were pyridylaminated, and the reducing terminal sugars of oligosaccharides larger than Galβ1-3GalNAc were analysed by HPLC. The enzyme was partially purified by gel chromatofocusing and subsequent DEAE-Toyopearl chromatography. Endo-enzyme activity eluted around pI 4.8 on a gel chromatofocusing column and eluted with 0.19-0.25 M NaCl from a DEAE-Toyopearl column. In the enzyme fraction obtained, no exo-glycosidases or proteases could be detected. The molecular weight of the enzyme was estimated as 105kDa by gel filtration, and the optimum pH was 5.5. Endo-GalNAc-ase-S hydrolyzed the *O*-glycosidic linkage between GalNAc and Ser (or Thr) in ^{3}H-labelled and unlabelled asialo fetuin, liberating both the disaccharide (Galβ1-3GalNAc) and the tetrasaccharide (Galβ1-3[Galβ1-4GlcNAcβ1-6]GalNAc), which have already been reported. When endo-α-*N*-acetylgalactosaminidase from *Alcaligenes* sp. (endo-GalNAc-ase-A) was incubated with asialo fetuin, only the disaccharide (Galβ1-3GalNAc) was liberated.

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S1.34

Characterization of Carboxypeptidase in β-Galactosidase Complex

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We have studied the properties of carboxypeptidase(CP) in lysosomal β-galactosidase(β-gal) complex. It has been known that β-gal exists as an enzyme complex with not only CP but also neuraminidase. CP protein is likely to be an essential factor to stabilize β-gal as well as to express neuraminidase activity by forming the complex in lysosome, while the function of CP itself remains unknown. In order to clarify the function of CP, we have, as a first step, characterized CP in the purified β-gal/CP complex from bovine liver. The purified complex gave four bands with 65K, 50K, 30K, and 20K on SDS-PAGE in the presence of DTT. Amino acid sequencing analyses showed *N*-terminal amino acid sequences of 65K and 50K proteins were identical and had high homology for human lysosomal β-gal. The result of SDS-PAGE without DTT suggested that 30K and 20K proteins exist as a 48K heterodimer and had high homology for human protective protein. CP activity was optimum at pH 6 and activated by divalent cations, but strongly inhibited by DIFP, a serine protease inhibitor. By affinity labelling with ^{3}[H]-DIFP, the catalytic site of CP activity was demonstrated on the 30K protein. The complex hydrolyzed only substrates with sequencial hydrophobic amino acid sequences on C-termini. Two forms of β-gal/CP complex(700K,110K) were separated by gel filtration. Although 700K complex were de-polymerized to 110K one at pH 7, it was associated to 700 K complex at pH 4.5. This conversion was observed even when CP activity was inactivated. 30K and/or 20K proteins, which are components of CP, may be necessary for forming high molecular weight β-gal complex to stabilize β-gal in lysosome, however, it seems that CP activity is not needed to form β-gal/CP complex.

S1.35

UDP-GalNAc can be Formed by a Novel Pyrophosphorylase of a Broad Specificity

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N-acetylgalactosamine (GalNAc) is an abundant constituent of *O*-linked glycoproteins as well as glycosphinogolipids. GalNAc is transferred to the latter compounds from UDP-GalNAc with the aid of various *N*-acetylgalactosaminyltransferases. It is known that UDP-GalNAc arises from UDP-GlcNAc by the action of a specific UDPGlcNAc 4′-epimerase which was demonstrated in crude or partially purified preparations from rat liver, rabbit skin, and Bacillus subtilis (1). This enzyme has not been studied in detail and its distinction from UDP-glucose-4′epimerase is not well-documented. Recently, we have found in pig liver a novel pyrophosphorylase of rather bread specificity that acts on the following "sugar nucleotides": UDP GalNAc >UDP-GlcNAc>UDP-Glc. This enzyme is distinct from the previously described UDP-GlcNAc pyrophosphorylase (2,3) since the latter enzyme did not react with UDP-GalNAc. The enzyme has been purified 9500 fold to homogeneity using the following steps: acid treatment, ammonium sulphate, DEAE-cellulose, Sephadex G-200, Phenyl-Sepharose, Heparin-Sepharose, Zn-IDA-Sepharose and Blue Sepharose. It has no UDP-GlcNAc 4′-epimerase activity. A study is in progress to use the enzyme for the synthesis of radioactive affinity probes labeling various GlcNAc-and GalNActransferases.

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S1.36

New Glycosyltransferase and Glucosidase Inhibitors

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Biosynthesis and degradation of the saccharide chain of glycoconjugates are accomplished by an array of enzymes. The metabolism of cell surface oligosaccharides and their function can be studied by means of inhibitors for glycosyltransferases and glycosidases.

The development of glycosyltransferase inhibitors might be of interest in diagnosis and therapy of cancer. α-Glycosidase inhibitors might be effective against virus diseases; the glycosidase inhibitors 1-deoxynojirimycin and castano-spermine, respectively, were found to have activity against HIV viruses. Another excellent glycosidase inhibitor, acarbose, is used in the therapy of diabetes.

A short chemical synthesis for a galactosyltransferase inhibitor with transition-state-analogue structure is reported. Some novel amino-substituted α- and β-C-glycosides with different configurations were synthesized and their inhibitory activity on glucosidase is reported and discussed in relation to the configuration of the anomeric residue[1].

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