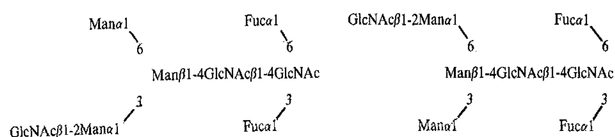

(II) The presence of GlcNAc transferase I (E.C. 2.4. 1. 101) activity in cultured Bm-N, Mb 0503, Sf-21 and Sf-9 cells was established using both Manα1-3(Manα1-6)Manβ1-octyl and Manα1-3(Manα1-6)Manβ1-4GlcNAc-pyridylamine as acceptor substrates.

GlcNAc was transferred in β1-2-linkage to the α1-3 arm of the pyridylaminated acceptor as shown by reversed-phase HPLC, exoglycosidase digestions and methylation analysis. The insect cell lines exhibited 5 to 20% of the specific GlcNAc transferase I activity found in the human hepatoma cell line HepG2.

S1.17

Inhibition of *O*-Glycan Core 2 UDP-GlcNAc: Galβ1-3GalNAc- β6-GlcNAc-Transferase in Acute Myeloid Leukemia Cells

M. A. Granovsky[1,2], W. J. Kuhns[1], F. Reck[1], M. A. Baker[3] and I. Brockhausen[1,2]

[1] *Department of Biochemistry, Hospital for Sick Children, Toronto, Canada;* [2] *Department of Biochemistry, University of Toronto, Toronto, Canada;* [3] *Department of Oncology and Haematology, Toronto General Hospital and Department of Medicine, University of Toronto, Toronto, Canada.*

The enzyme that introduces a branch into O-glycans, core 2 UDP-GlcNAc: Galβ1-3GalNAc- β6-GicNAc-transferase (core 2 β6-GlcNAc-T), is increased in leukocytes from patients with acute myelogenous leukaemia (AML). It has also been found to increase upon differentiation and activation of cells and in metastasis. The enzyme in mucin secreting tissues (M) appears to be different from the enzyme in leukocytes (L). Inhibitors for glycosyltransferases may be useful for functional studies of glycosyltransferases, for the development of therapy directed against pathological glycosylation, and for studies of the binding sites of these enzymes. A previously described weak competitive inhibitor for the core 2 β6-GlcNAc-T lacked the active 6-OH of GalNAc of the Galβ1-3GalNAc- substrate. We now report a specific and potent inhibitor for the core 2 β6-GlcNAc-T. Galβ1-3GalNAcα-p-nitrophenyl is a substrate for the enzyme, which upon uv irradiation is activated and acts as an irreversible inhibitor. With several other transferases only a low level of non-specific inactivation was observed. The uv-irradiated compound in the presence of uv-stable substrate inhibits specifically the L enzyme from AML cells, but not the M enzyme. The inhibitor can be labeled with radioactive Gal and may be useful as an affinity reagent for active site labeling of the L enzyme from leukocytes.

This work was supported by the MRC Canada.

S1.18

Purification, Properties and Specific Inactivation of Asparagine-*N*-Glycosyltransferase from Pig Liver

W. Breuer, M. Wesemann and E. Bause

Institut für Physiologische Chemie, Universität Bonn, Germany

Asparagine-*N*-glycosyltransferase catalyses the Dol-PP-mediated transfer of oligosaccharides from the glycolipid to asparagine residues in the triplet sequence Asn-Xaa-Thr/Ser/Cys (Bause, E. & Legler, G. (1981) Biochem. J. **195**, 639-644). We have purified the enzyme about 1000-fold from crude pig liver microsomes using lectin- and ion exchange chromatography. Analysis by SDS-PAGE of the purified enzyme revealed three prominent protein bands at 38, 48 and 66 kDa. Immunological evidence indicated that the 66 kDa protein, also identified in a purified transferase preparation from dog pancreas (Kelleher, D. J. *et al.* (1992) Cell 69, 55-65) is Ribophorin I suggesting that it may be part of the membrane-associated transferase complex. Purified *N*-glycosyltransferase has a pH optimum close to 7.0 and requires Mn^{2+} ions. The pH optimum is shifted to 8.2 when instead of *N*-Benzoyl-Asn-Gly-Thr-NH_2 (I) the non-physiological peptide *N*-Benzoyl-Asn-Gly-α, β-diamino butyric acid amide (II) is used as substrate, indicating that the non-protonated β-amino group in II can mimic the function of the hydroxy group during catalysis. Substitution of Asn by α,γ-diamino butyric acid yields a derivative which cannot be glycosylated, but inhibits competitively (Ki: 1 mM) the glycosylation of I. An active-site directed irreversible inactivation of the transferase is observed when the enzyme is incubated with a DNP-peptide derivative in which the threonine residue in the marker sequence is replaced by epoxyethylglycine. Several lines of evidence from double-labelling experiments suggest that this inactivation occurs, as previously proposed (Bause, E. (1983) Biochem. J. 209, 323-330), by a 'suicide mechanism', supporting our current view of the catalytic mechanism of Asn-*N*-glycosyltransferases.

S1.19

Linear Oligo-*N*-Acetyllactosaminoglycans with Two or Three Internally Located Galactoses are Efficiently Branched by Human Serum β1,6-GlcNAc Transferase

A. Leppänen, L. Penttilä, K. Juntunen and O. Renkonen

Laboratory of Carbohydrate Research, Institute of Biotechnology and Department of Biochemistry, University of Helsinki, Helsinki, Finland.

Previously we have shown that human serum contains a β1,6-GlcNAc transferase activity that forms middle-chain branches in linear oligo-*N*-acetyllactosaminoglycans (Leppänen *et al.*, (1991) *Biochemistry* **30**, 9287-9296). Further studies with longer oligo-*N*-acetyllactosaminoglycans as acceptors show that the preferred position of GlcNAcβ1-6-branch is dependent on the reducing end group and the non-reducing end group of the acceptor. Incubation of UDP-GlcNAc and radiolabeled heptasaccharides Galα1-3′LactNAcβ1-3′LactNAcβ1-3′LactNAc and GlcNAcβ1-3′LactNAcβ1-3′LactNAcβ1-3′LactNAc with human serum resulted in the preferred formation of branched octasaccharides Galα1-3′LactNAcβ1-3′LactNAcβ1-3(GlcNAcβ1-6)′LactNAc and GlcNAcβ1-3′LactNAcβ1-3(GlcNAcβ1-6′LactNAcβ1-3′LactNAc, respectively. The incubation of UDP-GlcNAc and radiolabeled hexasaccharides LactNAcβ1-3′LactNAcβ1-3′LactNAc and GlcNAcβ1-3′LactNAcβ1-3′LactNAcβ1-3Gal with serum resulted in the formation of LactNAcβ1-3(GlcNAcβ1-6)′LactNAcβ1-3′LactNAc and LactNAcβ1-3′LactNAcβ1-3(GlcNAcβ1-6)′LactNAc (equal amounts), and GlcNAcβ1-3′LactNAcβ1-3(GlcNAcβ1-6)′LactNAcβ1-3Gal,