S9.13

O-Glycopeptides from Urine of Patients with α-*N*-Acetylgalactosaminidase Deficiency Studied by Electrospray (ES) and Fast Atom Bombardment (FAB) Mass Spectrometry

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Electrospray mass spectrometry (ES-MS) has recently emerged as a new powerful technique for analysis of high molecular weight biopolymers (>100.000 Dal) at a subnanomolar level. The multiple charged molecules can be structurally attributed according to their state of charge. We used ES-MS for structural studies on glycopeptides. A superiority over well established FAB-MS methods was found in higher sensitivity as well as in ability to induce specific fragmentation of the carbohydrate portion. This allowed assignment of carbohydrate sequence and the binding site to the peptide portion. Using this approach, it is possible to avoid chemical derivatizations, which may influence alkali-labile groups, found frequently on carbohydrate chains. The positive and negative ion ES-MS was applied to analyse sialic acid containing *O*-glycopeptides from urine of patients suffering from inherited deficiency of lysosomal α-*N*-acetylgalactosaminidase. Beside the major species, Neu5Acα2-3Galβ1-(NeuAcα2-6)-3GalNAc-Ser(Thr), a number of mono-, di- and trisialylglycans were structurally characterized in fractions, obtained after several chromatographic steps, including gel permeation and anion exchange chromatography. After appropriate *N*- and/or *O*-derivatizations, the samples were also analysed by FAB-MS for sequencing of sugar chains and by methylation analysis for linkage sites.

S9.14

Precise Structural Determination of Unique Highly Branched Multiantennary *N*-Glycan Units Present in Fish Egg Hyosophorin

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Although glycoproteins containing high-molecular-weight glycan chains such as multiple *N*-acetyllactosamine units have been reported to occur on developing embryonic cell and erythrocyte surfaces, there reported almost no case where the precise structural elucidation of such glycoproteins including their glycan portion and apo-protein has been made. We have demonstrated the occurrence of heavily glycosylated polyproteins ("hyosophorin") in fish egg cortical alveolus, and we have been investigating their structures and functional role during early embryogenesis.

In this study, we found a new type of hyosophorin glycan chains in two medaka fish species, *Oryzias latipes* and *Oryzias melastigma* (1), and their structures were established by chemical degradation, methylation analysis, ^{1}H NMR spectroscopy, and FAB-mass spectrometry. Several crucial features of *Oryzias* L-hyosophorin molecules are **(a)** the presence of a novel *N*-linked pentaantennary structure in *O. latipes* and tetraantennary chain in *O. melastigma* with *M*r of 7 K in each case; **(b)** both hyosophorin molecules contain the clusters of β-linked Gal residues at the peripheral portions of the glycan units, i.e. Galβ1→4Galβ1→4Galβ1→; **(c)** the presence of uniquely branched Gal residues, i.e. →4GlcNAcβ1→3(→Galβ1→4)Galβ1→; **(d)** the sialic acid residues are linked exclusively α-2→3- to the terminal Gal residues of every antenna in *O. melastigma* while they are linked α-2→3- to either the penultimate Gal or the terminal Gal residues of every antenna in *O. latipes;* and **(e)** the *O. latipes* hyosophorin contains the Fuc residues in a form of β-galactosylated Lewis X antigenic epitope, Galβ1→4-Galβ1→4(Fucα1→3)GlcNAcβ1→.

Most recently, a detailed study on the complete structure of *Fundulus heteroclitus* hyosophorin has been performed and its characteristic features is also presented.

(1) T. Taguchi, A. Seko, K. Kitajima, S. Inoue, T. Iwamatsu, K.-H. Khoo, H. R. Morris, A. Dell and Y. Inoue (1993) *J. Biol. Chem.*, **268**, in press.

S9.15

Structural Elucidation of *O*-Glycosylation on Glycoproteins

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Structural definition of the array of oligosaccharides which are linked to Ser or Thr residues on proteins is a formidable analytical challenge. Any Ser or Thr residue in a glycoprotein may be glycosylated. We are developing a more efficient strategy to assign *O*-linked oligosaccharide chains to their respective peptide loci. Using LC-electrospray mass spectrometry (LC/ESIMS), we found five *O*-linked glycopeptides in a tryptic map of bovine fetuin. After specifically recovering >98% of the *O*-linked structures by Jacalin agarose chromatography, we detected more than 20 glycopeptide species. Ions from *O*-linked glycopeptides containing carbohydrate structures from NeuAcHexHexNAc to $NeuAc_5Hex_5HexNAc_5$ were observed. Some glycopeptides were formed by non-specific cleavages at Phe^{233} and Ser^{272}. The former cleavage yielded a glycopeptide bearing *N*-terminal glutamine, and species which eluted later with lower molecular masses (−17 Da) resulted from pyroglutamate formation. Studies are underway to isolate and characterize glycopeptides which bear only a single glycosylation site. High energy collision-induced dissociation analysis (tandem mass spectrometry) of these peptides can yield the size, composition, branching, location of the carbohydrate, and confirmation of the amino acid sequence of the peptide [1]

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1. K. F. Medzihradszky, B. L. Gillece-Castro, C. A. Settineri,