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Purification, properties and possible gene assignment of an α1,3-fucosyltransferase expressed in human liver

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α1,3-Fucosyltransferase solubilized from human liver has been purified 40 000-fold to apparent homogeneity by a multistage process involving cation exchange chromatography on CM-Sephadex, hydrophobic interaction chromatography on Phenyl Sepharose, affinity chromatography on GDP-hexanolamine Sepharose and HPLC gel exclusion chromatography. The final step gave a major protein peak that co-chromatographed with α1,3-fucosyltransferase activity and had a specific activity of ~ 5–6 μmol min^{-1} mg^{-1} and an M_r ~ 44 000 deduced from SDS-PAGE and HPLC analysis. The purified enzyme readily utilized Galβ1-4GlcNAc, NeuAcα2-3Galβ1-4GlcNAc and Fucα1-2Galβ1-4GlcNAc, with a preference for sialylated and fucosylated Type 2 acceptors. Fucα1-2Galβ1-4Glc and the Type 1 compound Galβ1-3GlcNAc were very poor acceptors and no incorporation was observed with NeuAcα2-6Galβ1-4GlcNAc. A polyclonal antibody raised against the liver preparation reacted with the homologous enzyme and also with the blood group Lewis gene-associated α1,3/1,4-fucosyltransferase purified from the human A431 epidermoid carcinoma cell line. No cross reactivity was found with α1,3-fucosyltransferase(s) isolated from myeloid cells. Examination by Northern blot analysis of mRNA from normal liver and from the HepG2 cell line, together with a comparison of the specificity pattern of the purified enzyme with that reported for the enzyme expressed in mammalian cells transfected with the *Fuc-TVI* cDNA, suggests a provisional identification of *Fuc-TVI* as the major α1,3-fucosyltransferase gene expressed in human liver.

Keywords: liver α1,3-fucosyltransferase, α1,3-fucosyltransferase genes, α1,3-fucosyltransferase antibodies, α1,3-fucosyltransferase mRNA

Introduction

The fucose-containing antigenic determinants Le^x (Galβ1-4[Fucα1-3]GlcNAc) and sialyl-Le^x (NeuAcα2-3Galβ1-4[Fucα1-3]GlcNAc) occur as cell surface structures on a wide variety of tissues and have been implicated as ligands for certain adhesion proteins involved in important physiological processes such as inflammation [1, 2] and metastasis [3, 4]. Le^x is also believed to participate in cell-cell recognition phenomena via homotypic carbohydrate-carbohydrate interactions [5, 6]. The final stage in the biosynthesis of both Le^x and sialyl-Le^x is the addition of fucose to the *O*-3 position of *N*-acetylglucosamine in, respectively, an unsialylated (Galβ1-4GlcNAc) or a sialylated (NeuAcα2-3Galβ1-4GlcNAc), Type 2 sequence (reviewed in [7, 8]). The fucosyltransferases responsible for the transfer of fucose to the *O*-3 position of *N*-acetylglucosamine are now known to comprise a multigene family of enzymes with closely related, but not identical, properties. Five α1,3-fucosyltransferase genes have been cloned, *Fuc-TIII* [9], *Fuc-TIV* [10–12], *Fuc-TV* [13], *Fuc-TVI* [14, 15] and *Fuc-TVII* [16, 17] and transient expression of these genes in mammalian cells has yielded enzymes differing in fine specificity, with one of the most prominent differences residing in their ability to utilize sialylated acceptors to make sialyl-Le^x [10–17]. The tissue expression of the various α1,3-fucosyltransferase genes, and the correlation of the kinetic and specificity properties of the

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encoded enzymes with those purified from natural sources, have, however, still to be clearly established.

This paper describes the isolation and properties of an α1,3-fucosyltransferase purified to apparent homogeneity from human liver and a provisional identification of the gene encoding the enzyme as *Fuc-TVI*. A brief account of the purification and specificity of the enzyme appeared earlier [18].

Materials and methods

SUBSTRATES

GDP-L-[^{14}C]fucose (200 mCi mmol^{-1}) was obtained from Amersham International, UK and unlabelled GDP-L-fucose was synthesized by the method of Nunez *et al.* [19]. 2'-Fucosyllactose (Fucα1-2Galβ1-4Glc), lacto-*N*-tetraose (Galβ1-3GlcNAcβ1-3Galβ1-4Glc), lacto-*N*-*neo*tetraose (Galβ1-4GlcNAcβ1-3Galβ1-4Glc), lacto-*N*-fucopentaose I (Fucα1-2Galβ1-3GlcNAcβ1-3Galβ1-4Glc) and lacto-*N*-fucopentaose II (Galβ1-3[Fucα1-4]GlcNAcβ1-3Galβ1-4Glc) were isolated from human milk as described [20]. 3-Sialyl-*N*-acetyllactosamine (NeuAcα2-3Galβ1-4GlcNAc) and 6-sialyl-*N*-acetyllactosamine (NeuAcα2-6Galβ1-4GlcNAc) were recovered from human urine by the method of Pärkkïnen and Finne [21], and supplied by Dr C.P.C. Soh, (formerly of the MRC Clinical Research Centre, Harrow, UK). *N*-acetyllactosamine (Galβ1-4GlcNAc) was synthesized by the method of Alais and Veyrières [22] and lacto-*N*-biose I (Galβ1-3GlcNAc) by the method of Flowers [23].

Phenyl β-D-galactoside, phenyl β-*N*-acetyl-D-glucosaminide and the glycoproteins fetuin and transferrin were purchased from the Sigma Chemical Co. Ltd UK. A purified blood group-precursor glycoprotein (No. 484) was isolated from an ovarian cyst of a blood group Le(a-b-), ABH non-secretor, patient by the method of Morgan [24]. Tamm-Horsfall glycoprotein purified as described [25] from the urine of an Sd^a positive donor was the gift of Dr C.P.C. Soh. Asialo-glycoproteins were prepared by treatment of a 1% solution of the glycoproteins with 0.05 M H_2SO_4 for 1 h at 80 °C. The products were thoroughly dialysed against distilled water and dried from the frozen state. Asialo-agalacto-fetuin was prepared by the digestion of asialo-fetuin with Jack bean β-galactosidase.

The glycolipids, lactosylceramide (Galβ1-4Glc-Cer), trihexaosylceramide (Galα1-4Galβ1-4Glc-Cer), globoside (GalNAcβ1-3Galα1-4Galβ1-4Glc-Cer) and lacto-*N*-*neo*tetraosyl ceramide (Galβ1-4GlcNAcβ1-3Galβ1-4Glc-Cer) were gifts from Dr A. Gardas, Medical Centre of Postgraduate Education, Warsaw, Poland.

GLYCOSIDASES

Neuraminidase from *Vibrio Cholerae* was purchased from Koch-Light, UK. α1,2- and α1,3/1,4-fucosidases from *Trichomonas foetus* were separated as described [26] and checked for specificity with lacto-*N*-fucopentaose I and lacto-*N*-fucopentaose II. Jack bean β-galactosidase was purchased from Sigma Chemical Co. Ltd, UK.

