glucurono hydrolase EC 3.2.1.31) was studied by sequential lectin affinity chromatography. β-glucuronidase glycopeptides were obtained by extensive pronase digestion followed by *N*-/14C/ acetylation and desialylation by neuraminidase treatment. According to the distribution of the radioactivity in the various fractions obtained by chromatography on different lectins, the relative distribution of glycan structure types is proposed.

The presence of complex bi-antennary and oligomannose type glycans was indicated by Concanavaline A-Sepharose chromatography. The absence of *O*-glycans, tri- and tetra-antennary type glycans was demonstrated by analysis of Concanavaline *A*-Sepharose unbound fraction by chromatography on immobilized soybean agglutinin, Ricinus communis agglutinin. The presence of fucosylated glycans was revealed by reaction with Lotus tetragonolobus lectin and Ulex europaeus lectin. The presence of hybrid or poly (*N*-acetyl-lactosamine) type glycans was examined by wheat germ agglutinin chromatography.

S1.31

A Novel Glycosphingolipid Deacylase Hydrolyzing Globoside Yields Lysoglobosides and Fatty Acid

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Glycosphingolipid deacylase cleaves the amide bond in ceramide moiety of glycosphingolipid and is distinguished from ceramidase because of their different substrate specificity. Only a strain of *Nocardia* has been reported to produce glycosphingolipid deacylase. The Nocardial enzyme is known to hydrolyze gangliosides GM3, GM2, GM1, and GDla (1).

We have found a novel glycosphingolipid deacylase acting on globoside (Gb_4Cer) in an actinomycete of *Rhodococcus* isolated from land soil in Japan. After porcine globoside was incubated with frozen-thawed cell of this microorganism, lipophilic compounds were recovered and desalted by reversed-phase cartridge technique. Analysis of the recovered compounds with thin-layer chromatography (TLC) showed that globoside had been converted to a new product which gave slower migration on TLC than the intact glycosphingolipid. The product was stained positively by ninhydrin reagent as well as orcinol-sulfuric acid reagent. The product was purified with reversed-phase HPLC followed by Iatrobeads HPLC. The sugar chain structure of the purified product was confirmed as globoside by stepwise hydrolysis with exo-glycosidases. The whole structure of the purified product was identified as lysoglobosides by means of 500MHz proton nuclear magnetic resonance spectroscopy and fast-atom bombardment-mass spectrometry. The release of fatty acid was ascertained using [^{14}C]-labeled globoside as the substrate. The enzyme from *Rhodococcus* sp. hydrolyzed the bond between sphingosine base and fatty acid in ceramide moiety of globoside to give lysoglobosides and fatty acid.

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S1.32

Substrate Recognition by Mammalian Heparanase

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Heparin and heparan sulphate are synthesized as proteoglycans with polysaccharide chains initially consisting of *D*-glucuronic acid (GlcA) and *N*-acetyl-*D*-glucosamine (GlcNAc) units in alternating sequence. These chains become partly modified through *N*-acetyl/*N*-sulphate exchange, C5-epimerization of GlcA to *L*-iduronic acid units and *O*-sulphation in various positions.

Endoglycosidases (heparanases) capable of degrading HS in the extracellular matrix are present in various mammalian cells and tissues. They have been implicated in processes such as extravasation of leucocytes, regulation of growth factor activity and metastasis (Bashkin *et al.*, 1990). The present study was aimed at defining the substrate recognition properties of a heparanase isolated from a human hepatoma cell line (SK-hep-1).

Capsular polysaccharide (PS) from *E. coli* K5 has the same -(GlcAβ1,4-GlcNAcα1,4)$_n$- structure as the initial, unmodified PS precursor in heparin and HS biosynthesis (Vann *et al.*, 1981). The product obtained by chemical *N*-deacetylation/*N*-sulphation of this PS was not cleaved by the hepatoma heparanase, as indicated by gel chromatography. However, incubation of the K5 PS with the sulphate donor, PAPS, in the presence of solubilized microsomal enzymes (*N*- and *O*-sulphotransferases, GlcA C5-epimerase) from a heparin-producing mouse mastocytoma, yielded a product that served as a substrate for the heparanase. Moreover, chemically *O*-desulphated heparin which is similar to *N*-sulphated K5 PS but contains, in addition, *L*-iduronic acid units, was cleaved by the enzyme. Substrate recognition by the heparanase thus appears to require both *N*-sulphate groups and *L*-iduronic acid units, but probably not *O*-sulphate groups.

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S1.33

Partial Purification and Characterization of an Endo-α-*N*-Acetylgalactosaminidase From the Culture Medium of *Streptomyces Sp.* OH-11242

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The presence of a new type of endo-α-*N*-acetylgalactosaminidase from a culture medium of *Streptomyces* sp. OH-11242 (endo-GalNAc-ase-S) has been previously reported. The crude enzyme preparation (80% ammonium sulfate precipitate) from the culture medium contained not only endo-α-*N*-acetylgalactosaminidase, but also other endo- or exo-glycosidases. To isolate the endo-GalNAc-ase-S from this glycosidase mixture and also to discriminate the endo-GalNAc-ase-S from the already reported endo-GalNAc-ase, which can liberate only Galβ1-3GalNAc, a new reliable method for assaying endo-GalNAc-ase-S activity was established. Using purified porcine gastric mucus glycoprotein (PGM) as the substrate, oligosaccharides liberated from PGM