containing *N*-linked oligosaccharides as described below. These *N*-linked oligosaccharides were mostly neutral, because sialic acid content was lower than 0.13 mol/mol of sugar chain, and sulfate was not detected. The oligosaccharides were released from pepsin-digested protein by glycoamidase A (from almond) digestion. The reducing ends of the oligosaccharide chains were aminated with a fluorescent reagent, 2-aminopyridine. The resulting mixture of pyridylamino derivatives of the oligosaccharides were separated by HPLC on an ODS column, and 15 oligosaccharides were isolated. The structure of each oligosaccharide fraction was analyzed by 2-D sugar mapping. The oligosaccharides did not elute coincidentally with any known standards on the 2-D map. However, upon digestion with β-*N*-acetylhexosaminidase, removing terminal GalNAc, all of them were converted to known standards on the map. Furthermore, the structures were confirmed by component sugar analysis, methylation analysis and ^{1}H NMR measurements. These oligosaccharides include five biantennary, seven triantennary, and three tetraantennary structures.

S9.22

N-Linked Carbohydrate Chains Derived from Human Urokinase Contain 4-*O*-Sulfated, α(2-6)-Sialylated or α(1-3)-Fucosylated *N*-Acetylgalactosamine-β(1-4)-*N*-Acetylglucosamine as Non-Reducing Terminal Units

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Urinary-type plasminogen activator (u-PA or urokinase) converts plasminogen into the fibrinolytic enzyme, plasmin. It is a potential clinical therapeutic agent in the treatment of thrombolytic occlusions. In addition, u-PA plays an important role in extracellular proteolytic processes, like those during gland involution and tumour-growth. Structural analysis of the enzymically released *N*-linked carbohydrate chains of human u-PA by ^{1}H-NMR spectroscopy demonstrated that the collection of *N*-glycans on the only *N*-glycosylation site (Asn-302) comprises di-, tri-, and tri'-antennary structures, which contain mainly GalNAcβ(1-4)GlcNAcβ instead of the conventional Galβ(1-4)GlcNAcβ elements. The unusual element is for the greater part substituted, namely the GlcNAc residue may be fucosylated at 0-3 [1], or the GalNAc may be sulfated at 0-4 or sialylated at 0-6. The major component, which accounts for about 30 mol% of the total oligosaccharide pool, consists of an α(1-6)-fucosylated diantennary *N*-linked carbohydrate chain, bearing terminal (SO_4-4)GalNAcβ(1-4)GlcNAcβ(1-2) units in both branches.

[1] Bergwerff, A. A., Thomas-Oates, J. E., Van Oostrum, J., Kamerling, J. P. & Vliegenthart, J. F. G. (1992) *FEBS Lett.*, **314**, 389–394.

S9.23

Improved Pyridylamination and Development of an Automated Carbohydrate Labeling Instrument

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The structures of the sugar chains of glycoconjugates are being elucidated in recent years. Along with the development of these studies, various functions of sugar chains have been identified. However, conventional methods for carbohydrate analysis have a number of disadvantages, including their being time-consuming and depending on difficult techniques. A fluorescence labeling method of sugar chains with the use of 2-aminopyridine, or pyridylamination, will be presented. We have developed a manual type carbohydrate labeling instrument, named the PALSTATION, which makes it possible to obtain PA-sugar chains simply and rapidly. Almost all of the excess amounts of reagents after pyridylamination are removed with blowing of nitrogen under reduced pressure in this device. Futhermore, we have produced a fully automated carbohydrate labeling instrument, named the ***GlycoTAG*** ™, which enables us to pyridylaminate samples hands-off operation and with highly reliable results.

When glycoproteins are produced by the techniques of genetic engineering, different kinds of host cells can produce sugar chains of different structures in spite of identical amino acid sequences. Because quality control of sugar chains is an important problem when glycoproteins are produced on a commercial scale, we set out to automate pyridylamination, and has made this possible. Here is presented the analysis of sugar chains of tPA as an example of analysis by this apparatus, the GlycoTAG.

S9.24

A New Analytical Method for Interaction Between Lectins and Oligosaccharides of Glycoproteins by Surface Plasmon Resonance

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The present study was undertaken to develop a simple analytical method for detecting interactions between lectins and oligosaccharides of glycoproteins by surface plasmon resonance using BIAcore. The interactions of four lectins, *Sambucus sieboldiana* agglutinin (SSA), *Ricinus communis* agglutinin I (RCA I), Concanavalin A (Con A) and *Datura stramonium* agglutinin (DSA) for fetuin and digested fetuin, asialo-, agalacto-, and aglucosamino-fetuin were investigated as a model system. These fetuins were immobilized to the matrix of the sensor chip and the lectins were injected into the sensor chip. The association and dissociation reactions could be monitored in real time. The association rate constants of SSA to fetuin, RCA I to asialofetuin, Con A to aglucosaminofetuin were estimated to be 1.8×10^3, 8.8×10^4 and 1.7×10^4 ($M^{-1} \cdot S^{-1}$), respectively. The binding amount and affinity of SSA decreased as the sialic acids of the oligosaccharide chains were removed, while those of RCA I and DSA increased. The binding ability of Con A increased as the sugar chains were trimmed. These changes of binding properties of lectins by trimming could be available to predict the structures of oligosaccharide chains. These results indicated that the interactions between lectins and glycoproteins could be well defined in real time and kinetically. The possibility of this method to elucidate the structures of sugar chains isolated from some glycoproteins is under investigation.

S9.25

Isolation, Purification and Partial Characterization of Multiple Forms of Bovine Bone OPN (Osteopontin)