COLUMN CHROMATOGRAPHY MEDIA

The column chromatography media, Phenyl Sepharose CL-4B, CNBr-activated Sepharose 4B, Sephadex G-50 and CM-Sephadex C-50 were obtained from Pharmacia Fine Chemicals Ltd, UK. GDP-hexanolamine, synthesized by the method of Beyer *et al.* [27], was a gift from Dr A. Betteridge (formerly of the MRC Clinical Research Centre, Harrow, UK) and was coupled to CNBr-activated Sepharose 4B by the procedure of Cuatrecasas [28]. The final product contained 3 μmol of GDP-hexanolamine per ml of packed gel. HPLC on a TSK G3000SW gel exclusion column was carried out on a Gilson apparatus. Dowex 1 × 8-200 was purchased from Sigma Chemical Co. Ltd, UK.

ANTIBODIES

A polyclonal antibody was raised in a rabbit against a preparation of the liver α1,3-fucosyltransferase purified (30 000-fold) up to Step 4. The eluate from the GDP-hexanolamine Sepharose 4B column was concentrated on an Amicon Model 8MC ultrafiltration unit with a PM 10 membrane and the solution was exhaustively washed through with 0.15 M NaCl to remove Triton X-100 and the sodium cacodylate buffer. Rabbits were injected intradermally at several sites along the back with enzyme preparation (~ 2 μg protein in 0.6 ml 0.15 M NaCl) emulsified with an equal volume of complete Freund's adjuvant (Difco, Detroit, USA). Three booster doses (~ 2 μg) of purified enzyme emulsified with incomplete Freund's adjuvant were given at monthly intervals. Bleedings were taken at weekly intervals and the separated serum tested for anti-α1,3-fucosyltransferase activity by mixing 20 μl of the serially diluted rabbit serum with 20 μl of the purified enzyme preparation. The mixtures were left at 4 °C for 16 h and then 10 μl of donkey anti-rabbit serum (Wellcome Diagnostics, Dartford, UK) was added and the mixtures were left at 4 °C for a further 5 h. The immunoprecipitate was spun down at 8000 × **g** for 30 min on a Sorvall RC-5B centrifuge at 4 °C. The supernatant was removed and precipitate was washed twice with 100 μl of 0.15 M NaCl at 4 °C. The supernatant and precipitate were then assayed for glycosyltransferase activity. The α1,3-fucosyltransferase activity in the immunoprecipitate resulted from antibody that had combined specifically with the enzyme protein but had not neutralized the catalytic activity. The neutralizing activity of the antiserum was calculated from the difference between the combined activity recovered in the immunoprecipitate and the supernatant and the activity present in the supernatant from reaction mixtures treated in the

same way except that the first antibody was replaced by pre-immune serum from the same rabbit.

A second polyclonal antibody, raised against a 200 000-fold purified α1,3/1,4-fucosyltransferase preparation from human milk [29] was also examined in parallel with the antibody raised against the liver enzyme. A rabbit was injected intradermally with ~ 100 μg protein in 0.6 ml 0.9% NaCl emulsified with an equal volume of complete Freund's adjuvant. A booster dose (~ 100 μg) in incomplete Freund's adjuvant was given after 4 weeks. The rabbit was bled at 2 weekly intervals and the separated serum tested as above for anti-α1,3/1,4-fucosyltransferase activity. The milk preparation used to immunize this rabbit was subsequently found to be a mixture of α1,3/1,4-fucosyltransferase that primarily utilized *N*-acetylglucosamine in Type 1 (Galβ1-3GlcNAc) structures and terminal reducing glucose residues in lactose-based oligosaccharides [30–32] and an α1,3-fucosyltransferase that utilized only Type 2 (Galβ1-4GlcNAc) acceptors. This antibody preparation reacted with the α1,3/1,4-fucosyltransferases purified from human milk [31, 32], and from the human epidermoid carcinoma cell line A431 [33] when tested with Type 1 substrates, as well as the Lewis-gene independent α1,3-fucosyltransferase purified from human milk [34] and the α1,3-fucosyltransferase purified from plasma [29] when tested with Type 2 substrates [35, 8].

PROTEIN ASSAYS

Protein concentrations were measured by the method of Read and Northcote [37] with bovine serum albumin as standard except for the fractions eluted from the HPLC column; the protein concentration in this eluate was calculated by the integration of the peaks recorded at 215 nm.

POLYACRYLAMIDE GEL ELECTROPHORESIS

Sodium dodecylsulphate-polyacrylamide-gel electrophoresis (SDS-PAGE) was performed by the method of Laemmli [38] with 10% acrylamide gels in Tris-HCl buffer pH 8.8 overlaid with a 3.5% stacking gel in Tris-HCl buffer pH 6.8. Gels were stained for protein by the silver staining method of Dubray and Bezard [39].

DETERMINATION OF MOLECULAR SIZE

The M_r of the purified α1,3-fucosyltransferase was estimated by comparison of the mobility of the enzyme on SDS-PAGE and HPLC gel exclusion chromatography with that of a range of standard proteins (Sigma Chemical Co. Ltd, UK; transferrin, M_r 76 000; bovine serum albumin, M_r, 66 000; ovalbumin, M_r, 45 000; carbonic anhydrase, M_r, 29 000; α-lactalbumin, M_r, 14 500; cytochrome C, M_r, 12 400).

HEPG2 CELLS

The hepatoma cell line, HepG2, was provided by Dr Naveenan Navaratnam, Hammersmith Hospital, London and grown in RPMI 1640 medium (Gibco Ltd, UK) supplemented with 10% heat inactivated (56 °C) foetal calf serum, 2 mM glutamine, 100 U ml^{-1} penicillin and 100 μg ml^{-1} streptomycin, in a 5% CO_2 atmosphere. The cells were grown to confluence, the medium was removed and the adherent cells were washed with PBS and detached from the flask by treatment for 5 min with 0.5% trypsin containing 0.2% EDTA. The detached cells were suspended in a small volume of RPMI, spun at 800 × **g**, washed with PBS, resuspended in PBS and counted in a haemocytometer. Immediately before fucosyltransferase assays were carried out, the cells were lysed by treatment of 8×10^7 cells in 1.5 ml PBS with 0.5 ml 1% Triton X-100 for 60 min at 4 °C. The resultant extract was spun briefly and the supernatant (20 μl) was used as the enzyme source.

FUCOSYLTRANSFERASE ASSAYS

Low molecular weight acceptors

Standard reaction mixtures for α1,2-, α1,3- and α1,4-fucosyltransferase assays contained in a total volume of 70 μl; 0.5 μmol acceptor substrate, 0.2 nmol (70,000 counts min^{-1}) GDP-[^{14}C]fucose, 1.0 μmol $MnCl_2$, 0.5 μmol neutral ATP, 5.0 μg sodium azide, 5.0 μmol sodium cacodylate/HCL buffer, pH 7.3; 5 μl 1% Triton X-100 and 20 μl enzyme preparation. Phenyl β-D-galactoside was used as the substrate for α1,2-fucosyltransferase and lacto-*N*-biose I the substrate for α1,4-fucosyltransferase activity. In routine assays *N*-acetyllactosamine was the substrate for α1,3-fucosyltransferase activity and when the purified enzyme was assayed, ATP was omitted from the reaction mixture and 250 μg bovine serum albumin was added. The reaction mixtures were incubated at 37 °C for between 30 min and 16 h depending on the activity of the preparations, and were worked up by one of two methods.

