Novel forms of protein glycosylation

Johannes FG Vliegenthart* and Florence Casset

A large number of new glycans, derived from glycoproteins, has been characterized in the past few years. *O*-linked fucose was found in epidermal growth factor-like domains of several proteins. For the *N*-linked glycans of *Helix pomatia* hemocyanin, novel types of antennae were identified. The positions of noncarbohydrate substituents were established in *N*-glycans. *C*-mannosylation of a tryptophan residue was discovered in human ribonuclease 2 and is the first example of *C*-glycosylation in glycoproteins.

Addresses
Department of Bio-organic Chemistry, Bijvoet Center, Utrecht University, Padualaan 8, NL-3584 CH Utrecht, The Netherlands
*e-mail: vlieg@accu.uu.nl

Current Opinion in Structural Biology 1998, 8:565–571

http://biomednet.com/elecref/0959440X00800565

© Current Biology Ltd ISSN 0959-440X

Abbreviations

EDN	eosinophil-derived neurotoxin
Fuc	fucose
Gal	galactose
GalNAc	*N*-acetylgalactosamine
Glc	glucose
GlcNAc	*N*-acetylglucosamine
Hex	hexose
HexNac	*N*-acetylhexosamine
HNK cell	human natural killer cell
Man	mannose
N-CAM	neural cell adhesion molecule
Neu	neuraminic acid
Neu5Ac	5-*N*-acetylneuraminic acid
RNase	ribonuclease

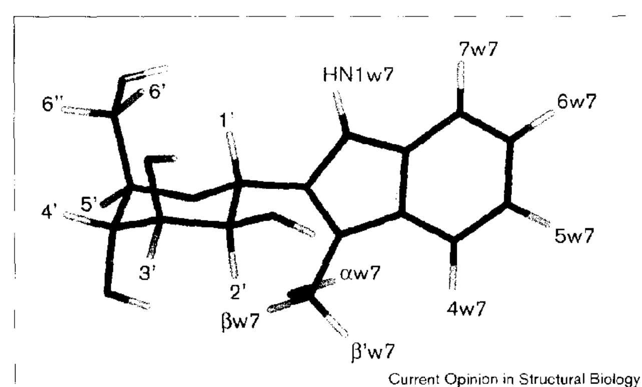
Introduction

Developments in structural glycobiology are proceeding very quickly, mainly due to improvements in the methodology for characterizing glycoconjugates. Small amounts of material, that is, below the nanomole level are sufficient for the unambiguous determination of primary structures. In particular, the advances in both isolation procedures and physical techniques, like mass spectrometry and NMR spectroscopy, have made this possible. For the primary structures of the glycans in glycoproteins, a large array of new structures has been established recently. Now CarbBank contains about 1000 unique *N*-linked chains and about 500 *O*-linked chains. New types of carbohydrate–protein linkages have been discovered and unusual constituents and noncarbohydrate substituents identified in terms of their chemical nature and localization in the carbohydrate chain. Here, we give an overview of the new forms of glycosylation of eukaryotic proteins and describe a selection of the glycan structures that have been determined. This review will highlight in particular the novel type of glycosylation that has been identified on human ribonuclease (RNase) 2, namely *C*-mannosylation.

C-glycosylation

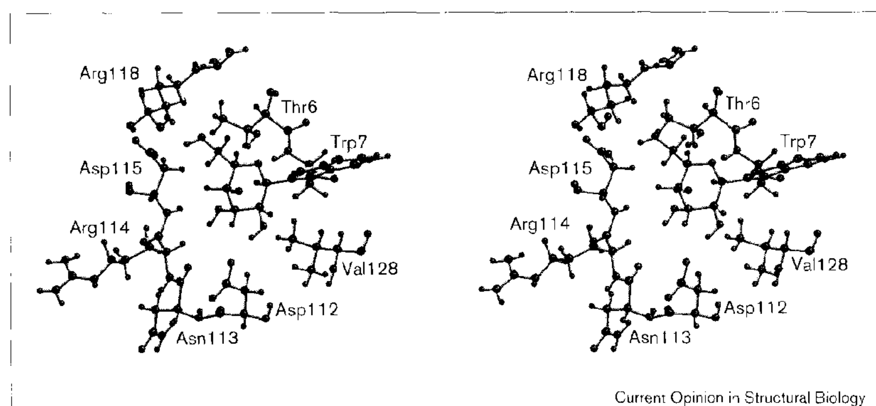
Recently, a new type of glycosylation, namely *C*-mannosylation, has been observed in human RNase 2 (formerly RNase Us) [1,2]. The anomeric carbon atom of an α-D-mannopyranose residue is directly linked to the C2 atom of Trp7 (Figure 1). Nonsecretory RNase 2, found in human urine, is an enzyme involved in the digestion of RNA. ^{1}H-NMR measurements on RNase 2 have shown that the mannose residue in the native protein adopts different orientations around its *C*-linkage compared to the denatured protein [1,2]. The three-dimensional structure of native RNase 2 seems to induce a specific orientation of the mannose residue. Currently, a study is being carried out using NMR spectroscopy, in combination with molecular modeling, in order to both identify the contacts between the *C*-linked mannose residue and the amino acids of the native protein and determine the influence of the mannose on the protein structure. Eosinophil-derived neurotoxin (EDN) is a member of the pyrimidine-specific RNase superfamily in vertebrates. It has the same amino acid sequence as RNase 2 and is also *C*-mannosylated [2]. The X-ray structure of recombinant EDN [3] expressed in *Escherichia coli* was used to model the *C*-linkage between mannose and Trp7 (Figure 2). The short distances between the amino acid protons and those of the mannose residue, as observed by molecular modeling, were compared to the NMR data on native RNase 2. It was concluded that the mannose residue interacts with loop residues 115–123, the end of β strand Met105–Arg114 and the beginning of β strand Pro124–Ile134. This interaction stabilizes Trp7 into a specific orientation. The interactions between H2 of mannose and the methyl group of Val128, and H3 of mannose and Hβ of Asp115 have been confirmed by NMR spectroscopy (F Casset, BR Leeflang, J Hofsteenge, JFG Vliegenthart, unpublished data). The main structural roles of the mannose residue seem to be

