

## MINI REVIEW

**“Unusual” modifications and variations of vertebrate oligosaccharides: are we missing the flowers for the trees?****Ajit Varki**

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The remarkable complexity of oligosaccharide structures found on vertebrate cells results from the concerted action of glycosyltransferase enzymes (Joziase, 1992; Stanley and Ioffe, 1995; Varki and Marth, 1995; Whitfield and Douglas, 1996), several of which were first identified and characterized by Robert Hill (Paulson *et al.*, 1977; Paulson *et al.*, 1978; Beyer *et al.*, 1979; Rearick *et al.*, 1979; Sadler *et al.*, 1979, 1982). Together with the structural characterization of naturally occurring oligosaccharides, the study of these enzymes has led to the definition of the major structural motifs of vertebrate sugar chains (Cummings, 1992; Kobata and Takasaki, 1992; Varki and Freeze, 1994; Varki and Marth, 1995). These motifs can be divided into (1) the core regions unique for each class of glycoconjugate (e.g., the Chitobiosyl-N-Asn-linkage region); and (2) the outer chains (e.g., type 1 and 2 lactosamine units with sialylation and/or fucosylation) that can be shared to varying extents by the different core classes (Cummings, 1992; Kobata and Takasaki, 1992; Varki and Freeze, 1994; Varki and Marth, 1995). Vertebrate cells utilize a relatively limited subset of the monosaccharides known to exist in nature, and in only some of the many possible combinations. However, several unusual variations of the typical structures exist, as well as a variety of specific modifications of the individual monosaccharide units. Hypothesizing that such subtle variations and modifications are more likely to mediate specific biological functions (Varki, 1993), we have paid special attention to their existence, structure and biosynthesis. Here I briefly describe some examples that my group has studied over the past decade.

**9(7)-O-Acetylation of sialic acids**

This was once considered an uncommon modification of sialic acids, only present on salivary mucins and neural gangliosides (Schauer, 1991; Varki, 1992). However, newly developed methods for the release, purification, and analysis of sialic acids from biological sources indicate that this is not the case (Manzi *et al.*, 1990; Klein *et al.*, 1994; Reuter and Schauer, 1994). For example, we found that the membrane glycoproteins of rat liver (one of the most commonly used model systems for the study of oligosaccharide biosynthesis) have ~20–30% of O-acetylation at the 7- or 9-positions of sialic acids

(Butor *et al.*, 1993). Moreover, the subcellular distribution of these modifications fits predictions derived from prior information on their enzymology and chemistry (7-O-acetyl groups are enriched in acidic internal compartments, while 9-O-acetyl group predominate on the cell surface).

Other studies using monoclonal antibodies have indicated that (7)9-O-acetylated sialic acids are selectively expressed on certain gangliosides in neural tissues and on some types of leukocytes (Schauer, 1991; Varki, 1992). Of recent interest is the identification that the CD60 antigens of human blood leukocytes are (7)9-O-acetylated forms of the ganglioside GD3 (Fox *et al.*, 1990b; Kniep *et al.*, 1993; Rieber and Rank, 1994; Zimmer *et al.*, 1994). It has been shown by others that ligation of this glycosphingolipid by specific monoclonal antibodies results in facilitation of T-cell activation (Fox *et al.*, 1990a). Interestingly, an antibody that preferentially bound the 7-O-acetyl form was the most potent at inducing this activation (Kniep *et al.*, 1995).

With the exception of these ganglioside antigens, it was previously not possible to directly detect 9-O-acetyl sialic acids on cell surfaces and tissue sections. We and others (Muchmore and Varki, 1987; Zimmer *et al.*, 1992, 1994) had suggested the use of the whole influenza C virion as a probe for this purpose. This virus carries a membrane-bound hemagglutinin-esterase (CHE), which can both detect and cleave 9-O-acetyl-residues on sialic acids, regardless of the underlying oligosaccharide structure. However, the whole virions are unstable, are subject to steric hindrance in binding, and are impractical for many applications. To address this deficiency, we prepared a recombinant soluble form of the CHE wherein the C-terminal transmembrane and cytoplasmic domains are replaced by the Fc portion of human IgG, and the potential fusion function of the protein is eliminated by a point mutation (Klein *et al.*, 1994). Irreversible inactivation of the esterase active site unmasks stable recognition activity, giving a molecule that binds specifically to 9-O-acetylated sialic acids. This probe demonstrates widespread but selective expression of 9-O-acetylated sialic acids in certain cell types, with patterns of polarized or gradient expression (Klein *et al.*, 1994). Using this probe, we also found that natural ligands of the B cell adhesion molecule CD22 can be masked by 9-O-acetylation of sialic acids (Sjoberg *et al.*, 1994), and that Chinese hamster ovary (CHO) cells have an endogenous O-acetyltransferase that becomes functionally activated upon transfection with a cDNA encoding the  $\alpha$ 2-6 sialyltransferase specific for type 2 lactosamines (Shi *et al.*, 1996). The latter work confirms prior predictions that the 9(7)-O-acetyltransferases of vertebrate cells are sialic acid linkage-specific in their action, and raises the possibility that there is a family of such enzymes yet to be isolated and cloned.

