ISOLATION AND CHARACTERIZATION OF GLYCOSYL TRANSFERASES. R. L. Hill, J. C. Paulson, J. E. Sadler, J. I. Rearick, T. A. Beyer and J.-P. Prieels (Durham, North Carolina).

Nucleoside diphosphohexanolamine agarose derivatives (1) have been particularly useful as specific adsorbents for purifying a variety of glycosyl transferases, most of which are normally present in only small amounts in tissues. At present, a UDP adsorbent has been used to prepare galactosyl (1), N-acetylgalactosaminyl (2) and glucuronyl transferases (3); a CDP adsorbent (4) to prepare three sialyl transferases with different acceptor specificities and a GDP adsorbent to purify fucosyl transferases from porcine submaxillary gland and human milk. The specificity of the adsorbents is provided by the pyrimidine or purine moiety of the nucleoside and reflects the structure of the donor substrate for a given transferase. For example, despite the close structural similarities between CDP and UDP-adsorbents, sialyl transferases adsorb only to CDP-agarose and galactosyl transferases only to UDP-agarose. Fucosyl transferases adsorb strongly to GDP-agarose but only weakly to UDP-agarose. Moreover, the fucosyl and sialyl transferases of submaxillary glands are easily separated on GDP-agarose.

The availability of pure or highly purified transferases permits more thorough characterization of their enzymic properties, including substrate specificities and possible modes of regulation. For example, the three sialyl transferases that have been highly purified show strikingly different acceptor substrate specificities. A homogeneous sialyl transferase from bovine colostrum incorporates sialic into the sequence, NeuAc $\alpha 2\rightarrow 6$ Gal $\beta 1\rightarrow 4$ GlcNAc, which is commonly found in glycoproteins (5). In contrast, a sialyl transferase preparation from procine submaxillary glands incorporates sialic acid into the sequences NeuAc $\alpha 2\rightarrow 6$ GalNAc-O-Ser/Thr and NeuAc $\alpha 2\rightarrow 3$ Gal $\beta 1\rightarrow 3$ GalNAc-O-Thr, respectively. The submaxillary activities have thus far resisted separation even after 10,000 fold purification on CDP-adsorbents, and by other methods as well. The structure function relationships between the two enzymes remain unclear.

The H blood group fucosyl transferase from porcine submaxillary glands has been purified 180,000 fold and is specific for transfer of fucose into $\alpha 1\rightarrow 2$ linkage with β-D-galactosides, including those found in milk oligosaccharides, defucosylated porcine mucin and asialo α_1-acid glycoprotein. In conjunction with the A^+ blood group N-acetylgalactosaminyl transferase purified to homogeneity from the same source, the fucosyl transferase has been used to demonstrate the absence of fucose in $\alpha 1\rightarrow 2$ linkage in α_1-acid glycoprotein.

When used in conjunction with pure glycosidases the glycosyl transferases are useful probes of the structure and function of specific oligosaccharides in glycoproteins. For example, the rabbit liver lectin that binds asialoglycoproteins is inactivated on removal of its sialic acid. It can be reactivated, however, by resialylation with CMP-NeuAc and the colostral sialyl transferase. This result and other observations, indicate that inactivation of the lectin on removal of sialic acid results from the binding of the lectin to its own exposed galactosyl residues. In addition, over 85% of the sialic acid could be reincorporated into the asialolectin by the transferase, suggesting that the oligosaccharides of the resialylated lectin contain the sequence NeuAc $\alpha 2\rightarrow 6$ Gal $\beta 1\rightarrow 4$ GlcNAc (5).

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