Figure 1



The structure of C2-α-D-mannopyranosyl–tryptophan. Carbon atoms, green; oxygen atoms, red; nitrogen atoms, blue; hydrogen atoms, gray.

Figure 2



Stereo representation of the C-linked α-D-mannopyranosyl unit with its surrounding amino acids modeled as in EDN.

to stabilize the N-terminal loop of the protein and to keep Trp7 in a specific orientation, compared to the non-*C*-glycosylated form.

In contrast to the processes of *N*-glycosylation and *O*-glycosylation, which are widely distributed and well established, very little is known about the biosynthetic aspects of *C*-mannosylation. It has been shown, however, that a microsomal transferase catalyzes the *C*-mannosylation of Trp7 [4•]. A minimal biosynthetic pathway could be defined as Man→GDP–Man→dolicholP–Man →(C2Man)–Trp. *C*-mannosylation occurs intracellularly, before the secretion of the protein, and can be carried out by a variety of mammalian cell cultures [5•]. Interestingly, pig kidney cells are capable of *C*-mannosylating Trp7 of human RNase 2, although the homologous RNase from pig kidney is not *C*-glycosylated, due to the absence of tryptophan at position 7. The recombinant RNase 2 preparations isolated from insect cells, plant protoplasts and *E. coli* were not *C*-mannosylated [5•]. Site-directed mutagenesis has revealed that the sequence Trp-x-x-Trp is required for the *C*-mannosylation of the first tryptophan residue [6••]. This amino acid motif is found in many mammalian proteins. The abundance of this motif in proteins suggests that *C*-glycosides could be part of the structures of more proteins [6••].

O-linked fucose

In recent years, a number of proteins have been shown to be modified with the monosaccharide L-fucose. *O*-linked fucose is an unusual form of glycosylation of the hydroxyl group of serine or threonine residues at consensus sites within epidermal growth factor-like domains of a number of serum proteins. A fucosyltransferase, which catalyzes the reaction that attaches fucose to the protein through an *O*-glycosidic linkage, has been identified in CHO cells and rat liver [7]. An assay for GDP–L-fucose:polypeptide fucosyltransferase has been established and the results suggest that the enzyme is membrane bound [7]. The pathway of this specific glycosylation reaction has been studied [8•]. It could be shown that CHO cells not only modify several endogenous proteins with *O*-linked fucose, but also that *O*-linked fucose becomes elongated for a subset of these proteins. Some proteins are modified with the monosaccharide only, whereas others are modified with either a monosaccharide or disaccharide, or a monosaccharide and an oligosaccharide. The major form of elongation is the disaccharide Glc(β1-3)Fuc. The occurrence of elongated forms of *O*-linked fucose suggests the presence of a novel glycosylation pathway in mammalian cells, with several potential end points all containing *O*-linked fucose as the core sugar [8•].

O-glycosylation

The post-translational modification of serine and threonine hydroxyl groups by glycosylation has experienced increasing interest, not only because the *O*-glycans are involved in many specific cell adhesion and recognition processes but also because they are involved in protein folding. The essentials of the function of *O*-glycans are not well elucidated and the determination of their structures remains an important first step. The O-GLYCBASE, which is a database of glycoproteins and their *O*-linked glycosylation sites, has been updated [9•]. It now contains 158 glycoprotein entries, with 903 experimentally determined *O*-glycosylation sites.

A mini-review has been published on the structure and function of GalNAc(α1-O)Ser/Thr protein glycosylation, highlighting the blood group antigens and related antigens of *O*-linked glycans and the core regions of serum, cell membrane and mucin glycoproteins [10]. The majority of *O*-linked chains found on serum and membrane glycoproteins consist of the sialylated trisaccharide and tetrasaccharide type, with core type 1 (Figure 3a). A novel glycosylation site has been identified on Ser248 of human plasminogen 2 [11]. The carbohydrate chain that is attached to Ser248 has the structure Neu5Ac(α2-3)Gal(β1-3)GalNAc, which is identical to that of the known glycan on Thr345 of the protein [11]. The structure of the *O*-linked