### De-N-acetyl sialic acids

In natural systems, the amino group at the 5-position of neuraminic acid residues is usually assumed to be either acylated (N-acetyl or N-glycolyl) or replaced by a hydroxyl group (as in keto-deoxy-nonulosonic acid, KDN) (Schauer, 1991; Troy, 1992; Varki, 1992; Sato *et al.*, 1993). However, earlier studies had suggested the possibility that a free amino group might be present on the sialic acids of some gangliosides (Hanai *et al.*, 1988; Nores *et al.*, 1989). To pursue this possibility, we collaborated with Tai and colleagues to raise specific monoclonal antibodies against semisynthetically prepared de-N-acetyl-GM3 and de-N-acetyl-GD3. These antibodies gave low and variably detectable levels in cultured human melanoma cells (Sjoberg *et al.*, 1995). Further studies showed that the tyrosine kinase inhibitor genistein and the microtubule inhibitor nocodazole caused an accumulation of these molecules on the cell surface, making them more easily detectable (Sjoberg *et al.*, 1995). This effect appears to be related to the cell shape change that occurs when these agents block cells in the G2M phase of the cell cycle. Studies are currently underway to conclusively demonstrate the structures of these gangliosides, and to elucidate their subcellular localization, biosynthesis and functions.

### Other novel sialic acids

Of all the monosaccharides in vertebrate oligosaccharides, the sialic acids appear most prone to substitution (Schauer, 1991; Troy, 1992; Varki, 1992; Sato *et al.*, 1993). The list of novel modifications of sialic acids continues to grow. An example is the recent demonstration by others of 9-O-sulfo-N-glycolyl-neuraminic acid in sea urchin eggs (Kitazume *et al.*, 1996). While exploring the biosynthetic function of the rat liver Golgi apparatus, we recently found evidence for even more diversity. When rat liver subcellular preparations enriched in Golgi compartments are incubated with CMP-[9-<sup>3</sup>H]N-acetyl-neuraminic acid, the label is efficiently transferred to endogenous acceptors that are only within the lumen of intact compartments, which are of correct topological orientation (Hayes and Varki, 1993b). Thus, this *in vitro* system recapitulates the *in vivo* glycosylation mechanism, but eliminates the dynamic variable of ongoing transport. Peptide:N-glycosidase F digestion released ~90% of the incorporated label, indicating that most of the sialylation had occurred on endogenous N-linked oligosaccharide acceptors. Upon HPLC analysis of sialic acids released from these oligosaccharides, two surprises were encountered (Hayes and Varki, 1993b). First, the expected level of (7)9-O-acetylation was not found. This was true even upon addition of excess of the acetyl donor acetyl-CoA, suggesting that the responsible O-acetyltransferases might be in a separate compartment. Second, ~20% of <sup>3</sup>H label released by sialidase did not elute at the position expected for [<sup>3</sup>H]N-acetyl-neuraminic acid, but was scattered through several aberrantly eluting peaks. Most peaks were unchanged by prior base treatment, indicating that they are not due to O-acetylation. Thus, during these short (10–20 min) incubations, a significant portion of the [<sup>3</sup>H]N-acetyl-neuraminic acid transferred from the sugar nucleotide donor is modified by unknown enzymes within the Golgi lumen. The nature of these novel sialic acids is under investigation.