Method 1. The products were separated by descending paper chromatography in propan-1:ol-ethyl acetate: pyridine:water (5:1:1:3 by vol) (Solvent 1) for 4 h. The chromatography papers were scanned in a Packard Radiochromatogram Scanner and the radioactive areas were cut out and counted in a Nuclear Chicago Scintillation Counter. The mobilities of the products were measured relative to lactose (R_{lac}). When lacto-*N*-*neo*tetraose was used as acceptor substrate, the product obtained after the first chromatographic separation was eluted and re-run in ethyl-acetate:pyridine:water (12:5:4 by vol) (solvent 2) for 3 days to enable separation to occur if products had been formed with fucose added to either the subterminal *N*-acetylglucosamine or to the terminal reducing glucose residues [32]; the mobilities of the products were measured relative to lacto-*N*-tetraose

(R_{LNT}). The reaction mixtures containing the sialylated oligosaccharides were first subjected to paper electrophoresis on Whatman 3MM paper in 40 mM pyridine-acetate buffer pH 5.4 on a Southern-Shandon electrophoresis apparatus at 4 kV and 80 mA for 2 h. The mobilities of the labelled products were measured relative to a picrate marker ($R_{picrate}$). After scanning the papers, the radioactive product areas were cut out, eluted with water and the concentrated eluate re-run on Whatman No. 40 paper in ethyl acetate:pyridine:water (2:1:2) (Solvent 3) with standard markers.

Method 2. The reaction products were separated on Dowex-1 columns (0.8 ml; formate form). The neutral oligosaccharides were eluted with 1.6 ml of water and the sialyl-products with 40 mM pyridine acetate. The eluants were mixed with 4 ml of water-miscible scintillant (Pico-Fluor 40, Canberra Packard, Pangbourne, UK) and counted in a Beckman LS 6800 scintillation counter. Control mixtures not containing acceptor substrates were included in all these experiments and any eluted counts representing breakdown of GDP-[^{14}C]fucose were subtracted from the product counts.

Glycoprotein acceptors

The reaction mixtures were the same as for the oligosaccharide acceptors except that 100 μg of glycoprotein was used as acceptor substrate. The reaction products were worked up by two methods.

Method 1. The radioactive products were separated by chromatography in propan-1:ol-ethyl acetate:pyridine: water (5:1:1:3 by vol) (Solvent 1) on Whatman No. 40 paper for 20 h. The papers were scanned and counted as above. Radioactive glycoprotein products remained at the origin and were separated from unused GDP-fucose and breakdown products which moved away from the origin.

Method 2. The radioactive products were separated on columns (0.7 × 16 cm) of Sephadex G-50. The columns were eluted with 0.2 M NaCl. The first 2 ml of eluate were discarded and the next 2 ml, which contained the product, were mixed with 5 ml of water-miscible scintillant and counted as above. In both procedures, reaction mixtures without added glycoprotein were included to correct for incorporation of [^{14}C]fucose into endogenous high molecular weight acceptors.

Glycolipid acceptors

The reaction mixtures were the same as for the oligosaccharide acceptors except that 0.5 μmol glycolipid was used as the acceptor and 5 μl of 1% Triton X-100 was included in the reaction mixture. At the end of the incubations, the glycolipids were extracted from the reaction mixture with 0.6 ml of chloroform:methanol (2:1 by vol) as described by Pacuszka and Koscielak [40], concentrated and subjected to chromatography for 16 h on Whatman 3MM paper in 1% sodium tetraborate pH 9.1. Radioactive areas were detected and counted as above. Control incubations lacked the glycolipid acceptors.

PRODUCT CHARACTERIZATION

Oligosaccharide products were characterized by comparison of their paper chromatographic mobilities with those of standards previously subjected to methylation and H^1NMR analysis [32] and by their susceptibility to degradation by α1,2- and α1,3/1,4-fucosidases isolated from *Trichomonas foetus* [26]. The sialylated products were first digested with neuraminidase to remove sialic acid before treatment with the α-fucosidases [32].

TREATMENT OF ENZYME WITH SULPHYDRYL REAGENTS

Aliquots of the purified liver α1,3-fucosyltransferase (10 μl diluted with 186 μl 50 mM sodium cacodylate buffer, pH 7.3) were mixed at 4 °C with 10 μl of *N*-ethylmaleimide (NEM) or p-hydroxymercuribenzoate (PMB) solutions, to give final concentrations of each of the inhibitors of 0.1 mM and 1.0 mM. The mixtures were kept at 4 °C and samples were removed and assayed for α1,3-fucosyltransferase activity at 0, 10, 20, 30 and 40 min.

NORTHERN BLOT ANALYSIS

A multiple tissue Northern (MTN) blot with mRNA from human tissues was purchased from Clontech Laboratories Inc., USA. The blot contained 2 μg of mRNA per lane. The liver sample from which the mRNA was isolated, contained all representative cell types found in a liver lobe, but was free from gall bladder tissue (information supplied by the manufacturers). Total RNA was isolated from HepG2 cells by the guanidinium-CsCl method [41]. Poly(A)$^+$RNA was isolated from the total RNA by oligo(dT)-cellulose column chromatography [42] and 5 μg of the poly(A)$^+$RNA, denatured in the presence of formaldehyde, were electrophoresed on a 1.25% agarose/formaldehyde gel for 3–4 h at 50 V. The RNA was transferred to a nylon membrane (Hybond N, Amersham, UK) by capillary blotting [43] and the membrane was baked for 2 h at 80 °C and prehybridized for 3 to 6 h at 42 °C in a freshly prepared hybridization solution: 50% deionised formamide (Gibco Ltd, UK), 1.0 M NaCl, 1% (w/v) SDS, 10% (w/v) dextran sulphate and 200 μg ml^{-1} denatured salmon sperm DNA (Sigma Chemical Ltd, UK).

Four α1,3-fucosyltransferase probes were prepared from plasmids kindly provided by Dr J.B. Lowe, (Howard Hughes Medical Centre, Ann Arbor, USA). The probes were: the 1.05 kb *Pvu* II fragment from the insert in pcDNA1-α-(1,3)-FT_{Mlu} for the detection of *Fuc-TIV* [11], the 1.9 kb *Xba* 1-*Eco* RI fragment from the insert in pcDNA1-Fuc-TV for the detection of

Fuc-TV [13], the 1.2 kb *Hind* III fragment from the insert in pcDNA1-Fuc-TVI for the detection of *Fuc-TVI* [14] and the 1.32 kb *Xho* I-*Xba* I fragment from the insert in pcDNA1-Fuc-TVII for the detection of *Fuc-TVII* [17]. A β-actin cDNA probe (Clontech Laboratories Inc., USA) was used as a control for degradation and even loading of the mRNA. [^{32}P]-labelled cDNA probes were prepared by the random oligonucleotide priming method according to the manufacturers instructions (Megaprime, Amersham, UK) to a specific activity equal to, or greater than, 1×10^8 cpm per μg DNA. The [^{32}P]-labelled cDNA probes (50 ng) were added to 5 ml of the hybridization solution and the membranes were then heated for 16 h at 42 °C. After hybridization the blots were washed once in 0.45 M NaCl, 0.045 M Na citrate, 1.0% SDS for 15 min at room temperature, twice with 0.3 M NaCl, 0.03 M Na citrate, 1.0% SDS for 30 min at 42 °C and once with 0.03 M NaCl, 0.003 M Na citrate, 1.0% SDS for 30 min at 42 °C. The membranes were air dried and then exposed to Kodak X-Omat film with an enhancing screen at −70 °C.

