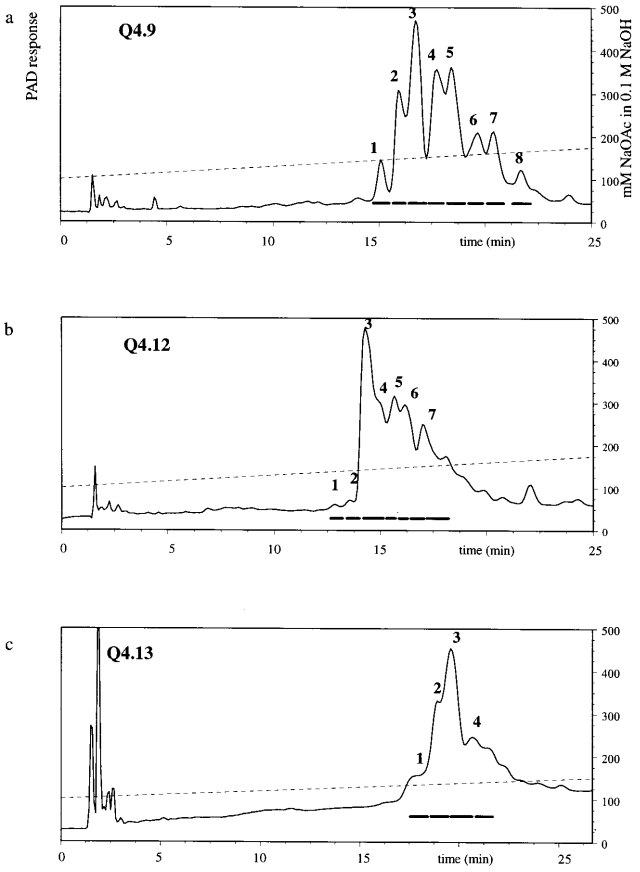


Fig. 6. Fractionation patterns of HPLC subfractions derived from FPLC fraction **Q4** containing oligosaccharides with additional *N*-acetyllactosamine units on CarboPac PA-1 with pulsed amperometric detection. Elutions were carried out with a concentration gradient of NaOAc in 0.1 M NaOH as indicated in the figures, at a flow rate of 4 ml/min. (**a**) **Q4.9**; (**b**) **Q4.12**; (**c**) **Q4.13**.

In general, an additional *N*-acetyllactosamine element attached to Gal-**8′** can be recognized by the characteristic Gal-**8′** H-1 signal at δ 4.467, while elongation at Gal-**6′** is characterized by the Gal-**6′** H-1 signal at δ 4.455 (Hokke *et al.*, 1991). Furthermore, elongation at Gal-**6′** or Gal-**8′** will shift H-4 of Gal-**6′** or Gal-**8′** downfield to δ 4.16. The anomeric proton of $GlcNAc_{ext}$ will resonate at about δ 4.70 and its NAc methyl signal at δ 2.037–2.038. So far, *N*-acetyllactosamine repeats are mainly found at the antennae containing Gal-**6′** and/or Gal-**8′**. In the following, ^{1}H-NMR data are presented that prove that compound **Q4.9.1** contains extensions only at the antennae containing Gal-**6′** and Gal-**8′**. This finding has been extrapolated to compounds **Q4.12.3** and **Q4.13.3** (extension at the antenna containing Gal-**6′**).

The Sd^a determinant, which is present in some of the carbohydrate chains of THp, produces a characteristic set of ^{1}H chemical shifts. The element Neu5Ac(α2–3)[GalNAc-(β1–4)]Gal(β1–4)GlcNAc(β- is recognized from the typical signals for Neu5Ac H-3a at δ 1.93, H-3e at δ 2.66 and H-6 at δ 3.48. Furthermore, GalNAc H-1 is observed at δ 4.73–4.76 (42 °C) while its NAc methyl signal resonates at δ 2.015–2.019. Due to the attachment of GalNAc to Gal via a (β1–4) linkage, Gal H-4 resonates at δ 4.11–4.12, H-3 at δ 4.15, and H-2 at δ 3.356–3.357 (Williams *et al.*, 1984; Donald and Feeney, 1986; Hård *et al.*, 1992).

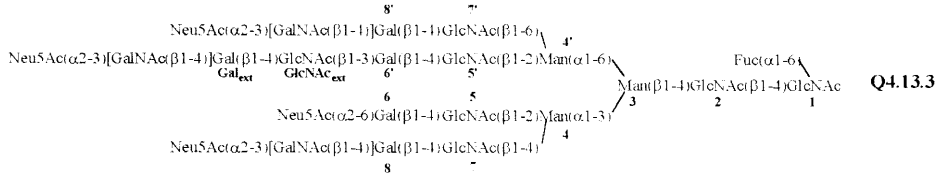
All glycans presented in this article are of the (α1–6)-fucosylated tetraantennary type. The (α1–6)-fucosylated *N,N′*-diacetylchitobiose unit is recognized by the anomeric signals of αGlcNAc-**1**, GlcNAc-**2**, and Fuc, together with the NAc signals of GlcNAc-**1** and GlcNAc-**2** as well as from the Fuc CH_3 signal (De Waard *et al.*, 1991). The set of chemical shifts of the H-1 signals of Man-**4** and Man-**4′**, together with those of the H-2 signals of Man-**3**, Man-**4**, and Man-**4′** reflect the branching pattern to be tetraantennary (Vliegenthart *et al.*, 1983; Hård *et al.*, 1992).

Fraction **Q4.13** contained one main component, **Q4.13.3** (Figure 7a). From the chemical shifts of Man-**4** H-1 at δ 5.137, Gal-**6** H-1 at δ 4.439 and Neu5Ac H-3a at δ 1.720, it can be concluded that the Gal-**6** residue is extended with an (α2–6)-linked Neu5Ac residue (Hård *et al.*, 1992).

The remaining three antennae are terminated with a Sd^a determinant, demonstrated by the ^{1}H characteristics as mentioned above (compare with **N4.9.2**; Hård *et al.*, 1992). In case of the antenna containing the Gal-**6′** residue, a novel feature is the additional *N*-acetyllactosamine unit terminated with a Sd^a determinant, as indicated by the signal of Gal-**6′** H-1 at δ 4.455.