### Mild acid labile GalNAc residues on N-linked oligosaccharides

When UDP-[6-<sup>3</sup>H]GalNAc is incubated with subcellular preparations enriched in Golgi compartments, some of the label is transferred to endogenous acceptors within the lumen of intact compartments, which are of correct topological orientation. A portion of this label can be released by PNGase F, indicating that [<sup>3</sup>H]GalNAc had been transferred to some N-linked oligosaccharides on endogenous acceptors. This is not entirely surprising, since α- and β-linked GalNAc have been previously reported on N-linked chains (Cummings, 1992; Kobata and Takasaki, 1992; Varki and Freeze, 1994; Baenziger, 1994). However, a major portion of this <sup>3</sup>H label was found to be mild acid labile, with acid release kinetics typical of a phosphodiester linkage. Direct analysis of the released label indicated that it remained in [<sup>3</sup>H]GalNAc, and thus could not be explained as previously described GlcNAc-P-Man linkages on high mannose oligosaccharides of lysosomal enzymes (Hayes and Varki, 1993a). Furthermore, unlike the case with GlcNAc-P-Man, this modification was found only on complex type chains (not binding to ConA), and some of them also carried sialic acids. The nature of these novel linkages and the proteins to which they are attached is under exploration. If this structure is confirmed, it would add to the list of previously described phosphodiester linkages in animal systems, that include GlcNAc-P-Man of lysosomal enzymes (Kornfeld and Mellman, 1989), Glc-P-Man on phosphoglucomutase (Dey *et al.*, 1994), GlcNAc-P-Ser on Dictyostelium (Freeze and Ichikawa, 1995), and Man-P-Man in yeast (Hernandez *et al.*, 1989).

### Unsubstituted glucosamine residues on endothelial heparan sulfates

Known biosynthetic pathways of heparan sulfate (HS) chains indicate that all glucosamine amino groups must be either N-acetylated or N-sulfated. This is because the de-N-acetylation and N-sulfation reactions are known to be catalyzed by the same polypeptides (Bame *et al.*, 1991, 1994; Wei *et al.*, 1993; Eriksson *et al.*, 1994; Mandon *et al.*, 1994; Orellana *et al.*, 1994). However, L-selectin binding HS chains from endothelial cells have some unsubstituted amino groups that could not be ascribed to any *in vitro* processing artifact (Norgard-Sumnicht and Varki, 1995). These free amino groups (present on about every 20–50 disaccharide units) are also found in HS chains from human umbilical vein endothelial cells. However, they are not found in HS chains from CHO cells, indicating that they are not random errors that routinely occur during the biosynthesis of HS chains. The mechanism of generation of these unusual groups, and their distribution and function remain unknown. Others have recently reported detection of the same modification using a specific monoclonal antibody (Van den Born *et al.*, 1995). Interestingly, the epitope showed highly selective distribution relative to the overall distribution of HS chains in tissues like the kidney.

### Unusual N-linked oligosaccharides from bovine lung

In earlier studies, we reported that calf pulmonary artery endothelial (CPAE) expressed a variety of sulfated N-linked sugar chains with unexpected structural features (Roux *et al.*, 1988; Sundblad *et al.*, 1988). To pursue this further, we examined total anionic N-linked oligosaccharides released by

PNGaseF from bovine lung, a rich source of endothelium (Norgard-Sumnicht *et al.*, 1995). We found that sialic acids, sulfates and phosphates account only for a minority of the anionic properties of these multi-antennary complex-type chains. The majority of the negative charge appears to be due to multiple carboxylic acid groups on as yet unidentified residues. The most highly charged N-linked oligosaccharides from the same source are similar in general structure to the corresponding ones we previously found in the cultured pulmonary artery endothelial cells (Sundblad *et al.*, 1988), carrying chondroitin sulfate, heparin/heparan sulfate, or keratan sulfate glycosaminoglycan chains. A significant proportion of these molecules seem to have more than one type of glycosaminoglycan chain associated with them, presumably on multiple branches of a single N-linked core region (Norgard-Sumnicht *et al.*, 1995). The structural elucidation of these novel chains is under way.