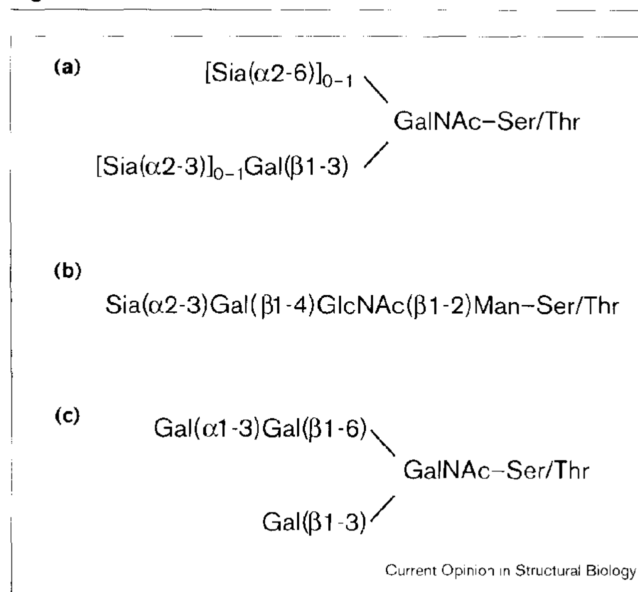
oligosaccharide on the 75 kDa neurotrophin receptor has also been reported [12]. This the glycoprotein modulates the affinity and activity of tyrosine kinases that promote neuronal survival. The glycan synthesized by cultured cells had a Gal(β1-3)GalNAc core structure, with $(Neu5Ac)_{1-2}$ at its nonreducing end.

The structure of a novel sialylated *O*-mannosyl-type oligosaccharide (Figure 3b) has been identified on bovine peripheral nerve α-dystroglycan, a heavily glycosylated protein. This oligosaccharide constitutes at least 66% of sialylated *O*-linked carbohydrate chains [13].

The *O*-glycosylation of high molecular mass precursors of insulin-like growth factor II, isolated from human plasma, has been characterized by matrix-assisted laser desorption/ionization (MALDI) mass spectrometry [14]. The *O*-linked carbohydrates were found to be associated with the C-terminal extension of the protein and comprised various sialylated forms of one and two HexNAc–Hex groups [14].

The structure of the *O*-linked glycans from a major calf thyroid cell-surface glycoprotein has also been determined [15]. In addition to known structures, a novel tetrasaccharide was identified (Figure 3c).

Figure 3



Structures showing **(a)** the majority of the O-linked chains found on serum and membrane glycoproteins, **(b)** an O-linked chain found on α-dystroglycan and **(c)** an O-linked chain found on calf thyroid cell-surface glycoproteins.

N-glycosylation

The structures of many *N*-linked oligosaccharides have been determined over the past two years. Thirty five kinds of complex oligosaccharide structures of integrin $\alpha5\beta1$ have been reported, with the help of a new, sensitive analytical method using different properties of HPLC [16]. The common oligosaccharide core Gal(β1-4)GlcNAc(β1-2)Man(α1-6)[Gal(β1-4)GlcNAc(β1-2)Man(α1-3)]Man(β1-4)GlcNAc(β1-4)[Fuc(α1-6)]GlcNAc was present, with different substitutions. More than 80% of the sialic acids present were (α2-3) linked to nonreducing terminal galactose residues [16].

The same type of core is also found in *N*-linked tetra-antennary oligosaccharides of the human Tamm–Horsfall glycoprotein, with two additional substitutions on the branched mannose. The nonreducing end shows additional *N*-acetyl-lactosamine units, sialylated with (α2-3) linkages. This is a donor-specific feature [17].

The polysialylglycans found in neural cell adhesion molecules (N-CAMs) from embryonic chicken brains showed two distinct types of multiantennary structures, triantennary and tetra-antennary [18]. The presence of GlcNAc(β1-6), linked on the Man(α1-6) arm, is required for the polysialylation of the core glycan. Gal(β1-3)GlcNAc and Gal(β1-4)GlcNAc sequences are both present in the peripheral part of the glycan. Sulfate is present, probably within the Gal(β1-4)$[SO_3$ -3]GlcNAc(β1-3)Gal(β1-4)GlcNAc structure. Interestingly, at least one terminal residue of the antennae was found to be not sialylated, indicating that polysialylation occurs asymmetrically on the antennae. The presence of *O*-acetyl groups on the N-CAM polysialic acid chain has been reported for the first time [18].

Ascorbate oxidase from *Acremonium* sp. HI-25 exclusively contains *N*-linked glycans. In addition to regular oligomannose-type glycans, a series of novel D-galactofuranose-containing oligomannose-type carbohydrate chains were identified [19].

In contrast to previous studies, the structural analysis of glycans from bovine pituitary membrane glycoproteins showed the presence of unsubstituted GalNAc(β1-4)GlcNAc and Gal(β1-4)GlcNAcβ structures at the nonreducing end of the *N*-linked glycans [20]. These results indicate that β-*N*-acetylgalactosaminylation is not unique to bovine pituitary glycoprotein hormones but occurs in most bovine pituitary glycoproteins.

