secreted). In order to elucidate their relationship, the gene encoding the "large" isoenzyme was cloned and sequenced for a comparison with the already known primary structure of the "small" sialidase (1). Oligonucleotides deduced from the *N*-terminal protein sequence allowed the detection of a 3.1 kb (Sau3A I) DNA-fragment, which encodes the main part of the gene including promoter sequences and an upstream ORF of unknown function. The missing *C*-terminus was found on a 2.1 kb Hind III-fragment. The correct linkage of both fragments resulted in *E. coli* clones which express sialidase activity. A comparison of the primary structures of both isoenzymes from *C. perfringens* revealed only poor similarity (21%). This is significantly lower than the homology (54%) between the "large" *C. perfringens* and the *C. septicum* sialidase sequence (2).

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S2.22

Cloning and Expression of the Rat Cytosolic Sialidase

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We have previously demonstrated that rat tissues contain at least four types of sialidase differing in subcellular location, catalytically, chromatographically and immunologically : they are intralysosomal, cytosolic and two membrane-associated sialidases. The cytosolic sialidase was purified (1) from rat skeletal muscle to apparent homogeneity and the amino acid sequence of peptides derived was analyzed. Degenerate oligonucleotides, based on the amino acid sequence data from the purified enzyme, were used as primers to amplify fragments of the gene from rat skeletal muscle cDNA by polymerase chain reaction. The cDNA thus isolated, as it lacked the 5′-end, was ligated to the downstream portion of a cDNA obtained with 5′-end amplification by polymerase chain reaction. The composite sequence encodes an open reading frame of 379 amino acids that includes all sequenced peptides. The deduced amino acid sequence is largely nonhomologous to those of bacterial and parasite sialidases, but there are two Asp boxes, the conserved sequence of the sialidases of these microorganisms (2). The cDNA was expressed in *Escherichia coli,* resulting in an appearance of sialidase activity, which was immunoprecipitated completely by an antiserum against the cytosolic sialidase of rat skeletal muscle. This is the first for a mammalian sialidase cDNA cloned.

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S2.23

Structure-Function Studies on Glycoprotein Processing-Specific Glucosidase I

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Glucosidase I initiates the processing of the oligosaccharide, $GlC_3Man_9GlcNAc_2$, in newly assembled *N*-linked glycoproteins by excising the distal α1,2-linked glucosyl moiety in the oligosaccharide. The enzyme is a resident protein in the endoplasmic reticulum (ER). The rat and bovine mammary enzyme is a tetramer of 320-340 kDa with a subunit M_r of 85 kDa, as examined by SDS-PAGE. It possesses a domain structure in which a 39 kDa lumenally-oriented region is anchored to the ER through a trans-membrane segment and a short cytoplasmic tail. Using anti-rat glucosidase I antibody as a probe and several biochemical parameters such as SDS-PAGE analysis, trypsin-catalyzed digestion, ConA-binding, endo H susceptibility and peptide mapping analysis, we have observed that the enzyme subunit from tissues of several animal species has the same M_r of 85 kDa and is cross-reactive to anti-rat glucosidase I antibody. The trypsin released fragments of the enzyme from bovine, rat, mouse and guinea pig tissues give a limit digest of 39 kDa that was previously shown to represent the catalytic domain of the enzyme. For the pig liver and kidney, the corresponding domain appears to be 29 kDa in size, indicative of an evolutionary variation. Sulfhydryl-specific reagents strongly inhibit glucosidase I. To test the hypothesis that a sulfhydryl group(s) is involved in the catalytic site of the enzyme, the purified enzyme from the rat mammary gland was loaded with 1-deoxynojirimycin, the competitive inhibitor of its substrate, to protect the putative -SH group(s), while the same groups on the remaining surface of the enzyme were blocked by alkylation. After removing the antibiotic from the active site cleft, the exposed -SH group(s) was tagged with 3-(*N*-maleimidyl-propionyl-biocytin) (MPB). The enzyme was SDS-PAGed, transblotted to nitrocellulose and probed with ^{125}I-streptavidin. It was unequivocally shown that an -SH group is present in the active site; the observation was confirmed with the 39 kDa domain. (Supported by N.I.H. grant DK-19682 and Project ANI-92-10 of the Maryland Agricultural Experiment Station.)

S2.24

Cloning Strategies for the Endoplasmic Reticulum Glycoprotein Glucosidase II

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Glucosidase II is a resident glycoprotein of the endoplasmic reticulum in hepatocytes. It catalyzes the removal of the two inner α1,3-linked glucose residues of the oligosaccharide precursor chains of *N*-glycosylated glycoproteins.

The aim of this study was to clone the cDNA encoding pig glucosidase II in order to elucidate its protein structure. Two different approaches were established. (i) a λgt11 cDNA library was screened with antibodies raised against the SDS-denatured glucosidase II. Five positive clones were purified to homogeneity and their fusion proteins further analyzed. One of them showed immunoreactivity with antibodies recognizing the native enzyme. The fusion protein of another clone and the purified glucosidase II were digested with different