### Conclusions and future prospects

Modern-day scientists are often accused of not being able to ‘see the forest for the trees’, that is, we spend so much time focused on the details of our particular systems that we fail to see the bigger picture. However, I wonder if the opposite might be the case among those studying the biology of glycosylation, that is, are we missing the flowers because we are focusing mainly on the trees? The complexities of the known major pathways of vertebrate glycosylation are already so great that there are numerous structures to be studied, enzymes to be isolated, and genes to be cloned. Thus, it is tempting to ignore further minor variations and modifications that occur less frequently. Many examples presented above from our own work fall into this category and, for the most part, have not been related to any specific function. The same is true in a variety of novel oligosaccharide modifications that have not been dealt with in this brief overview. However, scientists who study plant or bacterial polysaccharides are quite used to dealing with a much larger variety of oligosaccharide structures and modifications. Moreover, the history of discovery in glycobiology indicates that such unusual features can serve to generate the specificity of carbohydrate protein interactions that mediate specific biological functions. Examples include the addition of phosphate esters to mannose residues (critical for targeting lysosomal enzymes to lysosomes) (Kornfeld and Mellman, 1989), the addition of 3-O-sulfate esters to heparin (vital for high affinity binding to antithrombin III) (Lindahl *et al.*, 1979, 1980; Bourin and Lindahl, 1993; Razi and Lindahl, 1995), and the addition of sulfate esters to GalNAc residues on pituitary glycoprotein hormones (required for recognition by a specific liver receptor that controls plasma levels) (Green *et al.*, 1985; Baenziger, 1994, 1996). Of course, it does not follow that every unusual variation or modification will mediate a very specific biological function. For example, a particular unusual modification may well be an evolutionary relic left behind from a past time when the vertebrate organism in question evaded recognition by a now-extinct parasite. However, faced with the daunting task of exploring the complex world of vertebrate glycosylation, it might be worthwhile to focus some time and effort on such ‘flowers on the trees’.

### References

Baenziger, J.U. (1994) Protein-specific glycosyltransferases: how and why they do it. *FASEB J.*, **8**, 1019–1025.

Baenziger, J.U. (1996) Glycosylation: To what end for the glycoprotein hormones? *Endocrinology*, **137**, 1520–1522.

Bame, K.J., Reddy, R.V. and Esko, J.D. (1991) Coupling of N-deacetylation and N-sulfation in a Chinese hamster ovary cell mutant defective in heparan sulfate N-sulfotransferase. *J. Biol. Chem.*, **266**, 12461–12468.

Bame, K. J., Zhang, L., David, G. and Esko, J.D. (1994) Sulphated and under-sulphated heparan sulphate proteoglycans in a Chinese hamster ovary cell mutant defective in N-sulphotransferase. *Biochem. J.*, **303**, 81–87.

Beyer, T.A., Rearick, J.I., Paulson, J.C., Prieels, J.P., Sadler, J.E. and Hill, R.L. (1979) Biosynthesis of mammalian glycoproteins. Glycosylation pathways in the synthesis of the nonreducing terminal sequences. *J. Biol. Chem.*, **254**, 12531–12534.

Bourin, M.-C. and Lindahl, U. (1993) Glycosaminoglycans and the regulation of blood coagulation. *Biochem. J.*, **289**, 313–330.

Butor, C., Diaz, S. and Varki, A. (1993) High level O-acetylation of sialic acids on N-linked oligosaccharides of rat liver membranes. Differential subcellular distribution of 7- and 9-O-acetyl groups and of enzymes involved in their regulation. *J. Biol. Chem.*, **268**, 10197–10206.

Cummings, R.D. (1992) Synthesis of asparagine-linked oligosaccharides: pathways, genetics, and metabolic regulation. In Allen, H.J. and Kisailus, E.C. (eds), *Glycoconjugates: Composition, Structure, and Function*. Marcel Dekker, New York, pp. 333–360.

Dey, N.B., Bounelis, P., Fritz, T.A., Bedwell, D.M. and Marchase, R.B. (1994) The glycosylation of phosphoglucosyltransferase is modulated by carbon source and heat shock in *Saccharomyces cerevisiae*. *J. Biol. Chem.*, **269**, 27143–27148.

Eriksson, J., Sandbäck, D., Ek, B., Lindahl, U. and Kjellén, L. (1994) cDNA cloning and sequencing of mouse mastocytoma glucosaminyl N-deacetylase/N-sulfotransferase, an enzyme involved in the biosynthesis of heparin. *J. Biol. Chem.*, **269**, 10438–10443.

Fox, D.A., Davis, W., Zeldes, W., Kan, L., Higgs, J., Duby, A.D. and Holoshitz, J. (1990a) Activation of human T cell clones through the UM4D4/CDw60 surface antigen. *Cell Immunol.*, **128**, 480–489.