Results

PURIFICATION OF α1,3-FUCOSYLTRANSFERASE

All procedures were carried out at 4 °C unless otherwise stated. After Step 3 of the purification procedure, only siliconized glassware or plastic ware were used in the preparation. Connective tissue and fat were removed from post-mortem specimens of human liver and the gall bladder was carefully excised as this forms a source of contaminating *Le*-gene associated α1,3/1,4-fucosyltransferase [44]. The liver was cut into small cubes (~ 1.0 cm^3) which were allowed to drain of blood and then rinsed with one volume of cold distilled water. It is important to remove as much blood as possible at this stage to avoid contaminating fucosyltransferases [45] and haemoglobin which binds to the cation exchange gel and reduces the capacity of the adsorbant to bind α1,3-fucosyltransferase.

Step 1. Solubilization of the membrane bound α1,3-fucosyltransferase

The chopped, washed liver pieces (1200 g) were homogenized in batches of 100 g in 400 ml of cold distilled water in a Waring blender (two 30 s bursts at a low setting) in order to remove soluble proteins and lyse any remaining erythrocytes. The homogenate was spun at 7500 × **g** for 40 min in a Sorvall 4B centrifuge. The supernatant was removed and discarded and the insoluble pellet was subsequently suspended in two volumes of 25 mM sodium cacodylate, HCl buffer pH 6.3 and homogenized as above. The homogenate was made 20 mM with $MnCl_2$, stirred for 20 min and the insoluble material was separated by centrifugation at 7500 × **g** for 40 min. The pellet was re-extracted with two volumes of the same buffer, centrifuged at 7500 × **g** and the supernatant discarded. This extraction procedure resulted in the removal of a large proportion of the haemoglobin and other soluble proteins. Some α1,3-fucosyltransferase activity was released but subsequent steps revealed that more than 80% of total activity remained in the insoluble fraction. This fraction was resuspended in two volumes of 25 mM sodium cacodylate-HCl buffer, pH 6.3, and subjected to three 15 s bursts on the high setting of the Waring Blender with cooling in between. Triton X-100 (final concentration 1%) and $MnCl_2$ (final concentration 20 mM) were added to the homogenate and the mixture gently stirred for 30 min. Residual particulate material was removed by centrifugation at 7500 × **g** for 45 min and the supernatant which contained solubilized α1,3-fucosyltransferase was retained. Extraction of the particulate material was repeated with first two volumes and then one volume of the buffer containing Triton X-100 and $MnCl_2$ and the fractions containing the solubilized enzyme were pooled.

Step 2. Cation exchange chromatography on CM-Sephadex C-50

The pooled fractions (3100 ml) were loaded onto a column (8 cm × 30 cm) containing 600 ml CM-Sephadex C-50 equilibrated in 25 mM sodium cacodylate-HCl buffer pH 6.3. The gel was washed with 3500 ml of this buffer at a flow rate of 750 ml h^{-1} and then the bound α1,3-fucosyltransferase was eluted with sodium cacodylate-HCl buffer, pH 6.3, containing 0.25 M NaCl at a flow rate of 400 ml h^{-1} (Fig. 1). This step resulted in the removal of 80% of the total protein and also achieved removal of the Triton X-100 which was necessary before the enzyme could be bound to Phenyl Sepharose CL-4B.

Step 3. Ammonium sulphate precipitation and hydrophobic chromatography on Phenyl Sepharose CL-4B

The active fractions from the CM-Sephadex C-50 column were pooled (465 ml) and subjected to fractionation with ammonium sulphate. The fraction precipitating between 25–65% saturation was collected by centrifugation at 10000 × **g** for 30 min and redissolved in 25 mM sodium cacodylate-HCl buffer, pH 6.3, containing 0.1 M NaCl and 5% glycerol. The solution (465 ml) was loaded onto a column (1.5 × 20 cm) of Phenyl Sepharose CL-4B at a flow rate of 50 ml h^{-1}. The gel was washed with 300 ml of 25 mM sodium cacodylate-HCl buffer, pH 7.3, containing 0.1 M NaCl and 5% glycerol at a flow rate of 50 ml h^{-1} and the enzyme was subsequently eluted with the same buffer containing 0.1 M NaCl, 5% glycerol and 2% Triton X-100 (Fig. 2). This step resulted in only a modest purification but yielded a preparation that could be

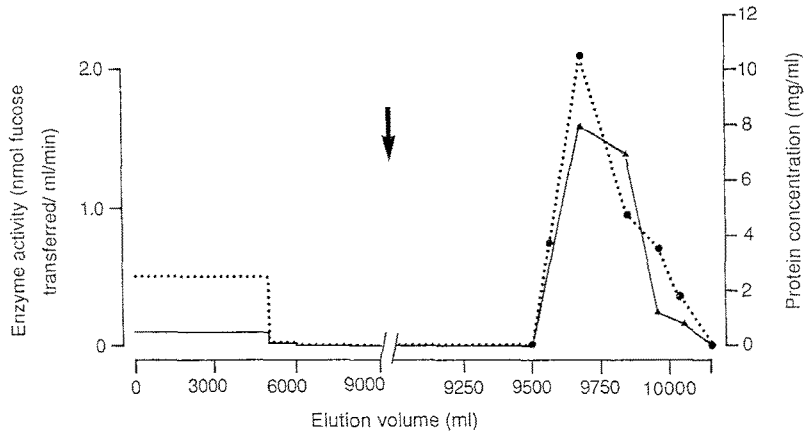

Figure 1. Elution profile of the liver α1,3-fucosyltransferase after cation exchange chromatography on CM-Sephadex C-50. The arrow indicates the point of application of 25 mM sodium cacodylate-HCl buffer, pH 6.3, containing 0.25 M NaCl. ●···● Protein concentration estimated by the method of Read and Northcote [37]; ▲—▲ α1,3-fucosyltransferase activity assayed by Method 1 with *N*-acetyllactosamine as substrate.