Fraction **Q4.12** contained two N-glycans containing a di-(*N*-acetyllactosamine) element, denoted **Q4.12.3** and **Q4.12.4** (Figure 7b and 7c, respectively). Both compounds bear the Sd^a determinant on each antenna, as indicated by its characteristic set of structural-reporter-group signals of Neu5Ac, GalNAc, and Gal.

Q4.12.3 and **Q4.12.4** are isomers and differ only in the branch position of the additional *N*-acetyllactosamine unit. In structure



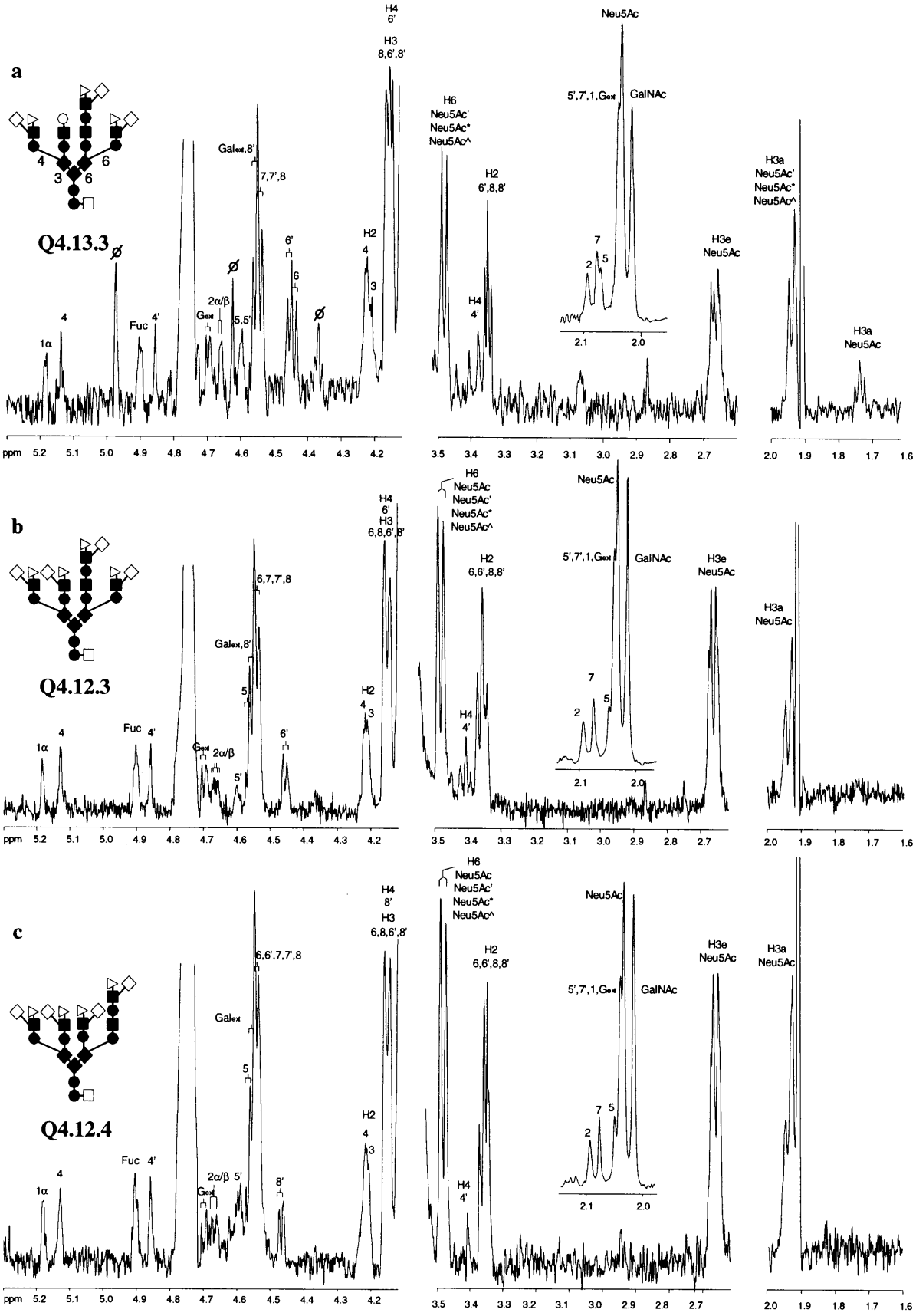
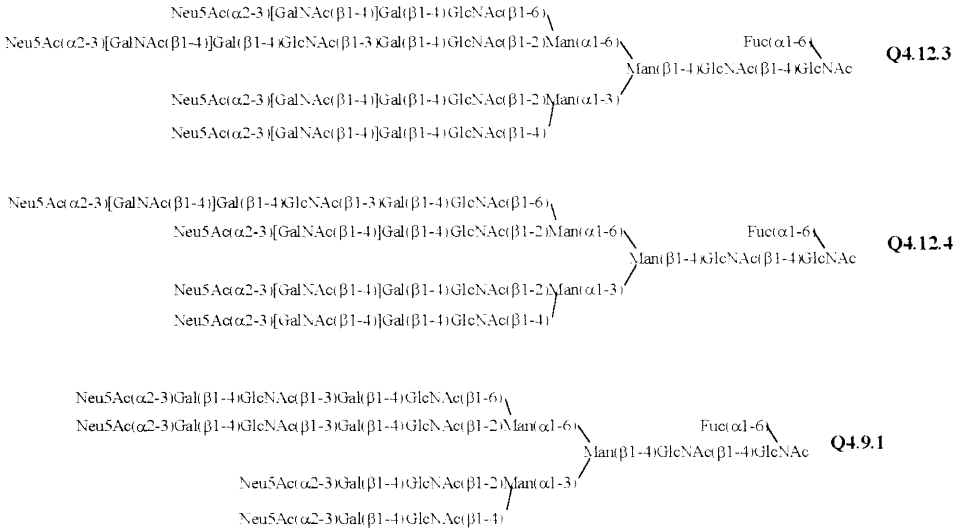


Fig. 7. Resolution-enhanced 600-MHz ^{1}H-NMR spectra at 300 K of oligosaccharides containing additional *N*-acetyllactosamine units, obtained from human Tamm-Horsfall glycoprotein. (**a**) fraction **Q4.13.3**; (**b**) fraction **Q4.12.3**; (**c**) fraction **Q4.12.4**. The numbering of the monosaccharide residues is exemplified in the structure of compound **Q4.13.3** in the text. Short-hand symbolic notations: open triangles, Neu5Ac(α2–3); open circles, Neu5Ac(α2–6); solid circles, GlcNAc; solid diamonds, Man; solid squares, Gal; open diamonds, GalNAc; open squares, Fuc. Slash-circle, contaminant; G_{ext}, $GlcNAc_{ext}$.