For hemocyanin, the high molecular mass copper-containing oxygen transporting protein that is freely dissolved in the hemolymph of several arthropod and mollusc species, the structures of 21 novel monoantennary and diantennary *N*-linked carbohydrate chains from the α_D-hemocyanin of *Helix pomatia* have been determined. Four novel types of antennae were identified, the most complex representative being 3MeGal(β1-6)3MeGal(β1-6)3MeGal(β1-3)[3MeGal(β1-6)]GalNAc(β1-4)GlcNAc(β1-, which is attached to O2 of α mannose residues of the trimannosyl-*N*,*N'*-diacetylchitobiose core element (see Figure 4). The core structures are generally β1,2-xylosylated and α1,6-fucosylated [21].

A glycoprotein carrying polylactosaminoglycans has been identified in Zajdela hepatoma cells and the structures of its *N*-glycans have been established. The carbohydrate chain is a tetra-antennary lactosaminoglycan of 6.6 kDa, containing galactose, GlcNAc, mannose and Neu5Ac in a 16:14:3:4 ratio, with an average of three repeating *N*-acetyllactosamine units per branch [22].

The *N*-glycosylation of proteins is a highly conserved process in eukaryotic evolution. The oligosaccharides $Glc_xMan_9GlcNAc_2$ (x = 1–3) are involved in a number of important steps during the biosynthesis and folding of glycoproteins. The conformation of the oligosaccharide Glc(α1-2)Glc(α1-3)Glc(α1-3)$Man_9GlcNAc_2$ has been studied by NMR spectroscopy [23]. The glucosyl cap has a single well-defined conformation, independent of the rest of the saccharide. The conformation of the mannose residues in $Man_9GlcNAc_2$, however, is largely unaffected by the presence of the glucosyl cap in comparison to the free oligosaccharide.

It is important to underline the fact that the regulation of *N*-linked core glycosylation using rabies virus glycoproteins has been described as a model system. It was demonstrated that amino acid X of the Asn-X-Ser sequon is an important determinant of the efficiency of *N*-glycosylation. The presence of proline at the X position completely blocked core glycosylation, whereas tryptophan, aspartate, glutamate and leucine were associated with inefficient *N*-glycosylation [24].

N-glycosylation and *O*-glycosylation

Transferrins are proteins involved in iron transport in body fluids and it is found in serum (serotransferrin) and in milk (lactotransferrin). The glycosylation sites of various transferrins have been characterized and it was shown that alterations of the structure of the glycans on human transferrin occur during pregnancy. Transferrin from the amniotic fluid of a pregnant woman suffering from hydramnion has been isolated and the structures of 14 *N*-linked and two *O*-linked carbohydrate chains have been determined [25••]. The *N*-glycans found were monosialylated, disialylated or trisialylated structures, including three carbohydrates containing sialyl Le^x {Neu5Ac(α2-3)Gal(β1-4)[Fuc(α1-3)]GlcNAc(β1-}. In comparison to human serum transferrin, a higher degree of (α1-6) fucosylation has been observed and there is also an increase in branching from diantennary to triantennary compounds. Furthermore, the presence of the *O*-glycans Gal(β1-3)GalNAc and Neu5Ac(α2-3)Gal(β1–3)GalNAc has been demonstrated for the first time in a transferrin [25••].

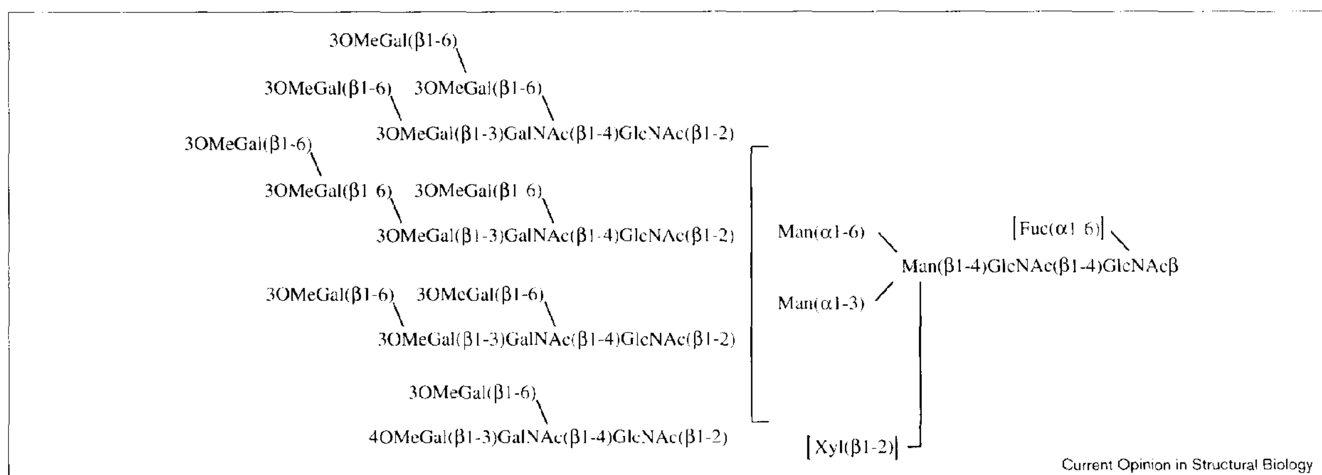
Sulfated glycans

The HNK-1 (human natural killer 1) cell's carbohydrate epitope [SO_3 -3GlcA(β1–3)Gal(β1-4)GlcNAc] is expressed by several neural recognition molecules. It is involved in the cellular interactions that control cell type-specific neurite outgrowth and regeneration. In the bovine peripheral myelin glycoprotein PO, the epitope was found for the first time on an asparagine-linked carbohydrate. The HNK-1 epitope is present in one of the major glycans of bovine PO (Figure 5a) and is attached to the (α1-6) arm of a diantennary core with a bisecting GlcNAc-residue [26].