Fox, D.A., Millard, J.A., Kan, L., Zeldes, W.S., Davis, W., Higgs, J., Emrich, F. and Kinne, R.W. (1990b) Activation pathways of synovial T lymphocytes. Expression and function of the UM4D4/CDw60 antigen. *J. Clin. Invest.*, **86**, 1124–1136.

Freeze, H.H. and Ichikawa, M. (1995) Identification of N-acetylglucosamine-1-phosphate transferase activity in *Dictyostelium discoideum*: an enzyme that initiates phosphoglycosylation. *Biochem. Biophys. Res. Commun.*, **208**, 384–389.

Green, E.D., van Halbeek, H., Boime, I. and Baenziger, J.U. (1985) Structural elucidation of the disulfated oligosaccharide from bovine lutropin. *J. Biol. Chem.*, **260**, 15623–15630.

Hanai, N., Dohi, T., Nores, G.A. and Hakomori, S. (1988) A novel ganglioside, de-N-acetyl-GM3, acting as a strong promoter for epidermal growth factor receptor kinase and as a stimulator for cell growth. *J. Biol. Chem.*, **263**, 6296–6301.

Hayes, B.K. and Varki, A. (1993a) The biosynthesis of oligosaccharides in intact Golgi preparations from rat liver. Analysis of N-linked and O-linked glycans labeled by UDP-[6-<sup>3</sup>H]N-acetylgalactosamine. *J. Biol. Chem.*, **268**, 16170–16178.

Hayes, B.K. and Varki, A. (1993b) Biosynthesis of oligosaccharides in intact Golgi preparations from rat liver. Analysis of N-linked glycans labeled by UDP-[6-<sup>3</sup>H]galactose, CMP-[9-<sup>3</sup>H]N-acetylneuraminic acid, and [acetyl-<sup>3</sup>H]acetyl-coenzyme A. *J. Biol. Chem.*, **268**, 16155–16169.

Hernandez, L.M., Ballou, L., Alvarado, E., Tsai, P.-K. and Ballou, C.E. (1989) Structure of the phosphorylated N-linked oligosaccharides from the mnn9 and mnn10 mutants of *Saccharomyces cerevisiae*. *J. Biol. Chem.*, **264**, 13648–13659.

Joziasse, D.H. (1992) Mammalian glycosyltransferases: genomic organization and protein structure. *Glycobiology*, **2**, 271–277.

Kitazume, S., Kitajima, K., Inoue, S., Haslam, S.M., Morris, H.R., Dell, A., Lenarz, W.J. and Inoue, Y. (1996) The occurrence of novel 9-O-sulfated N-glycolylneuraminic acid-capped  $\alpha 2 \rightarrow 5$ -Oglycolyl-linked oligo/polyNeu5Gc chains in sea urchin egg cell surface glycoprotein—identification of a new chain termination signal for polystylyltransferase. *J. Biol. Chem.*, **271**, 6694–6701.

Klein, A., Krishna, M., Varki, N.M. and Varki, A. (1994) 9-O-Acetylated sialic acids have widespread but selective expression: analysis using a chimeric dual-function probe derived from influenza C hemagglutinin-esterase. *Proc. Natl. Acad. Sci. USA*, **91**, 7782–7786.

Kniep, B., Flegel, W.A., Northoff, H. and Rieber, E.P. (1993) CDw60 glycolipid antigens of human leukocytes: structural characterization and cellular distribution. *Blood*, **82**, 1776–1786.