Q4.12.3 this unit is attached to Gal-**6′**, based on the structural-reporter-group signal of Gal-**6′** H-1 at δ 4.457, while **Q4.12.4** has the additional *N*-acetyllactosamine unit attached to Gal-**8′**, as follows from the Gal-**8′** H-1 signal at δ 4.467.

Fraction **Q4.9** contained two N-glycans with repeating *N*-acetyllactosamine units, **Q4.9.1** and **Q4.9.5**. The ^{1}H-NMR spectrum of fraction **Q4.9.1** revealed the presence of two additional *N*-acetyllactosamine units attached to Gal-**6′** and Gal-**8′**, respectively.

The typical structural-reporter-group signals were identical with those of reference compound **N4.8** (Hokke *et al.*, 1995) (Figure 8a). In order to gain independent evidence for the branch location of the additional *N*-acetyllactosamine units, **Q4.9.1** was subjected to treatment with endo-β-galactosidase and *N*-acetyl-β-glucosaminidase. This approach yielded a disialo N-glycan with characteristic ^{1}H chemical shifts identical to those of reference compound **D.Q3** (Hokke *et al.*, 1991), proving that the two repeating *N*-acetyllactosamine units were attached to Gal-**6′** and Gal-**8′**.

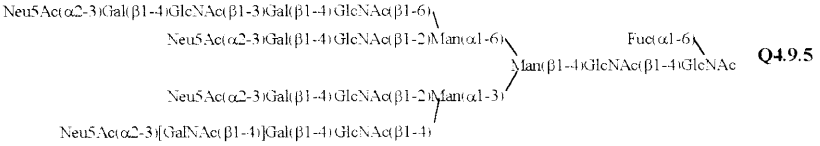
Compound **Q4.9.5** has a novel structure with one Sd^a element and three Neu5Ac(α2–3)Gal elements as was deduced from the integration of the two sets of Neu5Ac structural-reporter-group signals (H-3e, δ 2.660/2.756; H-3a, δ 1.927/1.802; ratio 1:3) (Figure 8b).

A repeating *N*-acetyllactosamine unit was attached to Gal-**8′**, as follows from Gal-**8′** H-1 at δ 4.467. The branch location of the Sd^a determinant was found by treating the oligosaccharide with neuraminidase (*Vibrio cholerae*), thereby removing only those Neu5Ac residues which are not involved in the Sd^a determinant (Donald *et al.*, 1983). The digest was separated on Resource Q affording a monosialo-fraction, denoted **Q4.9.5-ST**. From the chemical shift of Man-**4′** H-1, shifting from δ 4.858 in **Q4.9.5** to δ 4.867 in **Q4.9.5-ST**, it could be concluded that Gal-**6′** was desialylated (cf. compound **N2.10.2** and **N3.7.2A** in Hokke *et al.*, 1995), which means that the H-1 signal at δ 4.469 partly reflects desialylated Gal-**6′** (cf. compound **N3.7.2A** in Hokke *et al.*, 1995). Based on a comparison of the ^{1}H-NMR data of reference compounds **N4.6.2** and **N3.10** in Hokke *et al.* (1995), it can be stated that the H-1 signal at δ 4.481 reflects a desialylated Gal_{ext} residue. Consequently, the H-1 signal at δ 4.469 reflects also partly the corresponding Gal-**8′** residue (cf. compound **Q4.9.5**). In order to discriminate between a desialylated Gal-**6** or a desialylated Gal-**8** residue, use was made of reference compounds **N.3.7.1B** (desialylated Gal-**6**, δ 4.472) and **N3.7.1C** (desialylated Gal-**8**, δ 4.465) from Hokke *et al.* (1995). Based on the H-1 signal pattern between δ 4.4–4.5 (Figure 8b, inset) it is suggested that Gal-**6** is desialylated, which means that Gal-**8** is involved in the Sd^a element.

During this study, no indications were found for N-glycans containing more than one additional *N*-acetyllactosamine unit.

Discussion

The carbohydrate moieties of THp have been studied extensively (Hård *et al.*, 1992, and references therein; Williams *et al.*, 1984), showing an impressive repertoire of sialylated, sulfated, and GalNAc-containing N-glycans. A part of the carbohydrate moiety of THp, containing one or more repeating *N*-acetyllactosamine units, is a substrate for endo-β-galactosidase, resulting in the liberation of a tetra- and a Sd^a-antigen active pentasaccharide (Donald *et al.*, 1983; Donald and Feeney, 1986). In the present study, it was found that the content of repeating *N*-acetyllactosamine units is a donor-specific feature. This was shown from the investigation of the THp of four different donors resulting in a diversion of 42–718 μg tetra- plus pentasaccharide per 100 mg THp. It is interesting to know that THp of donor **PM** used for the glycan-structure determination by Hård *et al.* (1992) has an extremely low content of repeating *N*-acetyllactosamine units, explaining the negative results for identifying oligosaccharides with additional *N*-acetyllactosamine units. The same study also reported the absence of oligomannose-type carbohydrates, although THp has been shown to contain 1–20% oligomannose-type



