task. High resolving power of sodium dodecyl sulfate – polyacrylamide gel electrophoresis (SDS-PAGE) makes it a valuable tool for this purpose. However, often it is difficult to extract a glycoprotein from the gel, especially if its molecular weight is high. To overcome these problems, a set of model glycoproteins resolved by SDS-PAGE was transferred to polyvinylidene fluoride (PVDF) membrane and then eluted using either SDS/Triton X-100 mixtures or guanidinum hydrochloride/lysolecithin mixtures. Final recoveries ranged from 30 – 70% depending on the method of elution and on the glycoprotein type. The method was successfully applied for the preparation of pure glycoproteins from herpes viruses which were subsequently used for raising monospecific antibodies in rabbits.

S9.30

Enzymatic Analysis of Sugar Chain of Dermatan Sulfate Proteoglycan from Bovine Aorta

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Dermatan sulfate proteoglycans (DSPG) from bovine aorta were separated and subjected to structural analysis using several kinds of galactosaminoglycuronan-degrading enzymes, chondroitinases (Chases) ABC, AC-I, AC-II, AC-III, B and C.

There were some heterogeneity in their disaccharide compositions of each DSPG fraction. Major DSPG composed of five disaccharide isomers, ΔDi-0S, ΔDi-6S, ΔDi-4S, ΔDi-diSD and ΔDi-diSB, in the ratio of 1.2: 6.2: 86.0: 0.8 and 5.9, respectively. The digests of DSPG with each enzyme gave different degradation profiles on the gel permeation HPLC showing larger to smaller degradation in order of Chases ABC, B, AC I, AC III, AC II and C.

Digestion with chase B produced only two kinds of disaccharides ΔDi-4S and ΔDi-diSB in the ratio of 88 and 12, respectively, and also produced unsaturated tetra- and higher oligosaccharides which included GlcA-GalNAc(6S). To examine the structure of the linkage region, DSPG was digested with proteinase and the peptide portion was labelled with Fluorescamine (DSp*). Degradation profiles of DSp* with Chases were compared on the gel permeation HPLC equipped with RI- and fluoro-meters. The degradation pattern of DSp* monitored by RI were similar to those of DSPG, whereas the endolabelled oligosaccharides by fluorography showed mainly three different size species; 1. ΔUA-Gal-Gal-Xyl-p* (Chase C, AC-I, AC-II, AC-III), 2. ΔUA-GalNAc(S)-GA-Gal-Gal-Xyl-p*(Chase ABC, B), 3. ΔUA-GalNAc(S)-GA-GalNAc(S)-GA-Gal-Gal-Xyl-p*(Chase B). Chase C-digest gave a sharp peak, while each Chase AC-digest had a small shoulder corresponding to the oligosaccharide 2, and Chase B-digest gave a broad peak indicating the mixture of the oligosaccharides 2 and 3.

These results suggest that there are some heterogeneous structure in the oligosaccharides near the linkage region.

S9.31

Minor Carbohydrate Structures Responsible for Binding of Some Lectins by Glycophorin A

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Glycophorin A (GPA) reacts with *Evonymus europaea* lectin (EEL, reactive most strongly with blood group B and H structures) and the receptor was found in *N*-glycan fraction of GPA oligosaccharides [1]. *Moluccella laevis* lectin (MLL), which agglutinates blood group A or N erythrocytes, shows anti-Tn specificity and reacts weakly with normal GPA, more strongly with that of blood group N than M type [2]. The aim of our studies was to find out whether the structures expected to be involved in binding of these lectins (H, Tn) are detectable in GPA isolated from blood group O erythrocytes. The FAB-MS and methylation analyses of the pool of *N*-linked oligosaccharide alditols obtained from GPA showed the presence of several minor structures, including Fuc1-2Gal1-4GlcNAc- sequence. This finding confirmed that some antennas of GPA *N*-glycans are terminated at nonreducing end with structure able to bind EEL. The reduced *O*-glycans obtained from GPA by Carlson degradation were fractionated by gel filtration and in their low-molecular weight fraction a minor amount of free GalNAc-ol was systematically detected by GLC-MS. The comparison of the products derived from several preparations of blood group M- and N-type GPA showed that GPA-N degradation products contained on the average about 2 times more GalNAc-ol than those obtained from GPA-M. It remains to be established why GPA-N has a higher content of nonsubstituted GalNAc residues (Tn receptors) and whether this difference is the reason of stronger reactivity of MLL with GPA-N.

[1] Petryniak *et al.*, *Eur. J. Biochem.*, **105**, 335 – 341, 1980.
[2] Duk *et al.*, *Carbohydr. Res.*, **236**, 245 – 258, 1992.

S9.32

Specificity of Mistletoe (*Viscum album*) Lectin I Towards Oligosaccharides and Glycopeptides Related to *N*-Glysosyl-Proteins

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The behavior of *N*-acetyllactosamine-type oligosaccharides and glycopeptides on a column of mistletoe lectin I immobilized on Sepharose was examined. The immobilized lectin does not show any affinity for asialo-*N*-glycosylpeptides and related oligosaccharides, which possess one to four unmasked *N*-acetyllactosamine sequences. However, substitution of at least one of the *N*-acetyllactosamine sequences by a sialic acid residue, either at C-3 or C-6 of galactose slightly enhances the affinity of the lectin. Such sialylated *N*-glycosylpeptides or oligosaccharides are eluted from the lectin column by the starting buffer as retarded fractions. Surprisingly, the affinity of the mistletoe lectin I is higher for pentaantennary *N*-glycosylpeptides with P1-serologic activity isolated from turtle-dove ovomucoid. These glycopeptides possess two unmasked *N*-acetyllactosamine sequences, two *N*-acetyllactosamine sequences substituted each by an α-1,4-linked galactose residue and an *N*-acetyllactosamine sequence substituted by an α-NeuAc residue. Such glycans are strongly bound on the lectin column and their elution is obtained with a 0.15 M galactose solution in the starting buffer.

S9.33

A Secreted Glycoprotein from *Drosophila* Cells Possesses *O*-Glycans of Unusual Structure