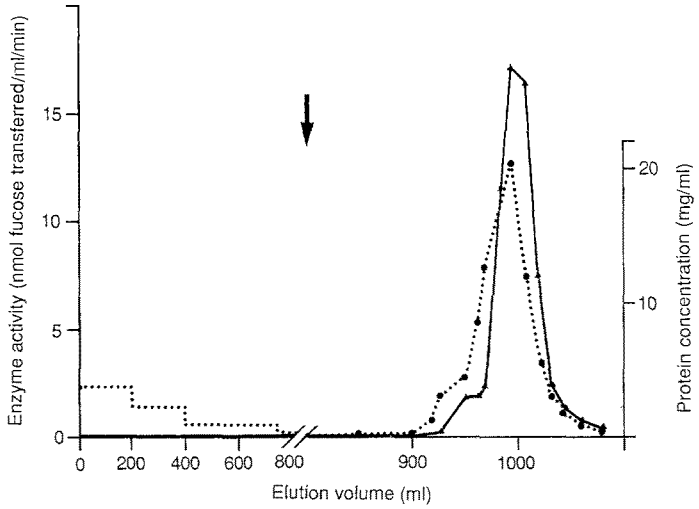

Figure 2. Elution profile of the liver α1,3-fucosyltransferase recovered from CM-Sephadex C-50 chromatography after hydrophobic interaction chromatography on Phenyl Sepharose CL-4B. The arrow indicates the point of application of 25 mM sodium cacodylate-HCl buffer pH 7.3 containing 0.1 M NaCl, 5% glycerol and 2% Triton X-100. ●···● Protein concentration estimated by the method of Read and Northcote [37]; ▲—▲ α1,3-fucosyltransferase activity assayed by Method 1 with *N*-acetyllactosamine as substrate.

absorbed by a small volume of GDP-hexanolamine Sepharose 4B.

Step 4. Affinity chromatography on GDP-hexanolamine Sepharose 4B

Fractions with α1,3-fucosyltransferase activity eluted from the Phenyl Sepharose CL-4B column were pooled (100 ml) and loaded at 20 ml h^{-1} onto a column (0.8 × 10 cm) containing 5 ml of GDP-hexanolamine Sepharose 4B. The gel was washed with 60 ml of 25 mM sodium cacodylate-HCl buffer, pH 7.3, containing 0.1 M NaCl and 25% glycerol at a flow rate of 15 ml h^{-1} and then the active enzyme was eluted with the same buffer mixture to which GDP-fucose (33 μM) and $MnCl_2$ (1.0 mM) had been added (Fig. 3).

Step 5. Concentration of enzyme fractions by hydrophobic chromatography on Phenyl Sepharose CL-4B

The α1,3-fucosyltransferase in the pooled active fractions eluted from the affinity column was concentrated by adsorption onto a mini-column (0.5 ml) of Phenyl Sepharose CL-4B packed into a plastic pipette tip. The gel was washed with 3 ml of 25 mM sodium cacodylate-HCl buffer, pH 7.3, containing 25% glycerol and the enzyme was then eluted with the same buffer containing 15% 1,2-ethanediol.

Step 6. HPLC gel exclusion chromatography

The active eluant from the Phenyl Sepharose CL-4B column (2 ml) was immediately subjected to gel exclusion HPLC on a TSK G3000 SW column (7.5 × 600 mm) in 4 × 0.5 ml aliquots. The enzyme was eluted with 25 mM sodium cacodylate-HCl buffer, pH 7.3, containing 0.2 M NaCl and 25% glycerol at a flow rate of 0.5 ml min^{-1} at room temperature. HPLC was carried out at room temperature instead of the lower temperatures at which the α1,3-fucosyltransferase is more stable because at room temperature a slower-running protein peak was more effectively separated from a strong peak of α1,3-fucosyltransferase activity that was detected in fractions emerging after 76 min. The peak of enzyme activity corresponded with a rise in the UV absorption at 215 nm (Fig. 4). For reasons that are not understood, material

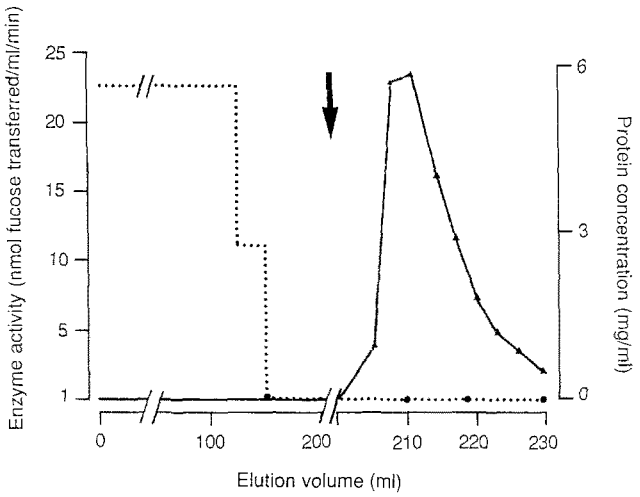

Figure 3. Elution profile of the liver α1,3-fucosyltransferase recovered from Phenyl Sepharose CL-4B chromatography after affinity chromatography on GDP-hexanolamine Sepharose 4B. The arrow indicates the point of application of 25 mM sodium cacodylate-HCl buffer, pH 7.3, containing 0.1 M NaCl, 25% glycerol (w/v), 33 μM GDP-fucose and 1 mM $MnCl_2$. ●···● Protein concentration estimated by the method of Read and Northcote [37]; ▲—▲ α1,3-fucosyltransferase activity assayed by Method 1 with *N*-acetyllactosamine as substrate.

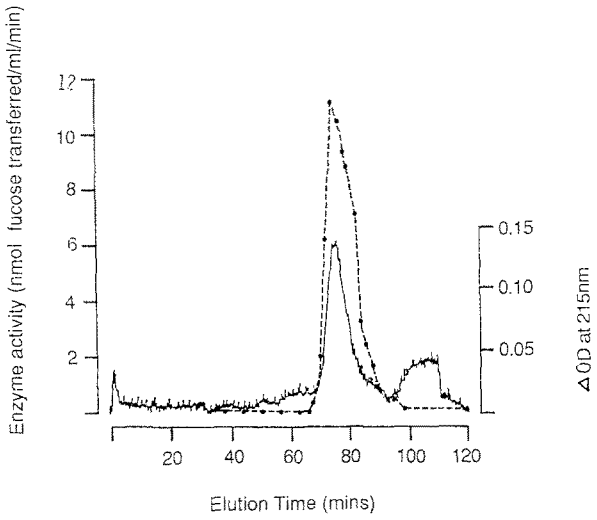

Figure 4. Elution profile of the liver α1,3-fucosyltransferase recovered from affinity chromatography on GDP-hexanolamine Sepharose 4B after HPLC gel exclusion chromatography on a TSK G3000 SW column. — Protein concentration measured by optical density at 215 nm: ●···● α1,3-fucosyltransferase activity assayed by Method 1 with *N*-acetyllactosamine as substrate.

staining with the silver reagent at ~ 66 K on SDS-PAGE of the pooled fractions collected after Step 5 (Fig. 5) did not appear in the eluate from the HPLC gel exclusion column. The same elution profile was observed consistently on several independent runs on the HPLC column.