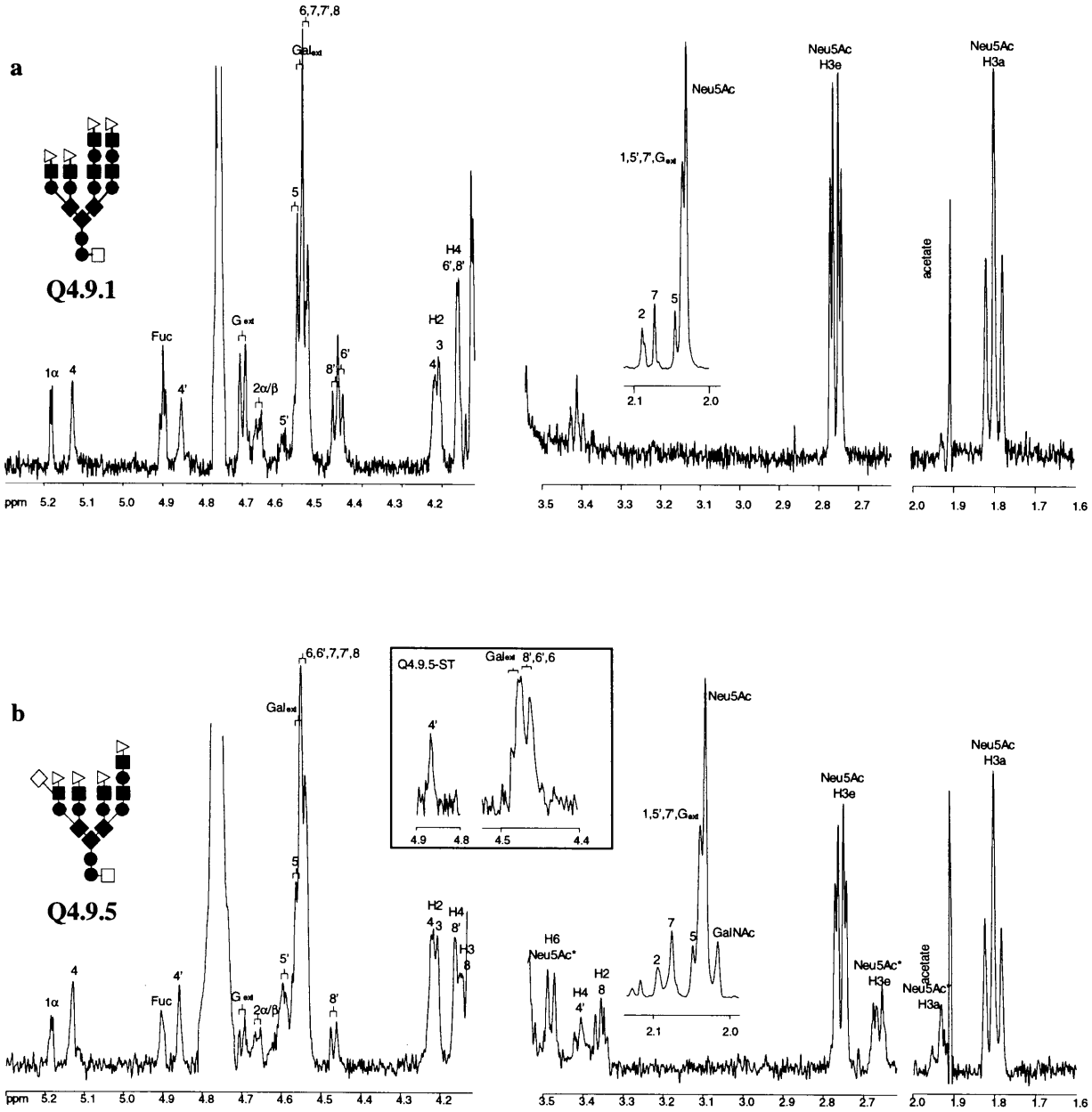


Fig. 8. Resolution-enhanced 600-MHz ^{1}H-NMR spectra at 300 K of oligosaccharides containing additional *N*-acetyllactosamine units, obtained from human Tamm-Horsfall glycoprotein. (**a**) Fraction **Q4.9.1**; (**b**) fraction **Q4.9.5**. For short-hand symbolic notation, see Figure 7.

carbohydrates (Dall' Olio *et al.*, 1988; Serafini Cessi *et al.*, 1984; Smagula *et al.*, 1990). These observations suggest that the synthesis of the carbohydrate repertoire of THp is donor specific.

The enzyme thought to be responsible for initiating the addition of a repeating *N*-acetyllactosamine unit is a i-β3-GlcNAc-transferase, which is normally participating in making the i blood group antigen on erythrocytes in early fetal life (Feizi, 1985). This enzyme is also present in adult kidneys, as concluded from the observation that the i-antigen was present on the membrane and in the cytoplasm of cells of the distal and collecting tubules (Rouger *et al.*, 1980). Therefore, the donor specificity in THp could be the result of a relative difference in expression of the i-β3-GlcNAc-transferase and other processing enzymes, involved in terminating the branches of carbohydrate chains.

The occurrence of the Sd^a determinant for a Gal(β1–4)GlcNAc(β1–3)Gal(β1–4)GlcNAc element is 28% to 50% (Table II), which is higher than the occurrence of the Sd^a determinant for a single Gal(β1–4)GlcNAc element (<5% based on the relative molar amount of tetraantennary N-glycans in Hård *et al.* [1992]). This result shows that the β-1,4-*N*-acetylgalactosaminyltransferase, which requires (α2–3)-linked sialic acid in the acceptor, is much more active when an additional *N*-acetyllactosamine unit is present. Therefore, it is tempting to suggest a role for repeating *N*-acetyllactosamine units in increasing the expression of the Sd^a determinant. In fact, it is known that fucosylated lactosaminoglycans use poly-*N*-acetyllactosamine units to present a variety of structures which appear to play a role in cell–cell interactions during development and differentiation, and possibly

in malignancy (Shur, 1983; Bird and Kimber, 1984; Smets and van Beek, 1984).

Although the biological role of the Sd^a antigen is still unknown, it was postulated that the Sd^a antigen could play a protective role in inhibiting the adhesion of *Escherichia coli* to the endothelium of the kidney and the intestine (Conte and Serafini Cessi, 1991). Adhesion of *E.coli* to the endothelium is mediated by enterotoxic and pyelonephritogenic strains, containing lectin-like regions with different substrate specificity (Korhonen *et al.*, 1983; Parkkinen *et al.*, 1983, 1988; Howard *et al.*, 1988). It has been shown that terminal Neu5Ac(α2–3)Gal and GalNAc-(β1–4)Gal elements are both substrates for these lectin-like regions. When combined into an Sd^a antigen, it might lose its substrate capacity, thereby being able to hinder the binding of *E.coli* adhesins to the endothelium (Watkins, 1995). This is supported by the observation of a strong Sd^a-expression on tissues in environments containing a rich bacterial flora like the kidney and the intestine. Expression of THp on the surface of the endothelium of the thick ascending limb could therefore help to protect the endothelium by capping the endothelium with its Sd^a determinant.