The primary structures of 32 sulfated diantennary, triantennary and tetra-antennary *N*-glycans of the human Tamm–Horsfall glycoprotein have been determined (Figure 5b, c). The glycans range from monosulfated to trisulfated *N*-glycans, the sulfate being attached to either position 3 of the terminal galactose or position 4 of a terminal GlcNAc [27].

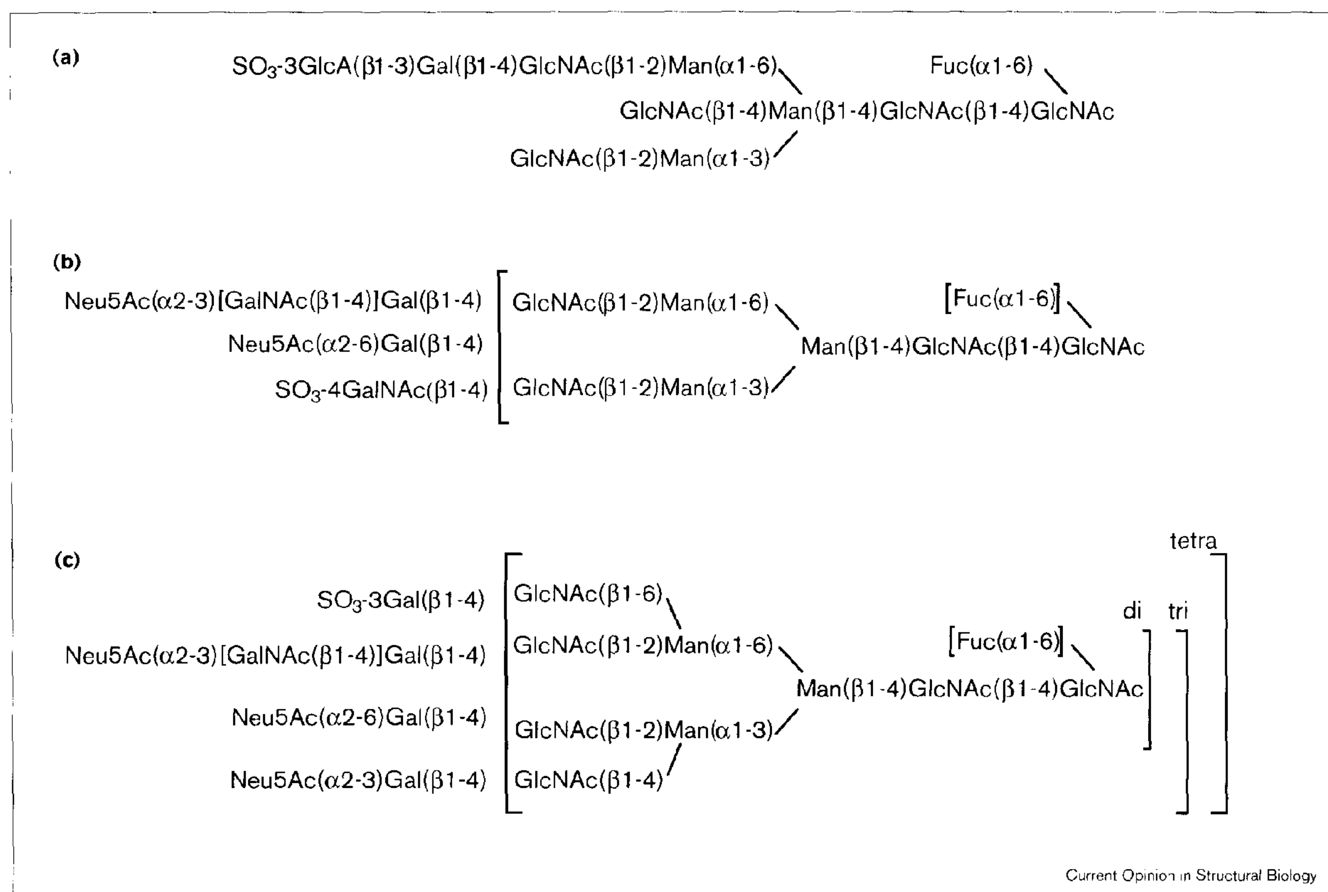
For the first time, a detailed investigation into the nature of highly sulfated (keratan sulfate-like), complex-type, asparagine-linked glycans with tetra-antennary core

Figure 4



Novel types of antennae in *N*-linked chains found on *Helix pomatia* hemocyanin.

