# DETERMINATION BY 360 MHZ $^1$H NMR SPECTROSCOPY OF THE TYPE OF BRANCHING IN COMPLEX ASPARAGINE-LINKED GLYCAN CHAINS OF GLYCOPROTEINS

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## 1. Introduction

Glycans chains of the *N*-acetyllactosamine type *N*-glycosidically linked to asparagine of glycoproteins are generally composed of the pentasaccharide core structure Manα(1→3)[Manα(1→6)]Manβ(1→4)GlcNAcβ(1→4)GlcNAcβ1→Asn, whereas the *N*-acetyllactosamine units can be attached to the α-mannose residues of this pentasaccharide [1]. In the frequently-occurring bi-antennary structure (fig.1, class A) *N*-acetyllactosamine units are β(1→2) linked to the α-mannose residues 4 and 4′ respectively. Addition of an extra *N*-acetyllactosamine unit in β(1→4) linkage to Man 4 of this bi-antennary glycan affords the so called tri-antennary glycan structure (fig.1, class B). Extension of the bi-antennary structure with 2 additional *N*-acetyl-lactosamine units, linked β(1→4) to Man 4 and β(1→6) to Man 4′ respectively, yields the tetra-antennary glycan (fig.1, class C). To one or more of the galactose units sialic acid residues can be linked via α(2→3) or α(2→6) linkages. Furthermore, in various positions of the glycan chain fucose residues might occur.

In general the structure determination of these glycan chains is carried out along chemical and enzymic routes [2–5]. However, recently it has been shown that high-resolution $^1$H NMR spectroscopy is a powerful tool in the identification of complex carbohydrate chains [3,6–9] having the advantages of being a fast and non-destructive method. In particular the anomeric, mannose H-2, sialic acid H-3, fucose $CH_3$ and *N*-acetyl protons give relevant structural information.

It is shown here that the above mentioned bi-, tri- and tetra-antennary structures of asparagine-linked asialo- and afuco-glycan chains can easily be distinguished on the basis of the chemical shifts of protons of the mannotriosido-branching core and of the *N*-acetyl protons of the GlcNAc residues.

## 2. Materials and methods

Asialo-glycopeptides of class A were obtained from human and rabbit serotransferrin [2,7]. Asialo-glycopeptides of classes A, B and C were obtained from human plasma $\alpha_1$-acid glycoprotein [10].

Solutions of glycopeptides were neutralized if

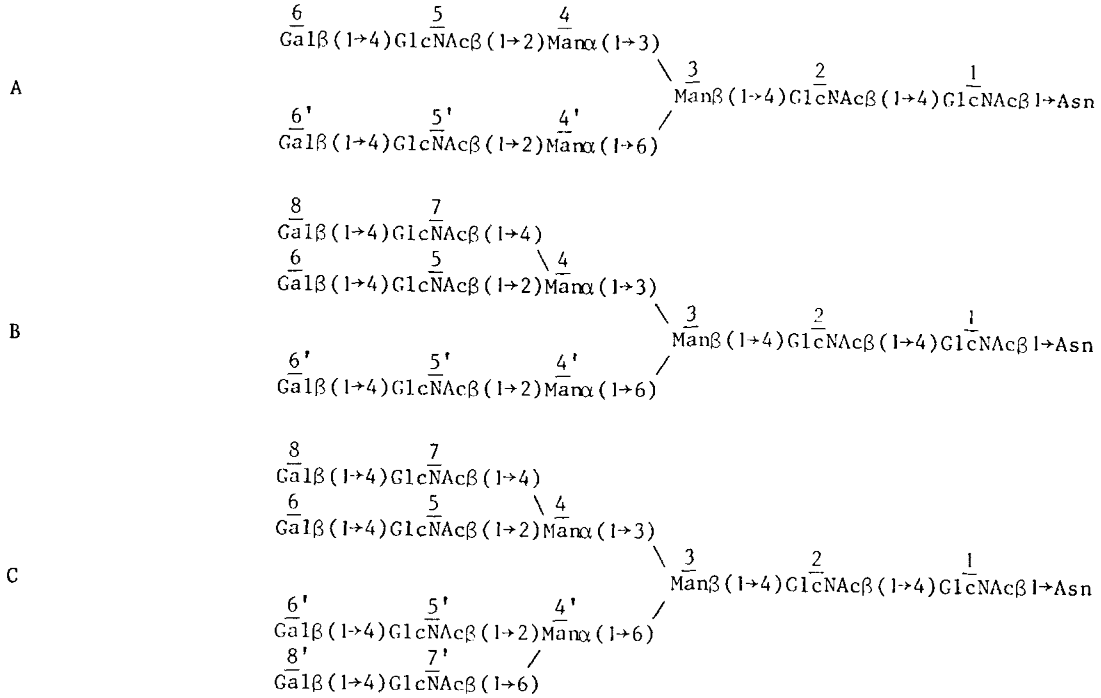


Fig.1. Structures of the asparagine-linked glycans of the *N*-acetyllactosamine type.

necessary and exchanged 3 times in $D_2O$ with intermediate lyophilization. Spectral analysis of 0.02–0.05 M solutions of the compounds in $D_2O$ (99.96 atom % D, Aldrich) was carried out on a Bruker HX-360 spectrometer, operating in the Fourier Transform mode at probe temp. 25°C or 60°C. Chemical shifts at 25°C are given relative to sodium 2,2-dimethyl-2-silapentane-5-sulphonate (indirectly to acetone in $D_2O$: $\delta = 2.225$ ppm).