Five N-glycans containing repeating *N*-acetyllactosamine units were isolated using enzymatic and chromatographic methods, and their characterization was performed using 600 MHz ^{1}H-NMR spectroscopy. One compound, containing two repeating *N*-acetyllactosamine units, was used in order to identify the branch location of these repeats. It is likely that only Gal-**6′** and Gal-**8′** were occupied by an additional *N*-acetyllactosamine unit. The location of the *N*-acetyllactosamine units in the branches attached to the (α1–6)-linked Man residue is in accordance with the reported specificity of i-β3-GlcNAc-transferase from Novikoff tumor cell ascites fluid which synthesizes the GlcNAc(β1–3)Gal linkage in repeating *N*-acetyllactosamine sequences (Van den Eijnden *et al.*, 1988).

The results presented here prove the existence of N-glycans containing additional *N*-acetyllactosamine units in THp, which, when treated with endo-β-galactosidase, release a Sd^a-active pentasaccharide and a tetrasaccharide. Furthermore, the presence of N-glycans containing repeating *N*-acetyllactosamine units in THp is a donor-specific feature. Further studies about the donor specificity of the N-glycans of THp as well as the possible biological role of THp are under current investigation.

Materials and methods

Materials

For each donor, THp was isolated from pooled morning urine as described (Serafini Cessi *et al.*, 1989). Recombinant peptide-N^4-(*N*-acetyl-β-glucosaminyl)asparagine amidase F (PNGase-F) from *Flavobacterium meningosepticum*, endo-β-galactosidase from *Bacteroides fragilis*, and neuraminidase from *Vibrio cholerae* were purchased from Boehringer Mannheim. *N*-Acetyl-β-glucosaminidase from jack beans was purchased from Sigma.

Endo-β-galactosidase digestion of Tamm-Horsfall glycoprotein

To 100 mg THp dissolved in 6.6 ml water was added 66.6 µl 1 M NaOAc buffer, pH 5.9, containing 22 mU endo-β-galactosidase. The mixture was incubated for 48 h at 37 °C, then fractionated on a column of Bio-Gel P-4 (50 × 1.0 cm, Bio-Rad) at a flow rate of 15 ml/h with 10 mM NH_4HCO_3, pH 7.0, as eluent. The effluent was monitored at 206 nm (Uvicord, LKB) and the fraction containing the released oligosaccharides (orcinol/H_2SO_4) was lyophilized, desalted by HiTrap (Pharmacia FPLC system; 4 columns connected, 4 × 5 ml; eluent, 5 mM NH_4HCO_3; flow rate, 3 ml/min; detection, 214 nm), and lyophilized again. The carbohydrate pool was separated by high-pH anion-exchange chromatography (HPAEC) as described below with a concentration gradient of NaOAc in 0.1 M NaOH as indicated in the figures. Collected fractions were immediately neutralized by 5 M HOAc, lyophilized, desalted by HiTrap, and lyophilized again.

Liberation of the carbohydrate chains

The N-linked carbohydrate chains were enzymatically released from THp according to a slightly modified version essentially as described (Hård *et al.*, 1992). However, for batches of 100 mg THp, the fractionation was performed on a Superdex 75 column (60 × 2.6 cm, Pharmacia), eluted with 100 mM NH_4HCO_3, pH 7.0, at a flow rate of 4 ml/min. The effluent was monitored at 206 nm (Uvicord, LKB). Carbohydrate-positive fractions (orcinol/H_2SO_4) and fractions containing the deglycosylated protein were each pooled, lyophilized, desalted by HiTrap (Pharmacia), and lyophilized again. Monosaccharide analyses of the liberated carbohydrates and the deglycosylated- and intact THp were performed as described (Kamerling and Vliegenthart, 1989).

FPLC fractionation

The enzymatically released carbohydrate pool was fractionated by anion-exchange chromatography on a Resource Q column (1 ml, Pharmacia, Pharmacia FPLC system) at a flow rate of 4 ml/min using a H_2O/NaCl-gradient as indicated in the figure. The effluent was monitored at 214 nm and carbohydrate-positive fractions, as detected by orcinol/H_2SO_4, were lyophilized, desalted by HiTrap (Pharmacia), and lyophilized again.

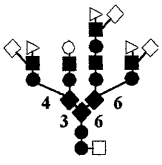
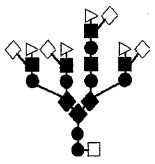
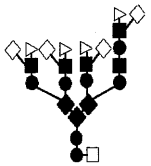
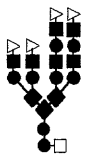
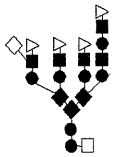
HPLC fractionation

HPLC was carried out as described previously (Hård *et al.*, 1992) using a Kratos SF 400 HPLC system (ABI Analytical, Kratos Division) equipped with a 5 µm Lichrosorb-NH_2 column (25 × 0.4 cm, Merck) at a flow rate of 1.5 ml/min. Elutions were performed with linear concentration gradients of 30 mM K_2HPO_4/KH_2PO_4, pH 6.8, and acetonitrile as indicated in the figure. The effluent was monitored at 206 nm, and relevant fractions were concentrated under a stream of nitrogen. Subsequently, these fractions were lyophilized, desalted by HiTrap (Pharmacia), and lyophilized again.

High-pH anion-exchange chromatography

Subfractionation of the main HPLC fractions was performed by high-pH anion-exchange chromatography (HPAEC) using pulsed amperometric detection (PAD) on a Dionex LC system consisting of a Dionex Bio LC quaternary gradient module, a PAD 2 detector, and a CarboPac PA-1 pellicular anion-exchange column (25 × 0.9 cm, Dionex). Elutions were carried out with a concentration gradient of NaOAc in 0.1 M NaOH as indicated in the figures at a flow rate of 4 ml/min. Detection was performed using the following pulse potentials and durations: E_1 = 0.05 V (300 ms); E_2 = 0.65 V (60 ms); E_3 = -0.95 V (180 ms). Collected fractions were immediately neutralized by 5 M HOAc, lyophilized, desalted by HiTrap (Pharmacia), and lyophilized again.