Figure 5



Structures showing **(a)** the HNK-1 epitope in the *N*-linked glycan of bovine peripheral myelin glycoprotein PO and **(b)** and **(c)** novel sulfated *N*-linked chains in the human Tamm–Horsfall glycoprotein.

structures has been reported [28]. The sulfated multiantennary *N*-linked glycan chains were derived from a fertilization-associated, carbohydrate-rich glycoprotein in unfertilized eggs of *Tribolodon hakonensis* [28]. A novel, repeating carbohydrate sequence Gal(β1-4)Gal(β1-4)β1[-4(SO_3 -6) GlcNAc(β1-3)(Gal(β1-4)Galβ1]$_n$ was observed. The GlcNAc linked to the mannose core structure was sulfated at position 6, in contrast to N-CAM, which was substituted at position 3 [18].

Sulfated oligosialic acid units have been described in *O*-linked glycans of the sea urchin egg receptor for sperm [29]. Sulfated oligosaccharide chains with the novel structure (SO_3 -9)NeuAc5Gcα2(-5O$_{glycolyl}$Neu5Gcα2-)$_n$ were identified [29].

New protein glycosylation sites

The positions of *N*-glycosylation sites have been identified on various proteins. For example, on human thyroglobulin, 16 out of the 20 putative sites for *N*-glycosylation have been confirmed as being carbohydrate-bearing sites [30]. It has also been shown that murine SR-BI, a high-density lipoprotein receptor that mediates selective lipid uptake, is highly *N*-glycosylated with multiple oligomannose chains [31].

In the human Tamm–Horsfall glycoprotein, isolated from the urine of a healthy male donor, it was established that seven out of the eight putative glycosylation sites were occupied. The oligomannose type of chain occurs exclusively at Asn251 in a donor-specific way. The (SO_3 -4)GalNAc(β1-4)GlcNAc determinant is present on the glycans only at Asn489, preferentially in diantennary structures. Asn14 is not occupied [32].

Novel *O*-linked glycosylation has been described, for example, on basic human parotid proteins [33] and on the Tau protein. The latter protein is important in modulating microtubule stability in neurons. It was found that normal bovine Tau protein is multiply modified by GlcNAc *O*-linked to serine or threonine residues [34]. This feature may play a role in the formation of paired helical filaments.

For serum immunoglobulins, the glycans of IgG are well known, whereas those from IgA_1 were not yet characterized.

Recently, however, the glycosylation of the IgA_1 Fab and Fc was unraveled. Over 90% of the *N*-glycans of IgA_1 are sialylated, as opposed to only 10% of the glycans of IgG. In contrast to IgG, which has only *N*-glycans, *N*-linked and *O*-linked oligosaccharides were observed on the Fab of IgA_1 [35].

The glycosylation of the complement regulatory protein human erythrocyte CD59 has been analyzed [36]. This cell-surface glycoprotein contains *N*-glycans, *O*-glycans and a glycosylphosphatidylinositol anchor. This study provides the most complete view of any cell-surface glycoprotein studied so far.

Conclusions

The past two years have shown that the identification of the variety of carbohydrate structures occurring on glycoproteins is still growing, thanks to the progress in developing analytical methodologies. SUGABASE [37], a carbohydrate NMR database that combines CarbBank Complex Carbohydrate Structure Data (CCSD) with proton and carbon chemical shift values, now includes information on 579 *N*-linked and 340 *O*-linked structures, derived from glycoproteins. The elucidation of primary glycan structures paves the way for studies on the integral structures of intact glycoproteins. This should ultimately lead to a molecular level understanding of the mode of action of these compounds in their natural environment. Here, we did not consider the function of glycosylation, although exciting experiments have been carried out in this area. In the context of structure/function relationships, the prion molecule, which is a sialoglycoprotein containing two *N*-linked glycans, should be mentioned. In recent studies, evidence has been provided that oligosaccharide chains may modulate the efficiency of the conversion process from the normal prion form into a pathogenic form [38]. These findings are an example of the many interesting results that can be expected from structural glycobiology.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. De Beer T, Vliegenthart JFG, Löffler A, Hofsteenge J: **The hexopyranosyl residue that is C-glycosidically linked to the side chain of tryptophan-7 in human RNase U_s is α-mannopyranose.** *Biochemistry* 1995, **34**:11785-11789.

2. Löffler A, Doucey M-A, Jansson AM, Müller DR, de Beer T, Hess D, Meldal M, Richter WJ, Vliegenthart JFG, Hofsteenge J: **Spectroscopic and protein chemical analyses demonstrate the presence of C-mannosylated tryptophan in intact human RNase 2 and its isoforms.** *Biochemistry* 1996, **35**:12005-12014.

3. Mosimann SC, Newton DL, Youle RJ, James MNG: **X-ray crystallographic structure of recombinant eosinophil-derived neurotoxin at 1.83 Å resolution.** *J Mol Biol* 1996, **260**:540-552.

4. Doucey M-A, Hess D, Cacan R, Hofsteenge J: **Protein**
• **C-mannosylation is enzyme-catalysed and uses dolichyl-phosphate-mannose as a precursor.** *Mol Biol Cell* 1998, **9**:291-300.
This paper defines the biosynthetic pathway of C-mannosylation, showing the presence of a C-mannosyltransferase.

