Structural Determination of Oligosaccharide in Glycoproteins

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Membrane and secreted proteins are often glycosylated during transit through the ER and Golgi apparatus in eukaryotic cells. These carbohydrates can be attached to the hydroxyl group on a Serine or Threonine (O-linked glycosylation), or the amine of an asparagine via an N-glycosidic bond (N-linked glycosylation). The addition of carbohydrate chains to the polypeptide backbone of a protein may have an impact on the structure, solubility, antigenicity, folding, secretion and stability of the protein (1). The carbohydrate may also affect the clearance rate and in vivo activity of the protein (2).

In recent years there has been a dramatic increase in reports of glycosylation of proteins in various Gram-negative systems including *Neisseria meningitidis, Neisseria gonorrhoeae, Campylobacter jejuni, Pseudomonas aeruginosa, Escherichia coli, Caulobacter crescentus, Aeromonas caviae* and *Helicobacter pylori.* Although this growing list contains many important pathogens and the glycosylations are found on proteins important in pathogenesis such as pili, adhesins and flagella the precise role(s) of the glycosylation of these proteins remains to be determined. Furthermore, the details of the glycosylation biosynthetic process have not been determined in any of these systems.

Human Erythropoietin is a 165 amino acid glycoprotein with 3 N-linked carbohydrates attached to asparagines at amino acid positions 24, 38, and 83, and one O-linked carbohydrate attached to Ser126 (3). The structure of rHuEPO and the function importance of the amino acids for activity is well studied (4). The importance of the carbohydrate on rHuEPO has been demonstrated by the ability to increase the in vivo activity and serum half-life of the protein by adding more N-linked carbohydrates to the molecule. The N-glycosylated oligosaccharides were structurally determined by HPLC (Figure 1) and MALDI MS (Figure 2).

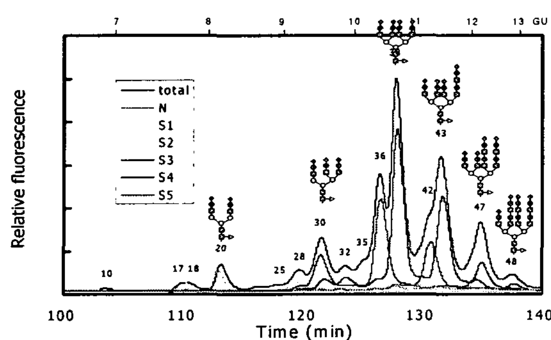

Figure 1. Oligosaccharide profiles of erythropoietin.

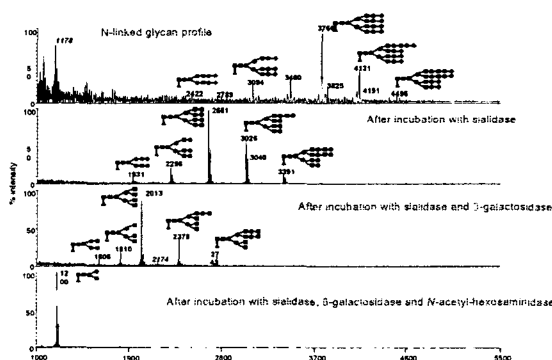

Figure 2. MALDI MS analysis of erythropoietin glycans.

The structures of the oligosaccharides attached to arylphorin from Chinese oak silkworm, Antheraea pernyi have been determined. Arylphorin, which is a storage protein present in 5th larval hemolymph, contained 4.8% (w/w) of carbohydrate that was composed of Fuc:GlcNAc:Glc:Man=0.2:4.0:1.4:13.6 moles per mole protein. Four moles of GlcNAc in oligomannose type oligosaccharides strongly suggest that the protein contains two N-glycosylation sites. Normal-phase HPLC (Figure 3) and mass spectrometry (Figure 4) oligosaccharide profiles confirmed that arylphorin contained mainly oligomannose type

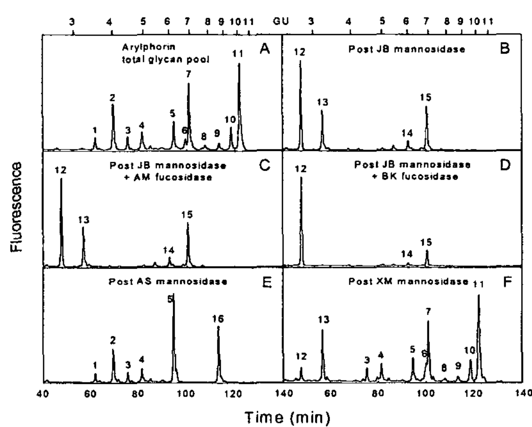

Figure 3. Oligosaccharide profiles of arylphorin with exoglycosidase array.

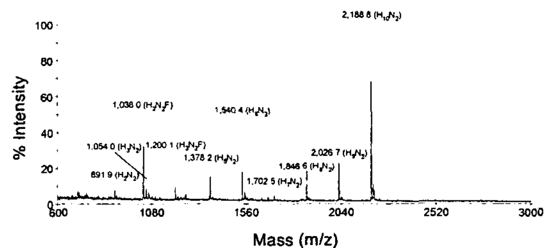

Figure 4. MALDI MS analysis of arylphorin glycan mixture.

glycans as well as truncated mannose type structures with or without fucosylation. Interestingly, the most abundant oligosaccharide was monoglucosylated Man9GlcNAc2 which was characterised by normal-phase HPLC, mass spectrometry, Aspergillus saitoi a-mannosidase digestion and 1H 600 MHz NMR spectrometry (Figure 5). This glycan structure is not normally present in secreted mammalian glycoproteins, however, it has been identified in avian species. The Glc1Man9GlcNAc2 structure was present only in arylphorin, while other hemolymph proteins contained only oligomannose and truncated oligosaccharides. The oligosaccharide was also detected in the arylphorin of another silkworm, Bombyx mori, suggesting a specific function for the Glc1Man9GlcNAc2 glycan. There were no processed glucosylated oligosaccharides such as Glc1Man5-8GlcNAc2. Furthermore, Glc1Man9GlcNAc2 was not released from arylophorin by PNGase F under non-denaturing conditions, suggesting that the N-glycosidic linkage to Asn is protected by the protein. Glc1Man9GlcNAc2 may play a role in the folding of arylphorin or in the assembly of hexamers.

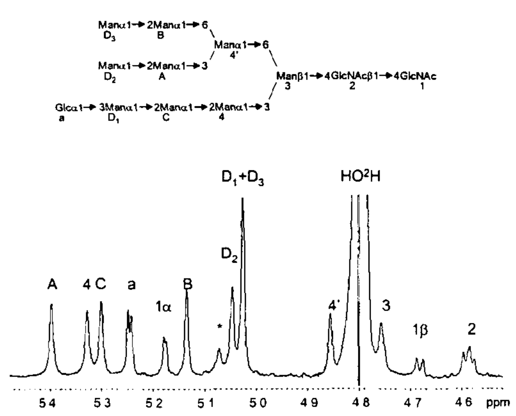

Figure 5. NMR analysis of arylphorin Glc1an9GlcNAc2.

References

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