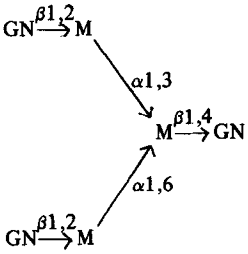
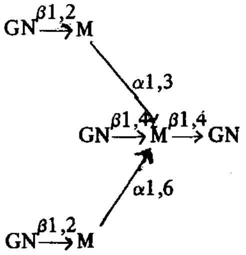
## 3. Results and discussion

360 MHz $^1H$ NMR spectra were recorded for a large number of representatives of each of the aforementioned classes (A,B,C, fig.1) of branched complex glycan chains in the asialo form.

The substitution pattern of the mannose residues in each class is reflected in the characteristic chemical shifts of the H-1 and H-2 protons of these mannose residues, summarized in table 1. The assignments were made by comparison with the spectrum of the asialo-glycan of human serotransferrin [7] and by selective homonuclear decoupling experiments for classes B and C glycans. For the detection of the H-2 proton signals selective irradiation experiments are in some cases essential, since in the 4.05–4.25 ppm region other protons might resonate. In particular extension of the peptide backbone may give rise to interfering proton resonances.

It has to be noted that addition of a GlcNAc residue via β(1→4) linkage to Man 3 of the mannotriosido-branching core gives rise to a set of mannose H-1 and H-2 values which can easily be distinguished from the values for classes A, B, C structures. The typical influence of this additional GlcNAc residue for oligosaccharide '6' isolated from urine of a patient with Sandhoff's disease [8] is shown in table 1. This type of structure was only available in the form of this oligosaccharide and not in the form of glycopeptides like those isolated from IgA [11] and ovomucoid [12]. However, the absence of the GlcNAc–Asn part does not alter significantly the resonance positions of the mannose H-1 and H-2 protons as can

Table 1
Chemical shifts[a] of mannose H-1 and H-2 protons for bi-, tri- and tetra-antennary asparagine-bound glycan chains of the *N*-acetyllactosamine type

| Structure | δ H-1 of residue | | | δ H-2 of residue | | |
|---|---|---|---|---|---|---|
| | 3 | 4 | 4' | 3 | 4 | 4' |
| Class A (bi-antenna)<br>*n*=4 | 4.764<br>±0.003 | 5.121<br>±0.002 | 4.928<br>±0.002 | 4.247<br>±0.003 | 4.189<br>±0.003 | 4.110<br>±0.003 |
| Class B (tri-antenna)<br>*n*=5 | 4.757<br>±0.004 | 5.119<br>±0.002 | 4.924<br>±0.003 | *4.215*<br>±0.004 | *4.215*<br>±0.004 | 4.109<br>±0.003 |
| Class C (tetra-antenna)<br>*n*=5 | 4.754<br>±0.004 | 5.127<br>±0.003 | *4.866*<br>±0.003 | *4.215*<br>±0.002 | *4.215*<br>±0.002 | *4.092*<br>±0.002 |
| oligosaccharide '5'[b]<br> | ~4.77 | 5.119 | 4.922 | ~4.25 | 4.192 | 4.112 |
| oligosaccharide '6'<br> | *~4.70* | *5.062* | *5.004* | *~4.18* | *4.250* | *4.151* |

[a] Mean values ± SD calculated from *n* independent samples
[b] GN, *N*-acetylglucosamine; M, mannose

be concluded from comparison of the data of class A glycopeptides with those of oligosaccharide '5' [8] (table 1).

Another characteristic feature of the spectra is formed by the resonance pattern of the *N*-acetyl protons of the GlcNAc residues as is shown in fig.2. The chemical shifts and peak intensities are indicative for the classes A, B, C structures.

These NMR data are that characteristic that they allow a rapid and reliable recognition of the bi-, tri- and tetra-antennary classes of complex asparagine-bound glycan chains.

For sialo-analogues of these structures a similar type of identification procedure can be set up [13]. The presence of sialic acid gives rise to small but significant changes in the chemical shift values for mannose H-1 and H-2 protons in comparison to the asialo-chains.

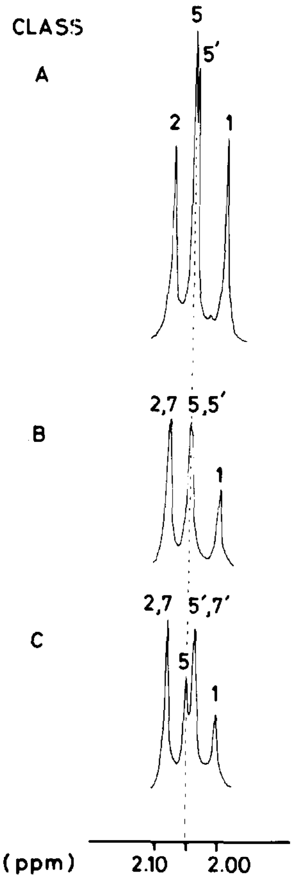


Fig.2. Characteristic resonance patterns of the *N*-acetyl protons for the classes A, B, C carbohydrate chains. The numbers in the figure refer to the corresponding GlcNAc residues.

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