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A specific glycoprotein secreted by follicle cells into a growing oocyte of *Drosophila melanogaster* is also produced and secreted by cultured *Drosophila* embryonic cells which were used to characterize it biochemically.

The biosynthesis of the glycoprotein, as revealed by ^{3}H-GlcN incorporation into the cultured cells, was not affected by tunicamycin, an inhibitor of *N*-glycosylation. The glycoprotein was also shown to be resistant to the action of glycopeptidase F (glycopeptide-*N*-glycosidase). The presence of *O*-glycans in the glycoprotein was corroborated by its sensitivity to mild alkaline hydrolysis. The glycoprotein does not possess a significant number of *Ser (Thr)-O-xyl* linkages because β-D-xylosides fail to disturb its biosynthesis. The most part of the glycoprotein's oligosaccharides (amounting up to 20% of the total molecular mass of 100 kD) seem to be attached to a polypeptide *via Ser (Thr)-O-GalNac* linkages as revealed by endo-α-*N*-acetylgalactosaminidase treatment. The glycan moiety of the glycoprotein enriched by partially sulfated *GlcN/GlcNac* residues, yielded tri-, di- and monosaccharides upon microbial chitinase digestion. Thus the glycoprotein in question seems to possess an unusual glycan structure not yet detected in insects, which combines the chitin-like stretches of *GlcN* residues and *GalNac-O* links of mucin type.

S9.34

Characterization of Oligosaccharide Structure of Human Arylsulfatase A

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Arylsulfatase A (ASA) is a lysosomal hydrolase responsible for metabolism of various sulfatides. In humans, a deficiency of enzymatic activity of ASA is responsible for a neurological disorder, known as metachromatic leukodystrophy. Electrophoretically homogeneous enzyme purified from human tissues is a glycoprotein composed of two non-identical subunits. According to its cDNA analysis ASA contains three potential *N*-glycosylation sites, two of which are usually utilized. It has recently been suggested that the difference in the molecular weights of the subunits results from different peptide chain lengths rather than variations in carbohydrate moiety.

The oligosaccharide structure of ASA was studied using the glycan differentiation method which is based on probing glycoproteins with the following digoxigenin-labeled lectins: Galanthus nivalis agglutinin (GNA), Sambucus nigra agglutinin (SNA), Maackia amurensis agglutinin (MAA), peanut agglutinin (PNA) and Datura stramonium agglutinin (DSA). The results allow us to conclude that the oligosaccharide structure of both subunits of ASA is mostly of high mannose type (GNA positive reaction). Only a minor part can be of hybride type (weak reaction with DSA). Lack of reaction with SNA and MAA as well as PNA demonstrated the absence of sialic acid in ASA glycan chains and *O*-linked oligosaccharides, respectively.

S9.35

Lectins Reactions with Haptoglobins Prepared from Normal and Cancer Sera

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Some lectins were used for characterization and comparison of carbohydrate moieties of human haptoglobins derived from normal and pathological sera.

Two monoclonal anti-haptoglobin antibodies (clones 2.36.71.41 and 7.60.66.55) were used for isolation of the human haptoglobin from biological fluids[1]. The sources of haptoglobins were: normal sera, and sera from women with ovarian cancer. Differences in haptoglobin binding to lectins (Concanavalin A, Sambuccus nigra lectin, Maackia amurensis lectin, Galanthus nivalis lectin, Datura stramonium lectin) were studied by lectin-enzyme immunosorbent assay[2] as well as by immunodotting and electroimmunoblotting. Our investigations suggest changes in haptoglobin glycosylation depending on pathophysiological states of human organism. The measurements of abnormally glycosylated forms of human haptoglobin could open exciting possibilities for diagnosis, prognosis, and monitoring of diseases.

[1]Katnik, I. *et al.*, *Hybridoma*, **8**, (1989), 551 – 560.
[2]Katnik, I., Dobryszycka, W., *J. Immunoassay*, **13**, (1992), 145 – 162.

S.10 HOST-PATHOGEN INTERACTIONS

S10.1

Glycobiology of Host-Trypanosomal Interactions

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African trypanosomes successfully evade the host immune system by antigenic variation of their glycosyl phosphatidylinositol (CPI) anchor bound variant surface glycoproteins (VSGs), which form a complete monolayer at their cell-surfaces. The abundance (~10^7/cell) and rapid synthetic rates of the GPI-linked VSGs have made the African trypanosome the best system for biochemical analyses of GPI biosynthesis by several labs. Using a cell-free system, the overall pathway of GPI assembly has been elucidated. GPIs are assembled stepwise, by adding GlcNAc from UDP-GlcNAc to PI, rapid deacetylation to yield GlcN-PI, transfer of a tri-α-mannosyl core sequentially from mannosylphosphoryldolichol, and finally the addition of a phosphoethanolamine (derived from phosphatidylethanolamine) to the 6-OH of the terminal mannose. Upon completion of the glycan portion, the fatty acids of the GPI are "remodeled" in a series of trypanosome specific reactions to yield exclusively di-myristoyl-GPI, which then is attached to the nascent polypeptide by a transpeptidation reaction involving the cleavage of a hydrophobic C-terminal domain from the VSG precursor. Understanding the myristate remodeling process has resulted in the characterization of several myristate analogs that are selectively, highly toxic to the bloodstream form of the parasite. Current work is largely focusing on the enzymes of this pathway.