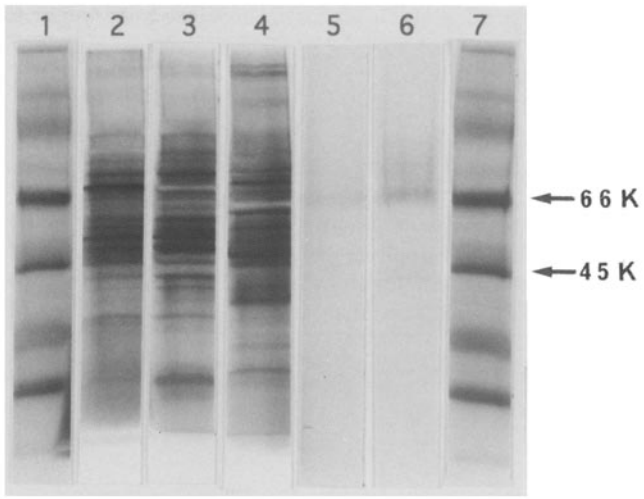

Figure 5. SDS-PAGE electrophoresis of fractions obtained at different stages of purification of liver α1,3-fucosyltransferase. Lanes 1 and 7, Molecular weight markers; Lane 2, crude liver extract; Lane 3, Eluate from CM-Sephadex G-50 column; Lane 4, Eluate from Phenyl-Sepharose CL-4B column; Lane 5, Eluate from GDP-hexanolamine Sepharose 4B column; Lane 6, Eluate from GDP-hexanolamine Sepharose 4B column concentrated on column of Phenyl-Sepharose CL-4B.

The fractions containing active enzyme were once again concentrated by absorption onto a Phenyl Sepharose CL-4B (0.5 ml) column packed in a plastic pipette tip, followed by elution with 25 mM Na cacodylate-HCl buffer, pH 7.3, containing 25% glycerol and 15% 1,2-ethanediol.

Details of the purification steps, specific activity, recovery and degree of purification of the α1,3-fucosyltransferase, are summarized in Table 1. A recovery of 38% was achieved after the second phenyl Sepharose CL-4B step (Step 5) and the 39000-fold purified product had a specific activity of 1.6 $\mu mol\,min^{-1}\,mg^{-1}$ measured with *N*-acetyllactosamine as substrate at the sub-optimal concentrations of GDP-fucose used to monitor enzyme fractions during the purification procedure. When the purified enzyme was tested with saturating concentrations of GDP-fucose the calculated specific activity increased to 5–6 $\mu mol\,min^{-1}\,mg^{-1}$ (see Table 3).

PURITY AND APPARENT M_r OF ENZYME PREPARATION

SDS-PAGE-electrophoresis

Multiple protein bands were visible on silver staining the gel tracks of the enzymically active fractions up to Step 3 of the purification procedure (Fig. 5). The active fractions eluted from GDP-hexanolamine Sepharose 4B (Step 4) showed only a faint band at 66 K corresponding to the albumin standard and a suggestion of an even fainter band with an M_r of approximately 44 K. After

Table 1. Purification of α1,3-fucosyltransferase from human liver

Purification step	*Volume (ml)*	*Protein (mg)*	*Total activity (μmol min^{-1})*	*Specific activity (μmol min^{-1} mg^{-1})*	*Recovery (%)*	*Purification (fold)*
1. Crude solubilized extract	3100	18500	0.77	4.2×10^{-5}	100	–
2. Cation exchange chromatography	465	2050	0.52	2.5×10^{-4}	68	6
3. Hydrophobic chromatography I	100	640	0.34	5.2×10^{-4}	44	13
4. Affinity chromatography	24	0.24	0.27	1.1	35	27000
5. Hydrophobic chromatography II	2	0.18	0.29	1.6	38	39000
6. HPLC gel exclusion chromatography	12	0.10	0.14	1.4	18	33000

Fucosyltransferase activity was assayed under standard conditions (Method 1) with a sub-optimal concentration of GDP-fucose as donor substrate. Protein was measured by the Read and Northcote [38] procedure up to Step 5 and by adsorption at 215 nm after HPLC gel exclusion chromatography.

concentration of the fractions on Phenyl Sepharose CL-4B (Step 5), both bands were more distinct and the band at ~ 44 K is believed to represent the enzyme protein. No silver staining bands were visible on electrophoresis of the pooled dilute enzyme fractions eluted from the HPLC gel filtration column and an attempt to concentrate this material on Phenyl Sepharose CL-4B at this stage led to loss of enzyme activity and the fraction was not further examined.

HPLC gel exclusion column

The purified α1,3-fucosyltransferase had an apparent molecular weight of ~ 41000 as estimated by HPLC gel exclusion chromatography (Fig. 4). This figure differs slightly from that of the putative enzyme staining band on SDS-PAGE (~ 44000) but the elution time on HPLC of the enzyme protein and of the fucosyltransferase activity both increased in parallel, to a slight extent, when the temperature was lowered from room temperature to 4 °C, or when glycerol was removed from the eluate, suggesting some interaction between the column matrix and the enzyme. The M_r of ~ 44000 is therefore considered a more accurate estimate of the molecular weight of the purified α1,3-fucosyltransferase.

EFFECT OF pH AND DIVALENT CATIONS

With *N*-acetyllactosamine as acceptor the α1,3-fucosyltransferase has a broad optimum between pH 7.0–8.0 (Fig. 6). The enzyme is activated by Mn^{2+}, Ca^{2+} and Mg^{2+} ions with the highest activation by Mn^{2+} ions (Fig. 7). The apparent K_m values for Mn^{2+}, Ca^{2+} and Mg^{2+} ions with *N*-acetyllactosamine as acceptor, were 12 mM, 16 mM and 22 mM respectively.

EFFECT OF SULPHYDRYL REAGENTS

The purified liver α1,3-fucosyltransferase enzyme was resistant to treatment with 0.1 mM NEM for up to 40 min at 4 °C but immediate inhibition occurred on mixing the enzyme with 1.0 mM NEM and the activity was reduced to 34% after 40 min exposure to this concentration of the

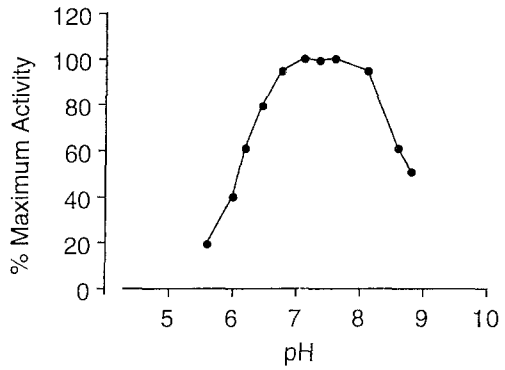

Figure 6. Effect of pH on the activity of the purified liver α1,3-fucosyltransferase. Fucosyltransferase activity was assayed with *N*-acetyllactosamine as acceptor substrate under standard conditions except that Tris-maleate buffer (3.3 μmol) was used over a range of pH values in place of sodium cacodylate buffer pH 7.3.

reagent (Table 2). The enzyme activity was also relatively resistant to 0.1 mM PMB but activity was rapidly lost on exposure to 1.0 mM PMB (Table 2).

APPARENT K_m VALUES FOR GDP-FUCOSE

When the purified liver α1,3-fucosyltransferase was tested with *N*-acetyllactosamine as acceptor substrate and a range of GDP-fucose concentrations, the apparent K_m value for the donor substrate derived from a Lineweaver-Burk plot was 2.6 μM.