5. Krieg J, Gläsner W, Vicentini A, Doucey M-A, Löffler A, Hess D,
• Hofsteenge J: **C-mannosylation of human RNase 2 is an intracellular process performed by a variety of cultured cells.** *J Biol Chem* 1997, **272**:26687-26692.
This article describes the fact that the C-mannosylation of Trp7 occurs intracellularly in human HL-60 cells. This C-mannosylation occurs in different cell types in man, green monkey, pig, mouse and hamster but the recombinant RNase isolated from insect cells, plant protoplasts and *E. coli* was not C-mannosylated.

6. Krieg J, Hartmann S, Vicentini A, Gläsner W, Hess D, Hofsteenge J:
•• **Recognition signal for C-mannosylation of Trp-7 in RNase 2 consists of sequence Trp-x-x-Trp.** *Mol Biol Cell* 1998, **9**:301-309.
This is one of the most recent articles about C-mannosylation. The authors used site-directed mutagenesis to identify the sequence Trp-x-x-Trp as the specific determinant for C-mannosylation, whereby the first tryptophan becomes mannosylated. It has been shown that this sequence motif occurs in 336 mammalian proteins present in the Brookhaven Protein Data Bank.

7. Wang Y, Lee GF, Kelley RF, Spellman MW: **Identification of GDP-L-fucose:polypeptide fucosyltransferase and enzymatic addition of O-linked fucose to EGF domains.** *Glycobiology* 1996, **6**:837-842.

8. Moloney DJ, Lin AI, Haltiwanger RS: **The O-linked fucose**
• **glycosylation pathway.** *J Biol Chem* 1997, **272**:19046-19050.
This article revealed that some proteins containing O-linked fucose are modified with either a monosaccharide or disaccharide, or a monosaccharide and oligosaccharide. An O-linked glycosylation pathway has also been proposed.

9. Hansen JE, Lund O, Nilsson J, Rapacki K, Brunak S: **O-GLYCBASE**
• **Version 3.0: a revised database of O-glycosylated proteins.** *Nucleic Acids Res* 1998, **26**:387-389.
Version 3.0 of O-GLYCBASE is accessible via the internet at http://www.cbs.dtu.dk/databases/OGLYCBASE/. O-GLYCOBASE is important for tracing regularities in the amino acid sequences around O-glycosylation sites.

10. Hounsell EF, Davies MJ, Renouf DV: **O-linked protein glycosylation structure and function.** *Glycoconj J* 1996, **13**:19-26.

11. Pirie-Shepherd SR, Stevens RD, Andon NL, Enghild JJ, Pizzo SV: **Evidence for a novel O-linked sialylated trisaccharide on Ser-248 of human plasminogen 2.** *J Biol Chem* 1997, **272**:7408-7411.

12. Chapman BS, Eckart MR, Kaufman SE, Lapointe GR: **O-linked oligosaccharide on 75-kDa neurotrophin receptor.** *J Neurochem* 1996, **66**:1707-1716.

13. Chiba A, Matsumura K, Yamada H, Inazu T, Shimizu T, Kusunoki S, Kanazawa A, Kobata A, Endo T: **Structures of sialylated O-linked oligosaccharide of bovine peripheral nerve α-dystroglycan.** *J Biol Chem* 1997, **272**:2156-2162.

14. Jespersen S, Koedam JA, Hoogerbrugge CM, Tjaden UR, Van der Greef J, Van den Brande JL: **Characterization of O-glycosylated precursors of insulin-like growth factor II by matrix-assisted laser desorption/ionization mass spectrometry.** *J Mass Spect* 1996, **31**:893-900.

15. Edge ASB, Spiro RG: **Structure of the O-linked oligosaccharides from a major thyroid cell surface glycoprotein.** *Arch Biochem Biophys* 1997, **343**:73-80.

16. Nakagawa H, Zheng M, Hakomori S-I, Tsukamoto Y, Kawamura Y, Takahashi N: **Detailed oligosaccharide structures of human integrin α5β1 analyzed by a three-dimensional mapping technique.** *Eur J Biochem* 1996, **237**:76-85.

17. van Rooijen JJM, Jeschke U, Kamerling JP, Vliegenthart JFG: **The abundance of additional *N*-acetyllactosamine units in N-linked tetraantennary oligosaccharides of human Tamm-Horsfall glycoprotein is a donor specific feature.** *Glycobiology* 1998, in press.

18. Kudo M, Kitajima K, Inoue S, Shiokawa K, Morris HR, Dell A, Inoue Y: **Characterization of the major core structures of the α2→8-linked polysialic acid-containing glycan chains present in neural cell adhesion molecule in embryonic chick brains.** *J Biol Chem* 1996, **271**:32667-32677.

19. Ohta M, Emi S, Iwamoto H, Hirose J, Hiromi K, Itoh H, Shin T, Murao S, Matsuura F: **Novel β-D-galactofuranose-containing high-mannose type oligosaccharides in ascorbate oxidase from *Acremonium* sp. HI-25.** *Biosci Biotechnol Biochem* 1996, **60**:1123-1130.

20. Taka J, Sato T, Sakiyama T, Fujisawa H, Furukawa K: **Bovine pituitary membrane glycoproteins contain β-*N*-acetylgalactosaminylated N-linked sugar chains.** *J Neurochem* 1996, **66**:852-859.