Kniep, B., Claus, C., Peter-Katalinic, J., Monner, D.A., Dippold, W. and

- Nimtz, M. (1995) 7-O-Acetyl-GD3 in human T-lymphocytes is detected by a specific T-cell-activating monoclonal antibody. *J. Biol. Chem.*, **270**, 30173–30180.
- Kobata, A. and Takasaki, S. (1992) Structure and Biosynthesis of Cell Surface Carbohydrates. In Fukuda, M. (ed), *Cell Surface Carbohydrates and Cell Development*. CRC Press, Boca Raton, pp. 1–24.
- Kornfeld, S. and Mellman, I. (1989) The biogenesis of lysosomes. *Annu. Rev. Cell Biol.*, **5**, 483–525.
- Lindahl, U., Backstrom, G., Hook, M., Thunberg, L., Fransson, L.A. and Linker, A. (1979) Structure of the antithrombin-binding site in heparin. *Proc Natl. Acad. Sci. USA*, **76**, 3198–3202.
- Lindahl, U., Backstrom, G., Thunberg, L. and Leder, I.G. (1980) Evidence for a 3-O-sulfated D-glucosamine residue in the antithrombin-binding sequence of heparin. *Proc. Natl. Acad. Sci. USA*, **77**, 6551–6555.
- Mandon, E., Kempner, E.S., Ishihara, M. and Hirschberg, C.B. (1994) A monomeric protein in the Golgi membrane catalyzes both N-deacetylation and N-sulfation of heparan sulfate. *J. Biol. Chem.*, **269**, 11729–11733.
- Manzi, A.E., Diaz, S. and Varki, A. (1990) High-pressure liquid chromatography of sialic acids on a pellicular resin anion-exchange column with pulsed amperometric detection: a comparison with six other systems. *Anal. Biochem.*, **188**, 20–32.
- Muchmore, E. and Varki, A. (1987) Inactivation of influenza C esterase decreases infectivity without loss of binding: a probe for 9-O-acetylated sialic acids. *Science*, **236**, 1293–1295.
- Nores, G.A., Hanai, N., Levery, S.B., Eaton, H.L., Salyan, M.E.K. and Hakomori, S. (1989) Synthesis and characterization of ganglioside GM3 derivatives: lyso-GM3, de-N-acetyl-GM3, and other compounds. *Methods Enzymol.*, **179**, 242–252.
- Norgard-Sumnicht, K. and Varki, A. (1995) Endothelial heparan sulfate proteoglycans that bind to L-selectin have glucosamine residues with unsubstituted amino groups. *J. Biol. Chem.*, **270**, 12012–12024.
- Norgard-Sumnicht, K.E., Roux, L., Toomre, D.K., Manzi, A., Freeze, H.H. and Varki, A. (1995) Unusual anionic N-linked oligosaccharides from bovine lung. *J. Biol. Chem.*, **270**, 27634–27645.
- Orellana, A., Hirschberg, C.B., Wei, Z., Swiedler, S.J. and Ishihara, M. (1994) Molecular cloning and expression of a glycosaminoglycan N-acetylglucosaminyl N-deacetylase/N-sulfotransferase from a heparin-producing cell line. *J. Biol. Chem.*, **269**, 2270–2276.
- Paulson, J.C., Rearick, J.I. and Hill, R.L. (1977) Enzymatic properties of beta-D-galactoside alpha-2-6 sialyltransferase from bovine colostrum. *J. Biol. Chem.*, **252**, 2363–2371.
- Paulson, J.C., Prieels, J.P., Glasgow, L.R. and Hill, R.L. (1978) Sialyl- and fucosyltransferases in the biosynthesis of asparaginyl-linked oligosaccharides in glycoproteins. Mutually exclusive glycosylation by beta-galactoside alpha-2-6 sialyltransferase and N-acetylglucosaminidase alpha-1-3 fucosyltransferase. *J. Biol. Chem.*, **253**, 5617–5624.
- Razi, N. and Lindahl, U. (1995) Biosynthesis of heparin/heparan sulfate. The D-glucosaminyl 3-O-sulfotransferase reaction: target and inhibitor saccharides. *J. Biol. Chem.*, **270**, 11267–11275.
- Rearick, J.I., Sadler, J.E., Paulson, J.C. and Hill, R.L. (1979) Enzymatic characterization of beta-D-galactoside alpha-2-3 sialyltransferase from porcine submaxillary gland. *J. Biol. Chem.*, **254**, 4444–4451.
- Reuter, G. and Schauer, R. (1994) Determination of sialic acids. *Methods Enzymol.*, **230**, 168–199.
- Rieber, E.P. and Rank, G. (1994) CDw60: a marker for human CD8+ T helper cells. *J. Exp. Med.*, **179**, 1385–1390.
- Roux, L., Holoyda, S., Sundblad, G., Freeze, H.H. and Varki, A. (1988) Sulfated N-linked oligosaccharides in mammalian cells I: complex-type chains with sialic acids and O-sulfate esters. *J. Biol. Chem.*, **263**, 8879–8889.