Table III. ^{1}H-Chemical shifts of structural-reporter-group protons of the constituent monosaccharides of tetraantennary N-linked oligosaccharides containing additional *N*-acetyllactosamine units, derived from human Tamm-Horsfall glycoprotein

Reporter group	Residue	Chemical shift (δ, ppm) in				
		Q4.13.3	**Q4.12.3**	**Q4.12.4**	**Q4.9.1**	**Q4.9.5**
						
H-1	GlcNAc-**1**α	5.183	5.183	5.182	5.182	5.182
	GlcNAc-**2**	4.66[a]	4.66	4.66	4.66	4.66
	Man-**4**	5.137	5.127	5.129	5.128	5.129
	Man-**4'**	4.854	4.859	4.857	4.854	4.858
	GlcNAc-**5**	4.59	4.568	4.568	4.56	4.56
	GlcNAc-**5'**	4.60	4.60	4.592	4.60	4.60
	Gal-**6**	4.439	4.54	4.54	4.541	4.54
	Gal-**6'**	4.455	4.457	4.54	4.453	4.54
	GlcNAc-**7**	4.54	4.54	4.54	4.541	4.54
	GlcNAc-**7'**	4.54	4.54	4.54	4.546	4.54
	Gal-**8**	4.54	4.54	4.54	4.541	4.54
	Gal-**8'**	4.556	4.554	4.467	4.467	4.467
	GalNAc	nd[b]	nd	nd	-	nd
	$GlcNAc_{ext}$	4.698	4.699	4.699	4.698[c]	4.700
	Gal_{ext}	4.556	4.554	4.555	4.556[c]	4.558
	Fuc_{α}	4.898	4.897	4.899	4.896	4.898
	Fuc_{β}	4.904	4.906	4.912	4.904	4.907
H-2	Man-**3**	4.21	4.20	4.20	4.20	4.20
	Man-**4**	4.22	4.22	4.22	4.22	4.22
	Man-**4'**	4.08	4.08	4.08	4.08	4.08
H-3e[g]	Neu5Ac	2.66	2.66	2.66	2.756	2.756
	Neu5Ac'	2.66	2.66	2.66	2.756	2.756
	Neu5Ac*	2.66	2.66	2.66	2.756	2.660
	Neu5Ac^	2.66	2.66	2.66	2.756	2.756
H-3a	Neu5Ac	1.720	1.93	1.93	1.800	1.802
	Neu5Ac'	1.93	1.93	1.93	1.800	1.802
	Neu5Ac*	1.93	1.93	1.93	1.800	1.927
	Neu5Ac^	1.93	1.93	1.93	1.800	1.802
NAc	GlcNAc-**1**	2.038	2.038	2.038	2.036	2.038
	GlcNAc-$\mathbf{2}_{\alpha/\beta}$	2.093	2.092	2.094	2.089	2.094
	GlcNAc-**5**	2.069	2.048	2.048	2.047	2.048
	GlcNAc-**5'**	2.038	2.038	2.038	2.036	2.038
	GlcNAc-**7**	2.076	2.076	2.076	2.074	2.075
	GlcNAc-**7'**	2.038	2.038	2.038	2.036	2.038
	$GlcNAc_{ext}$	2.038	2.038	2.038	2.036[f]	2.038
	GalNAc	2.015[d]	2.015[e]	2.015[e]	-	2.015
	Neu5Ac	2.031[e]	2.032[e]	2.032[e]	2.032[e]	2.031[e]
CH_3	Fuc_{α}	1.210	1.210	1.210	1.210	1.210
	Fuc_{β}	1.222	1.222	1.222	1.222	1.222

Chemical shifts are given at 300 K and were measured in $^{2}H_2O$ relative to internal acetone (δ 2.225). Compounds are represented by short-hand symbolic notation: open triangles, Neu5Ac(α2-3); open circles, Neu5Ac(α2-6); solid circles, GlcNAc; solid diamonds, Man; solid squares, Gal; open diamonds, GalNAc; open squares, Fuc. For numbering of the monosaccharide residues, see text. α and β stand for the anomeric configuration of GlcNAc-**1**.

[a]Some values are given with only two decimals because of spectral overlap.

[b]nd, Not determined.

[c]Signal stemming from two protons.

[d]Signal stemming from three NAc groups.

[e]Signal stemming from four NAc groups.

[f]Signal stemming from two NAc groups.

[g]Neu5Ac is linked to Gal-**6**; Neu5Ac′ is linked to Gal-**6′**; Neu5Ac* is linked to Gal-**8**, and Neu5Ac^ is linked to Gal-**8′**.

Branch location of repeating N-acetyllactosamine units

Oligosaccharides, containing repeating *N*-acetyllactosamine units, were digested with endo-β-galactosidase, and the products were purified by anion-exchange chromatography and treated with *N*-acetyl-β-glucosaminidase as described previously (Hokke *et al.*, 1991).

Neuraminidase treatment

The oligosaccharide was dissolved in 100 µl 100 mM NaOAc buffer, pH 5.6, containing 1 mM $Ca(OAc)_2$ and 100 mM NaCl, and incubated with 5 mU neuraminidase for 4 h at 37°C. After fractionation by FPLC on Resource Q as described, the obtained fractions were lyophilized, desalted by HiTrap (Pharmacia), and lyophilized again.

^{1}H-NMR spectroscopy

Prior to ^{1}H-NMR analysis, samples were exchanged twice in 99.9% ^2H_2O with intermediate lyophilization and finally dissolved in 450 µl 99.96% ^2H_2O (Isotec Inc); 600 MHz ^{1}H-NMR spectra were recorded on a Bruker AMX 600 spectrometer (SON hf-NMR facility, Department of Biophysical Chemistry, University of Nijmegen, The Netherlands) essentially as described previously (Hård *et al.*, 1992). 500 MHz ^{1}H-NMR spectra were recorded on a Bruker AMX-500 spectrometer (Bijvoet Center, Department of NMR Spectroscopy, Utrecht University).

The 2D-clean TOCSY experiments were carried out at 300 K, using MLEV-17 mixing sequence cycles with a spin-lock mixing pulse of 100 ms at a field strength (9 kHz) corresponding to a 90° ^{1}H pulse width of 26.5 µs. The spectral width was 4000 Hz in each dimension and a number of 512 × 2048 data points were recorded. The carrier frequency was placed on the waterline in all cases. Phase-sensitive handling of the data in the ω_1 dimension became possible by the time-proportional phase increment method implemented in the Bruker software. The time domain data were zero-filled to a 1024 × 2048 data matrix prior to multiplication with a squared-bell function phase shifted by π/3.

