3-3-13. Structural Determination of the *N*-Glycans of a Lepidopteran Arylphorin Reveals the Presence of a Monoglucosylated Oligosaccharide in the Storage Protein

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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 and mass spectrometry oligosaccharide profiles confirmed that arylphorin contained mainly oligomannose type 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. 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.