fold increase in both specific activities. SDS-PAGE of active protein fractions from the purification procedure showed a prominent band corresponding to an apparent molecular weight of 70-80 000. A partially purified protein fraction was subjected to SDS-PAGE (under mild conditions), and the gel was cut to 3 mm pieces which were extracted with buffered Triton X-100/sucrose for 48 h at 4°C. GlcA- and GlcNAc-transferase activities were both detected in extracts corresponding to the 70-80 000 band.

S1.8

The Use of Nucleoside Diphosphate Sugar Photoaffinity Probes to Isolate Glycosyltransferases

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Glycosylation is the most frequent post-translational modification that occurs on proteins and therefore the glycosyltransferases that mediate these reactions are important to isolate and characterize in order to understand how these events are regulated. However, many of these enzymes, especially those involved in the lipid-linked saccharide pathway of *N*-linked glycosylation, are low-abundance proteins and are quite unstable during purification. Thus, purifying these enzymes to homogeneity to prepare molecular probes is a difficult and major undertaking. One of the approaches that we have taken to aid in the purification is to prepare azido-radiolabeled nucleoside diphosphate sugars that react with the appropriate glycosyltransferase and allow us to identify subunits of the enzymes on SDS gels. Thus, we purified the GDP-mannose pyrophosphorylase from pig liver to homogeneity, and have shown that this enzyme will produce azido-GDP[^{32}P]mannose from azido-GTP [^{32}P] and mannose-1-P. This enzyme is interesting in its own right since it has a broad specificity and will also produce GDP-glucose from GTP and glucose-1-P. The subunits of this enzyme have been isolated and partially sequenced. We also purified the pig liver UDP-GlcNAc pyrophosphorylase to homogeneity and found that this enzyme would convert azido-UTP [^{32}P] and [^{14}C]GlcNAc-1-P to azido-UDP [^{32}P or ^{14}C]GlcNAc. This enzyme also shows a broad specificity and will form UDP-GalNAc from UTP and GalNAc-1-P, and UDP-glucose from UTP and glucose-1-P. We also have purified the UDP-glucuronic acid carboxylyase from wheat germ to homogeneity and can use this enzyme to convert azido-UDP [^{32}P]-glucuronic acid to azido-UDP[^{32}P]-xylose. These probes have been used with crude extracts of plant and animal cells and found to react with several proteins in each case. We anticipate that they will be useful to obtain homogeneous preparations of some of these enzymes.
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S1.9

Hydrolysis of the *N*-glycosidic Linkage by Glycosylasparaginase and Inhibition of the Enzyme by Diazo-Oxonorvaline

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Glycosylasparaginase (N^4-(β-*N*-acetyl-D-glucosaminyl)-L-asparaginase, EC 3.5.1.26), a lysosomal enzyme that cleaves the *N*-glycosidic linkage, reacts as an exo-hydrolase toward the L-asparagine moiety with free α-amino and α-carboxyl groups of its substrates. The substrates include both high mannose and complex type glycoasparagines (1) in contrast to endo-β-*N*-acetylglucosaminidase (2), which prefers the di-*N*-acetylchitobiose moiety in high mannose type structures. This suggests that in the genetic deficiency of glycosylasparaginase activity (aspartylglycosaminuria) the major accumulating compound N^4-(β-*N*-acetyl-D-glycosaminyl)-L-asparagine mostly originates from high mannose type glycoasparagines and a series of glycoasparagines with structurally different *N*-glycans are likely to be derived from complex type carbohydrate structures. Glycosylasparaginase catalyzes three symmetrical reactions and the mechanism of action involves a β-aspartyl enzyme intermediate (1). The enzyme is irreversibly inhibited by the L-asparagine analogue diazo-oxonorvaline (DONV). N^4-(β-*N*-acetyl-D-glucosaminyl)-L-asparagine competitively protects the enzyme against inactivation with DONV suggesting that the compound is an active site-directed inhibitor. DONV reacts with the hydroxyl group of the *N*-terminal threonine residue of the light subunit of glycosylasparaginase (3) in a protein sequence that is highly conserved among mammalian glycosylasparaginases suggesting its role in the reaction mechanism of the enzyme.

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S1.10

Further Studies on the Primer Formation of Glycogen Biosynthesis

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Analysis of the synthesis of the glucans covalently linked to protein by a rat brain fraction at pHs: 7.2, 7.9 and 9.0 showed they differ in the glucosyl length. Also the pattern of the proteoglucan obtained under denaturing P.A.G.E. was different.

With a rat heart fraction after Mn^{++} addition only one radioglucosylated protein band of Mr: 42 KDa was evident. One or two glucoses were found linked to protein.

In the absence of Mn^{++}, two bands were obtained (60,7 and 64,4 KDa) indicating elongation of the glucan chains and that this step was inhibited by Mn^{++}. After Glc6P addition a family of glucosylated proteins with higher Mr was obtained. Mn^{++} inhibition in the second step is reversed by PMSF + Glc6P, and a family of radioglucosylated protein bands with Mr far in excess of 42 KDa was obtained.

Therefore, we have identified intermediate steps for the synthesis of proteoglucans from UDP-Glc either from brain or from rat heart fractions. Selected incubation conditions showed that between the first glucose transferred to protein and that of the appropriate substrate for glycogen synthase, it