Acknowledgments

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References

Bird,J.M. and Kimber,S.J. (1984) Oligosaccharides containing fucose α(1–3) and α(1–4) to *N*-acetylglucosamine cause decompaction of mouse morulae. *Dev. Biol.*, **104,** 449–460.

Conte,R. and Serafini Cessi,F. (1991) Comparison between the erythrocytes and urinary Sd^a antigen distribution in a large number of individuals from Emilia Romagna, a region of northern Italy. *Transfus. Med.*, **1,** 47–49.

Dall' Olio,F., Kanter,F.J.J., van den Eijnden,D.H. and Serafini Cessi,F. (1988) Structural analysis of the preponderant high-mannose oligosaccharides of human Tamm-Horsfall glycoprotein. *Carbohydr. Res.*, **178,** 327–332.

Dall' Olio,F., Chiricolo,M., Malagolini,N., Franceschi,C. and Serafini Cessi,F. (1991) Immunosuppressive activity of Tamm-Horsfall glycoprotein oligosaccharides: effect of removal of outer sugars and conjugation with a protein carrier. *Cell. Immunol.*, **137,** 303–315.

De Waard,P., Koorevaar,A., Kamerling,J.P. and Vliegenthart,J.F.G. (1991) Structure determination by ^{1}H-NMR spectroscopy of (sulfated) sialylated N-linked carbohydrate chains released from porcine thyroglobulin by peptide-N^4-(*N*-acetyl-β-glucosaminyl)asparagine amidase-F. *J. Biol. Chem.*, **266,** 4237–4243.

Donald,A.S.R., Yates,A.D., Soh,C.P.C., Morgan,W.T.J. and Watkins,W.M. (1983) A blood group Sd^a-active pentasaccharide isolated from Tamm-Horsfall urinary glycoprotein. *Biochem. Biophys. Res. Commun.*, **115,** 625–631.

Donald,A.S.R. and Feeney,J. (1986) Oligosaccharides obtained from a blood-group-Sd(a+) Tamm-Horsfall glycoprotein. *Biochem. J.*, **236,** 821–828.

Dubois,M., Gilles,K.A., Hamilton,J.K., Rebers,P.A. and Smith,F. (1956) Colorimetric method for determination of sugars and related substances. *Anal. Chem.*, **28,** 350–356.

Duncan,J.L. (1988) Differential effect of Tamm-Horsfall protein on adherence of *Escherichia coli* to transitional epithelial cells. *J. Infect. Dis.*, **158,** 1379–1382.

Feizi,T. (1985) Demonstration by monoclonal antibodies that carbohydrate structures of glycoproteins and glycolipids are onco-developmental antigens. *Nature*, **314,** 53–57.

Hård,K., van Zadelhoff,G., Moonen,P., Kamerling,J.P. and Vliegenthart,J.F.G. (1992) The Asn-linked carbohydrate chains of human Tamm-Horsfall glycoprotein of one male: novel sulfated and novel *N*-acetylgalactosamine-containing N-linked carbohydrate chains. *Eur. J. Biochem.*, **209,** 895–915.

Hokke,C.H., Kamerling,J.P., van Dedem,G.W.K. and Vliegenthart,J.F.G. (1991) Determination of the branch location of extra *N*-acetyllactosamine units in sialo N-linked tetraantennary oligosaccharides. *FEBS Lett.*, **286,** 18–24.

Hokke,C.H., Damm,J.B.L., Penninkhof,B., Aitken,R.J., Kamerling,J.P. and Vliegenthart,J.F.G. (1994) Structure of the O-linked carbohydrate chains of porcine zona pellucida glycoproteins. *Eur. J. Biochem.*, **221,** 491–512.

Hokke,C.H., Bergwerff,A.A., van Dedem,G.W.K., Kamerling,J.P. and Vliegenthart,J.F.G. (1995) Structural analysis of the sialylated N- and O-linked carbohydrate chains of recombinant human erythropoietin expressed in Chinese hamster ovary cells. Sialylation patterns and branch location of dimeric *N*-acetyllactosamine units. *Eur. J. Biochem.*, **228,** 981–1008.

Horton,J.K., Davies,M., Topley,N., Thomas,D.B.L. and Williams,J.D. (1990) Activation of the inflammatory response of neutrophils by Tamm-Horsfall glycoprotein. *Kidney Int.*, **37,** 717–726.

Howard,C.K., Roberts,D.D. and Ginsburg,V. (1988) Many pulmonary bacteria bind specifically to the carbohydrate sequence GalNAcβ1–4Gal found in some glycolipids. *Proc. Natl. Acad. Sci. USA*, **85,** 6157–6161.

Kamerling,J.P. and Vliegenthart,J.F.G. (1989) Carbohydrates. In Lawson,A.M. (ed.), *Clinical Biochemistry; Principles, Methods, Applications. Mass Spectrometry.* Walter de Gruyter, Berlin, pp. 175–263.

Korhonen,T.K., Vaisanen-Rhen,V., Rhen,M., Pere,A., Parkkinen,J. and Finne,J. (1983) *Escherichia coli* fimbriae recognising sialyl galactosides. *J. Bacteriol.*, **159,** 762–766.

Muchmore,A.V. and Decker,J.M. (1985) Uromodulin: a unique 85-kilodalton immunosuppressive glycoprotein isolated from urine of pregnant woman. *Science*, **229,** 479–481.

Muchmore,A.V. and Decker,J.M. (1987) Evidence that recombinant IL-1α exhibits lectin-like specificity and binds to homogeneous uromodulin via N-linked oligosaccharides. *J. Immunol.*, **138,** 2541–2546.

Muchmore,A.V., Shifrin,S. and Decker,J.M. (1987) *In vitro* evidence that carbohydrate moieties derived from uromodulin, an 85,000 dalton immunosuppressive glycoprotein isolated from human pregnancy urine, are immunosuppressive in the absence of intact protein. *J. Immunol.*, **138,** 2547–2553.

Muchmore,A.V., Decker,J.M., Shaw,A. and Wingfield,P. (1990a) Evidence that high mannose glycopeptides are able to functionally interact with recombinant tumor necrosis factor and recombinant interleukin-1. *Cancer Res.*, **50,** 6285–6290.