ACCEPTOR SUBSTRATE SPECIFICITY

The acceptor specificity of the purified α1,3-fucosyltransferase was tested with a range of low-molecular-weight oligosaccharide substrates with a saturating concentration (14 μM) of GDP-fucose (Table 3). The enzyme transferred fucose to the *N*-acetylglucosamine residues in structures containing terminal, or subterminal, Galβ1-4GlcNAc sequences. Substitution of the terminal galactosyl residue with α1,2-linked fucose or α2,3-linked sialic acid lowered the apparent K_m values for these substrates

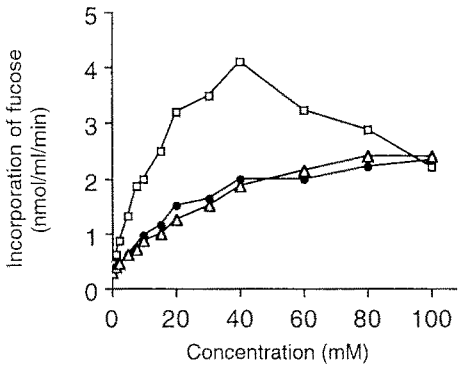

Figure 7. Effect of divalent cations on the activity of the purified liver α1,3-fucosyltransferase. Fucosyltransferase activity was assayed with *N*-acetyllactosamine as acceptor substrate under standard conditions except the concentrations of divalent cations were varied as shown. □—□ Mn^{2+} ions; ●···● Ca^{2+} ions; △—△ Mg^{2+} ions.

Table 2. Action of sulphydryl reagents on purified liver α1,3-fucosyltransferase

Time[a]	*% Activity*[b]			
	NEM		*PMB*	
(min)	*0.1 (mM)*	*1.0 (mM)*	*0.1 (mM)*	*1.0 (mM)*
0	100	57	74	8
40	91	34	66	8

[a]Time of exposure of enzyme to sulphydryl reagent at 4 °C.
[b]Percentage activity of control enzyme sample left at 4 °C for the same length of time.
α1,3-fucosyltransferase activity was assayed by Method 1 with *N*-acetyllactosamine as substrate.

in comparison with the apparent K_m for *N*-acetyllactosamine, with a similar V_{max}, and thus enhanced the incorporation. The specific activity with *N*-acetyllactosamine as substrate was ~5 μmol min^{-1} mg^{-1} and with α2,3-sialyl-*N*-acetyllactosamine was ~ 6 μmol min^{-1} mg^{-1}. The apparent K_m for the tetrasaccharide, lacto-*N*-*neo*tetraose, was also lower than for the unsubstituted disaccharide. No activity was detected when the sialic acid was α2,6-linked to *N*-acetyllactosamine and minimal activity was observed with the Type 1 disaccharide, Galβ1-3GlcNAc, or lactose-based structures such as Fucα1-2Galβ1-4Glc (Table 3). The enzyme did not transfer fucose to the *N*-acetylglucosamine residue in phenyl *N*-acetyl-β-D-glucosaminide. Phenyl β-D-galactoside was not a substrate demonstrating the absence of α1,2-fucosyltransferase activity.

Glycolipids and glycoproteins carrying Type 2 structures also functioned as acceptors for the transferase (Table 4). Of the glycolipids tested, lacto-*N*-*neo*tetraosyl ceramide was the only effective acceptor substrate. The glycoprotein, transferrin, is known to have N-linked chains with Type 2 sequences terminating with α2,6-linked sialic acid residues [46] and, from the reactivity with α1,3/1,4-fucosyltransferase [32], the O-linked chains in the precursor ovarian cyst glycoprotein are inferred to have predominantly α2,6-linked sialic acid residues. These glycoproteins gave greater incorporation of fucose when the sialic acid residues have been removed. In contrast, Tamm-Horsfall glycoprotein [47] and fetuin [48] which carry Type 2 chain endings with predominantly α2,3-linked sialic acid, were better substrates before the removal of sialic acid. The results obtained with glycoprotein acceptors therefore supported the findings with low-molecular-weight acceptors that indicate a preference of the enzyme for α2,3-sialic acid substituted Type 2 structures. Fetuin from which both the sialic acid and galactosyl residues had been removed, did not function as an acceptor indicating that the preparation was free from α1,6-fucosyltransferase which transfers fucose to this substrate [49].

Acceptor substrate specificity of α1,3-fucosyltransferase in HepG2 cells

Extracts of the HepG2 cells assayed by Method 2 (See Materials and methods section) also utilized both sialylated and unsialylated acceptors and incorporation of fucose into α2,3-sialyl-*N*-acetyllactosamine and fetuin was greater (121% in each case) than the incorporation into, respectively, *N*-acetyllactosamine or asialofetuin. Lacto-*N*-biose 1 and 2′-fucosylactose were poor acceptors (incorporation respectively, 2% and 7% of that into *N*-acetyllactosamine).

CHARACTERIZATION OF PRODUCTS

N-*acetyllactosamine*

The radioactive product formed with *N*-acetyllactosamine had a mobility R_{lac} = 0.78 in Solvent 1 and co-chromatographed with the product earlier synthesized by the milk α1,3/1,4-fucosyltransferase [32] that was rigorously characterized by methylation and H^1NMR as the Le^x trisaccharide Galβ1-4[Fucα1-3]GlcNAc. Radioactive fucose was released from the product by treatment with α1,3/1,4-fucosidase and not by treatment with α1,2-fucosidase.

Fucosyl-N-*acetyllactosamine*

The tetrasaccharide product synthesized with Fucα1-2Galβ1-4GlcNAc had a mobility of R_{lac} 0.4 in Solvent 1. Radioactive fucose was released by treatment with α1,3/1,4-fucosidase and not by treatment with α1,2-fucosidase.

Sialyl-N-*acetyllactosamine*

The reaction mixture containing the product formed with NeuAcα2-3Galβ1-4GlcNAc was first subjected to paper

Table 3. Acceptor specificity of the purified liver α1,3-fucosyltransferase with low-molecular-weight acceptors

Substrate	*Apparent* K_m *(mM)*	V_{max} *(μmol min^{-1} mg^{-1})*	*Relative activity*[a]
Galβ1-4GlcNAc	2.9	5.1	100
Galβ1-3GlcNAc	–	0.05	1
Fucα1-2Galβ1-4Glc	–	0.04	1
Fucα1-2Galβ1-4GlcNAc	1.3	4.9	120
NeuAcα2-3Galβ1-4GlcNAc	0.9	5.9	133
NeuAcα2-6Galβ1-4GlcNAc	–	0	0
Galβ1-4GlcNAcβ1-3Galβ1-4Glc	1.5	5.4	127
Phenyl β-D-Gal	–	0	0
Phenyl β-D-GlcNAc	–	0	0

Acceptor substrates were tested in standard reaction mixtures over a concentration range of 0.25–5.0 mM with a saturating concentration (14 μM) of GDP-fucose. Assays were worked up by Method 1. Apparent K_m values were obtained from Lineweaver-Burk reciprocal plots.
[a]Relative activity was measured at 10 mM acceptor substrate concentration.