21. Lommerse JPM, Thomas-Oates JE, Gielens C, Préaux G, Kamerling JP, Vliegenthart JFG: **Primary structure of 21 novel monoantennary and diantennary N-linked carbohydrate chains from α_D-hemocyanin of *Helix pomatia*.** *Eur J Biochem* 1997, **249**:195-222.

22. Goulut-Chassaing C, Bourrillon R: **Expression and characterization of a lactosaminoglycan-carrying glycoprotein of Zajdela hepatoma cell surface.** *Eur J Biochem* 1997, **247**:1091-1101.

23. Petrescu AJ, Butters TD, Reinkensmeier G, Petrescu S, Platt FM, Dwek RA, Wormald MR: **The solution NMR structure of glucosylated N-glycans involved in the early stages of glycoprotein biosynthesis and folding.** *EMBO J* 1997, **16**:4302-4310.

24. Shakin-Eshleman SH, Spitalnik SL, Kasturi L: **The amino acid at the *X*- position of an Asn-*X*-Ser sequon is an important determinant of N-linked core-glycosylation efficiency.** *J Biol Chem* 1996, **271**:6363-6366.

25. van Rooijen JJM, Jeschke U, Kamerling JP, Vliegenthart JFG:
•• **Expression of N-linked sialyl Le^x determinants and O-glycans in the carbohydrate moiety of human amniotic fluid transferrin during pregnancy.** *Glycobiology* 1998, in press.

This paper reports the isolation of transferrin from the amniotic fluid of a pregnant woman with hydramnion. The structures of 14 *N*-glycan and two *O*-glycan chains have been elucidated and compared to the structure of human serum transferrin.

26. Voshol H, Van Zuylen CWEM, Orberger G, Vliegenthart JFG, Schachner M: **Structure of the HNK-1 carbohydrate epitope on bovine peripheral myelin glycoprotein PO.** *J Biol Chem* 1996, **271**:22957-22960.

27. Van Rooijen JJM, Kamerling JP, Vliegenthart JFG: **Sulfated di-, tri- and tetraantennary N-glycans in human Tamm-Horsfall glycoprotein.** *Eur J Biochem* 1998, **256**:471-487.

28. Taguchi T, Iwasaki M, Muto Y, Kitajima K, Inoue S, Khoo K-H, Morris HR, Dell A, Inoue Y: **Occurrence and structural analysis of highly sulfated multiantennary N-linked glycan chains derived from a fertilization-associated carbohydrate-rich glycoprotein in unfertilized eggs of *Tribolodon hakonensis*.** *Eur J Biochem* 1996, **238**:357-367.

29. Kitazume-Kawaguchi S, Inoue S, Inoue Y, Lennarz WJ: **Identification of sulfated oligosialic acid units in the O-linked glycan of the sea urchin egg receptor for sperm.** *Proc Natl Acad Sci USA* 1997, **94**:3650-3655.

30. Yang S-X, Pollock HG, Rawitch AB: **Glycosylation in human thyroglobulin: location of the N-linked oligosaccharide units and comparison with bovine thyroglobulin.** *Arch Biochem Biophys* 1996, **327**:61-70.

31. Babitt J, Trigatti B, Rigotti A, Smart EJ, Anderson RGW, Xu S, Krieger M: **Murine SR-BI, a high density lipoprotein receptor that mediates selective lipid uptake, is N-glycosylated and fatty acylated and colocalizes with plasma membrane caveolae.** *J Biol Chem* 1997, **272**:13242-13249.

32. van Rooijen JJM, Voskamp AF, Kamerling JP, Vliegenthart JFG: **Glycosylation sites and site-specific glycosylation in human Tamm-Horsfall glycoprotein.** *Glycobiology* 1998, in press.

33. Proctor GB, Carpentier GH, Pankhurst CL, Shori DK: **Novel O-linked glycosylation on basic human parotid proteins.** *Biochem Soc Trans* 1997, **25**:11S.

34. Shane Arnold C, Johnson GVW, Cole RN, Dong DL-Y, Lee M, Hart GW: **The microtubule-associated protein Tau is extensively modified with O-linked *N*-acetylglucosamine.** *J Biol Chem* 1996, **271**:28741-28744.

35. Mattu TS, Pleass RJ, Willis AC, Kilian M, Wormald MR, Lellouch AC, Rudd PM, Woof JM, Dwek RA: **The glycosylation and structure of human serum IgA1, Fab and Fc regions and the role of N-glycosylation on Fcα receptor interactions.** *J Biol Chem* 1998, **273**:2260-2272.

36. Rudd PM, Morgan BP, Wormald MR, Harvey DJ, van den Berg CW, Davis SJ, Ferguson MAJ, Dwek RA: **The glycosylation of the complement regulatory protein, human erythrocyte CD59.** *J Biol Chem* 1997, **272**:7229-7244.

37. Sugabase on World Wide Web URL: http://www.boc.uu.nl/sugabase/sugabase.htm

38. Lehmann S, Harris DA: **Blockade of glycosylation promotes acquistion of scrapie-like properties by prion protein in cultured cells.** *J Biol Chem* 1997, **272**:21479-21487.