is resistant to Prep. 1 in the molar ratio of 20 – 31: 69 – 80. Variably sulfated tetrasaccharides were isolated from this oligosaccharide fraction of each isomer by h.p.l.c. on an amine-bound silica column, and were characterized by chemical analysis and enzymatic analysis in conjunction with h.p.l.c. Four components were characterized also by 500 MHz 1D and 2D ^{1}H n.m.r. spectroscopy. Thus, structures of 10 components have been determined. They included five disulfated, four trisulfated and one tetrasulfated tetrasaccharides with the common core structure, $\Delta_{4,5}$GlcAβ1-3GalNAcβ1-4GlcAβ1-3GalNAc. Each isolated tetrasaccharide was resistant to Prep. 1, but susceptible to Prep. 2. Prep. 1 was demonstrated to be also inactive towards $\Delta_{4,5}$GlcAβ1-3GalNAcβ1-4GlcAβ1-3GalNAc, GlcAβ1-3GlcNAcβ1-4GlcAβ1-3GlcNAc, and $\Delta_{4,5}$GlcAβ1-3GalNAc(4-*O*-sulfate)β1-4IdoAα1-3GalNAc(4-*O*-sulfate) isolated from chondroitin, hyaluronan, and dermatan sulfate, respectively. These results indicate that unlike Prep. 2 Prep. 1 cannot degrade tetrasaccharides irrespective of their sulfation patterns. The enzymatic action of Prep. 1 is size-dependent. An extra protein band (100 K) was observed by SDS-PAGE for Prep. 2 in addition to the 98 K band observed for Prep. 1. The results may indicate that the 98 K component cannot degrade tetrasaccharides while the 100 K component may be an isomer of the 98 K component which can degrade tetrasaccharides into disaccharides.

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S9.19

Glycan Structure of a Major Glycopeptide from the S-layer Glycoprotein of *Clostridium thermosaccharolyticum* E207-71

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Clostridium thermosaccharolyticum E207-71 is covered by a square S-layer lattice[1]. By SDS-PAGE the glycoprotein subunits of the S-layer show molecular masses in the range of 100 to 210 kDa. After deglycosylation with trifluoromethanesulfonic acid a molecular mass of *ca.* 83 kDa was obtained. Proteolytic degradation of the purified S-layer glycoprotein followed by several purification steps including chromatofocusing between pH values of 9.2 to 5.7 yielded at least four different glycopeptide fractions. One and two-dimensional nuclear magnetic resonance experiments at 600 MHz, together with methylation analysis and periodate oxidation established the following structure for the polysaccharide chain of one of the major glycopeptides:

$$[\rightarrow 4)-\beta-Glc_p-(1\rightarrow 4)-\alpha-Man_p-(1\rightarrow 4)-\beta-Gal_p-(1\rightarrow]_n$$
$$\begin{array}{c} 3 \\ \uparrow \\ 1 \end{array}$$
$$\beta-Qui_p3NAc-(1\rightarrow 6)-\beta-Gal_f-(1\rightarrow 4)-\alpha-Rha_p$$

Qui3NAc = 3-acetamido-3,6-dideoxy-glucopyranose

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S9.20

Characterization of ASN-linked Oligosaccharides on the Immunoglobulins from Egg-Yolk of Japanese Quail

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Avian immunoglobulin G (IgG) differ in many important aspects from any mammalian IgG (1) as shown by our previous studies of the structures of Asn-linked oligosaccharides on IgG isolated from chicken serum and hen egg yolk (2, 3). It was not known whether the observed differences were characteristic of other members of the Phasianidae family.

Thus, in this investigation, structures of Asn-linked oligosaccharides on IgG were studied in samples of egg yolk obtained from Japanese quail. Asn-linked oligosaccharides were cleaved from IgG by hydrazinolysis and labelled with *p*-aminobenzoic acid ethyl ester after *N*-acetylation. The labelled oligosaccharides were then fractionated by a combination of Con-A agarose column chromatography and anion exchange, normal phase and reversed phase HPLC. Structures were determined by sequential exoglycosidase digestion, methylation analysis, two-dimensional HPLC and 500 MHz proton NMR. Japanese quail IgG was found to contain only neutral oligosaccharides of the following categories: the glucosylated oligomannosetype (0.6%, Glcα1-3Glcα1-3Man$_9$GlcNAc$_2$; 35.6%, Glcα1-3Man$_{7\text{-}9}$GlcNAc$_2$), oligomannosetype (15.0%, with the structure Man$_{5\text{-}9}$GlcNAc$_2$) and biantennary complex-type with core structures of -Manα1-3(-Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAc (9.9%), -Manα1-3(GlcNAcβ1-4)(-Manα1-6)Manβ1-4GlcNAcβ1-4GlcNAc (25.1%) and -Manα1-3(GlcNAcβ1-4)(-Manα1-6)Manβ1-4GlcNAcβ1-4(Fucα1-6)GlcNAc (11.4%). These results suggest that chicken and Japanese quail are similar and differ from mammalian IgG oligosaccharides. In avian species, a unique processing route may convert glucosylated oligosaccharide to complex-type oligosaccharide.

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S9.21

Application of the 2-D Sugar Mapping Technique to Analyze a Variety of Galnac-Containing *N*-Linked Oligosaccharides from Human Urinary Kallikrein

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To analyze the structures of *N*-linked oligosaccharides, we have established a powerful 2-D sugar mapping technique for even very similar structures of oligosaccharides. More than 180 pyridyl aminated oligosaccharides have been chromatographed on 2 HPLC columns (ODS and amide-silica), and the elution positions on both columns were used as coordinates in the 2-D map. The structure of unknown oligosaccharide can be estimated by comparing the elution positions of an unknown sample with those of the known oligosaccharides.

Human urinary kallikrein is glycosylated at Asn-78, Asn-84, and Asn-141. Using the 2-D mapping technique, we discovered the presence of 15 different structures of terminal GalNAc-