- Sadler, J.E., Rearick, J.I., Paulson, J.C. and Hill, R.L. (1979) Purification to homogeneity of a beta-galactoside alpha-2-3 sialyltransferase and partial purification of an alpha-N-acetylgalactosaminidase alpha-2-6 sialyltransferase from porcine submaxillary glands. *J. Biol. Chem.*, **254**, 4434–4442.
- Sadler, J.E., Beyer, T.A., Oppenheimer, C.L., Paulson, J.C., Prieels, J.P., Rearick, J.I. and Hill, R.L. (1982) Purification of mammalian glycosyltransferases. *Methods Enzymol.*, **83**, 458–514.
- Sato, C., Kitajima, K., Tazawa, I., Inoue, Y., Inoue, S. and Troy, F.A., II (1993) Structural diversity in the alpha-2-8-linked polysialic acid chains in salmonid fish egg glycoproteins. Occurrence of poly(Neu5Ac), poly(Neu5Gc), poly(Neu5Ac, Neu5Gc), poly(KDN), and their partially acetylated forms. *J. Biol. Chem.*, **268**, 23675–23684.
- Schauer, R. (1991) Biosynthesis and function of N- and O-substituted sialic acids. *Glycobiology*, **1**, 449–452.
- Shi, W.X., Chammass, R. and Varki, A. (1996) Linkage-specific action of endogenous sialic acid O-acetyltransferase in Chinese hamster ovary cells. *J. Biol. Chem.*, **271**, 15130–15138.
- Sjoberg, E.R., Powell, L.D., Klein, A. and Varki, A. (1994) Natural ligands of the B cell adhesion molecule CD22 $\beta$  can be masked by 9-O-acetylation of sialic acids. *J. Cell Biol.*, **126**, 549–562.
- Sjoberg, E.R., Chammass, R., Ozawa, H., Kawashima, I., Khoo, K.-H., Morris, H.R., Dell, A., Tai, T. and Varki, A. (1995) Expression of de-N-acetyl-gangliosides in human melanoma cells is induced by genistein or nocodazole. *J. Biol. Chem.*, **270**, 2921–2930.
- Stanley, P. and Ioffe, E. (1995) Glycosyltransferase mutants: Key to new insights in glycobiology. *FASEB J.*, **9**, 1436–1444.
- Sundblad, G., Holoyda, S., Roux, L., Varki, A. and Freeze, H.H. (1988) Sulfated N-linked oligosaccharides in mammalian cells II: identification of glycosaminoglycan-like chains attached to complex-type glycans. *J. Biol. Chem.*, **263**, 8890–8896.
- Troy, F.A., II (1992) Polysialylation: from bacteria to brains. *Glycobiology*, **2**, 5–23.
- Van den Born, J., Gunnarsson, K., Bakker, M.A., Kjellen, L., Kusche-Gullberg, M., Maccarana, M., Berden, J.H. and Lindahl, U. (1995) Presence of N-unsubstituted glucosamine units in native heparan sulfate revealed by a monoclonal antibody. *J. Biol. Chem.*, **270**, 31303–31309.
- Varki, A. (1992) Diversity in the sialic acids. *Glycobiology*, **2**, 25–40.
- Varki, A. (1993) Biological roles of oligosaccharides: all of the theories are correct. *Glycobiology*, **3**, 97–130.
- Varki, A. and Freeze, H.H. (1994) The major glycosylation pathways of mammalian membranes, a summary. In Maddy, A.H. and Harris, J.R. (eds), *Subcellular Biochemistry*. Plenum Press, New York, pp. 71–100.
- Varki, A. and Marth, J. (1995) Oligosaccharides in vertebrate development. *Semin. Dev. Biol.*, **6**, 127–138.
- Wei, Z., Swiedler, S.J., Ishihara, M., Orellana, A. and Hirschberg, C.B. (1993) A single protein catalyzes both N-deacetylation and N-sulfation during the biosynthesis of heparan sulfate. *Proc. Natl. Acad. Sci. USA*, **90**, 3885–3888.
- Whitfield, D.M. and Douglas, S.P. (1996) Glycosylation reactions—present status future directions. *Glycoconjugate J.*, **13**, 5–17.
- Zimmer, G., Reuter, G. and Schauer, R. (1992) Use of influenza C virus for detection of 9-O-acetylated sialic acids on immobilized glycoconjugates by esterase activity. *Eur. J. Biochem.*, **204**, 209–215.
- Zimmer, G., Suguri, T., Reuter, G., Yu, R.K., Schauer, R. and Herrler, G. (1994) Modification of sialic acids by 9-O-acetylation is detected in human leukocytes using the lectin property of influenza C virus. *Glycobiology*, **4**, 343–350.

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