Muchmore,A.V., Sathyamoorthy,N., Decker,J.M. and Sherblom,A.P. (1990b) Evidence that specific high mannose oligosaccharides can directly inhibit antigen driven T-cell responses. *J. Leuk. Biol.*, **48,** 457–464.

Parkkinen,J., Finne,J., Achtman,M., Vaisanen,V. and Korhonen,T.K. (1983) *Escherichia coli* strains binding neuraminyl α2–3 galactosides. *Biochem. Biophys. Res. Commun.*, **111,** 456–461.

Parkkinen,J., Virkola,R. and Korhonen,T.K. (1988) Identification of factors in human urine that inhibit the binding of *Escherichia coli* adhesins. *Infect. Immun.*, **56,** 2623–2630.

Rindler,M.J., Naik,S.S., Li,N., Hoops,T.C. and Peraldi,M.N. (1990) Uromodulin/Tamm-Horsfall glycoprotein/uromucoid is a phosphatidylinositol-linked membrane protein. *J. Biol. Chem.*, **265,** 20784–20789.

Rouger,R., Gane,P., Homberg,J.C. and Salmon,C. (1980) Developmental aspects of A, B, H, lewis, I, i and Pr antigens in human kidneys. *Rev. Fr. Transfus. Immunohematol.*, **23,** 553–562.

Sathyamoorthy,N., Decker,J.M., Sherblom,A.P. and Muchmore,A.V. (1991) Evidence that specific high mannose structures directly regulate multiple cellular activities. *Mol. Cell Biochem.*, **102,** 139–147.

Scudder,P., Uemura,K., Dolby,J., Fukuda,M.N. and Feizi,T. (1983) Isolation and characterisation of an endo-β-galactosidase from *Bacteroides fragilis*. *Biochem. J.*, **213,** 485–494.

Serafini Cessi,F., Dall' Olio,F. and Malagolini,N. (1984) High-mannose oligosaccharides from human Tamm-Horsfall glycoprotein. *Biosci. Rep.*, **4,** 269–274.

Serafini Cessi,F., Bellabarba,G., Malagolini,N. and Dall' Olio,F. (1989) Rapid isolation of Tamm-Horsfall glycoprotein (uromodulin) from human urine. *J. Immunol. Methods*, **120,** 185–189.

Sherblom,A.P., Decker,J.M. and Muchmore,A.V. (1988) The lectin-like interaction between recombinant tumor necrosis factor and uromodulin. *J. Biol. Chem.*, **263,** 5418–5424.

Shur,B.D. (1983) Embryonal carcinoma cell adhesion: role of surface galactosyl transferase and its 90 kDa lactosaminoglycan substrate. *Dev. Biol.*, **99,** 360–372.

Sikri,K.L., Foster,C.L., Bloomfield,F.J. and Marshall,R.D. (1979) Localization by immunofluorescence and by light- and electron-microscopic immunoperoxidase techniques of Tamm-Horsfall glycoprotein in adult hamster kidney. *Biochem. J.*, **181,** 525–532.

Smagula,R.M., van Halbeek,H., Decker,J.M., Muchmore,A.V., Moody,C.E. and Sherblom,A.P. (1990) Pregnancy-associated changes in oligomannose oligosaccharides of human and bovine uromodulin (Tamm-Horsfall glycoprotein). *Glycoconjugate J.*, **7,** 609–624.

Smets,L.A. and van Beek,W.P. (1984) Carbohydrates of the tumor cell surface. *Biochim. Biophys. Acta*, **738,** 237–249.

Tamm,I. and Horsfall,F.L. (1950) Characterization and separation of an inhibitor of viral hemagglutination present in urine. *Proc. Soc. Exp. Biol. Med.*, **74,** 108–114.

Thomas,D.B.L., Davies,M., Peters,J.R. and Williams,J.D. (1993a) Tamm-Horsfall glycoprotein bind to a single class of carbohydrate specific receptors on human neutrophils. *Kidney Int.*, **44,** 423–429.

Thomas,D.B.L., Davies,M. and Williams,J.D. (1993b) Release of gelatinase and superoxide from human mononuclear phagocytes in response to particulate Tamm-Horsfall protein. *Am. J. Pathol.*, **142,** 249–260.

Toma,G., Bates Jr,J.M. and Kumar,S. (1994) Uromodulin (Tamm-Horsfall glycoprotein) is a leukocyte adhesion molecule. *Biochem. Biophys. Res. Commun.*, **200,** 275–282.

Van den Eijnden,D.H., Koenderman,A.H.L. and Schiphorst,W.E.C.M. (1988) Biosynthesis of blood group i-active polylactosamine glycans. Partial purification and properties of an UDP-GlcNAc: *N*-acetyllactosaminide β1–3-*N*-acetylglucosaminyltransferase from Novikoff tumor cell ascites fluid. *J. Biol. Chem.*, **263,** 12461–12471.

Vliegenthart,J.F.G., Dorland,L. and van Halbeek,H. (1983) High resolution, ^{1}H-nuclear magnetic resonance spectroscopy as a tool in the structural analysis of carbohydrates related to glycoproteins. *Adv. Carbohydr. Chem. Biochem.*, **41,** 209–374.

Watkins,W.M. (1995) Sd^a and Cad Antigens. In Cartron,J.P. and Rouger,P. (eds.), *Blood Cell Chemistry; Molecular Basis of Major Human Blood Group Antigens*. Plenum Press, New York, pp. 351–375.

Williams,J.D., Marshall,R.D., van Halbeek,H. and Vliegenthart,J.F.G. (1984) Structural analysis of the carbohydrate moieties of human Tamm-Horsfall glycoprotein. *Carbohydr. Res.*, **134,** 141–155.

Yu,C.L., Lin,W.M., Liao,T.S., Tsai,C.Y., Sun,K.H. and Chen,K.H. (1992) Tamm-Horsfall glycoprotein (THG) purified from normal human pregnancy urine increases phagocytosis, complement receptor expression and arachidonic acid metabolism of polymorphonuclear cells. *Immunopharmacology*, **24,** 181–190.

Yu,C.L., Tsai,C.Y., Lin,W.M., Liao,T.S., Chen,H.L., Sun,K.H. and Chen,K.H. (1993) Tamm-Horsfall urinary glycoprotein enhances monokine release and augments lymphocyte proliferation. *Immunopharmacology*, **26,** 249–258.