Table 4. Acceptor specificity of the purified liver α1,3-fucosyltransferase with glycoprotein and glycolipid acceptors

Substrate	*Relative activity**		
	Untreated	*Sialic acid removed*	*Sialic acid and galactose removed*
Glycoproteins			
Fetuin	100	74	1.5
α_1-Acid glycoprotein	93	81	ND
Tamm-Horsfall glycoprotein	185	125	ND
Transferrin	33	52	ND
Ovarian cyst glycoprotein	59	222	ND
Glycolipids			
Lacto-*N-neo*tetraosyl ceramide	81	ND	ND
Trihexosyl ceramide	0	ND	ND
Globoside	0	ND	ND
GM_1	0	ND	ND

Reaction mixtures containing glycolipids worked up by Method 1.
*Relative activity calculated from incorporation into 0.5 μmol glycolipid or 100 μg glycoprotein relative to the incorporation into 100 μg fetuin.
ND, not determined.

electrophoresis and the compound with mobility $R_{picrate} = 0.57$ was eluted and chromatographed in Solvent 3 to yield a single product $R_{lac} = 0.30$. This product was initially resistant to hydrolysis by either the α1,2- or α1,3/1,4-fucosidase, but after neuraminidase treatment a radioactive trisaccharide product was formed that co-chromatographed with the Le^x trisaccharide in Solvent 1 ($R_{lac} = 0.78$) and was then susceptible to hydrolysis by α1,3/1,4- and not α1,2-fucosidase.

Lacto-N-neo*tetraose*

The reaction mixture containing the product formed with lacto-*N-neo*tetraose was subjected to paper chromatography in Solvent 1 and the radioactive product $R_{lac} = 0.2$ was eluted and re-run for 3 days in Solvent 3. A single radioactive spot, $R_{LNT} = 0.5$, that co-chromatographed with authentic lacto-*N*-fucopentaose III was clearly distinguished from lacto-*N*-fucopentaose VI (Galβ1-3GlcNacβ1-3Gal[Fucα1-3]Glc ($R_{lac} = 0.55$), the compound that would have been present if the fucose had been transferred to the terminal reducing glucose residue [32]. Labelled fucose was released from the compound by the α1,3/1,4-fucosidase and not by the α1,2-fucosidase. The failure of Jack bean β-galactosidase to degrade the labelled pentasaccharide provided further proof that the added fucose was attached to the subterminal *N*-acetylglucosamine unit since this enzyme does not release terminal galactose from compounds that have substituents on the adjacent sugar [32].

POLYCLONAL ANTIBODIES RAISED AGAINST THE α1,3-FUCOSYLTRANSFERASE PURIFIED FROM HUMAN LIVER

The antiserum from the rabbit immunized with the purified liver α1,3-fucosyltransferase did not neutralize the activity of the homologous enzyme when the enzyme-antiserum mixture was assayed in standard tests with

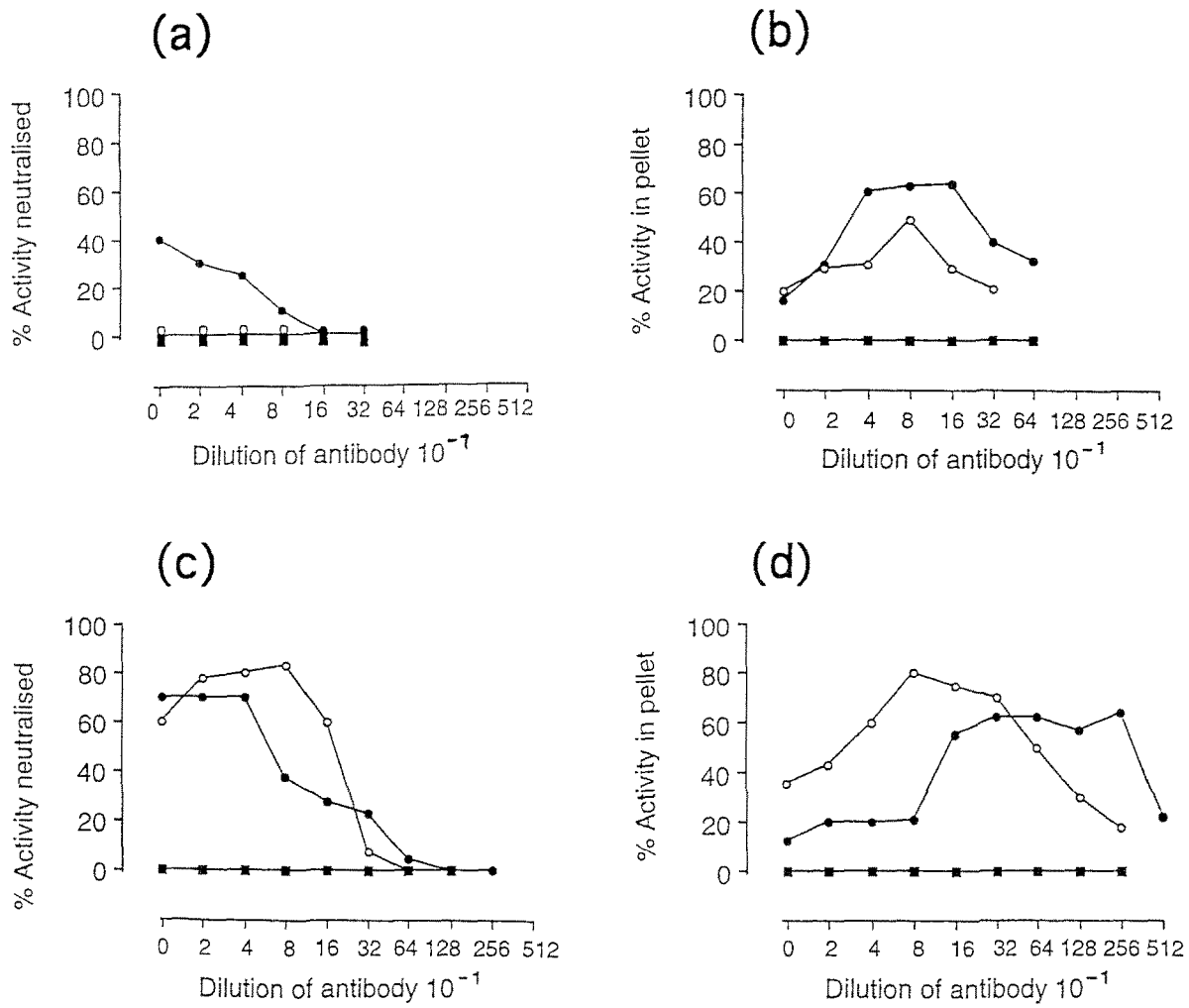

Figure 8. Inhibitory activities of antibodies raised against purified α1,3-fucosyltransferase. a. Percentage neutralizing activity of the antiserum from rabbit immunized with the purified α1,3-fucosyltransferase. b. Percentage of α1,3 or α1,4-fucosyltransferase activity in the immunoprecipitate following the addition of anti-rabbit globulin serum to the antigen-antibody mixtures in (a). c. Percentage neutralizing activity of the antiserum from rabbit immunized with a mixed α1,3/1,4- and α1,3-fucosyltransferase purified from human milk. d. Percentage of α1,3 or α1,4-fucosyltransferase activity in immunoprecipitate following the addition of anti-rabbit globulin to the antigen-antibody mixtures in (c). The antibodies were tested with; ○—○ purified liver α1,3-fucosyltransferase; ●—● purified α1,3/1,4-fucosyltransferase from A431 cells; ■—■ purified α1,3-fucosyltransferase from CML myeloid cells. The activity of the liver and myeloid α1,3-fucosyltransferase was measured with *N*-acetyllactosamine as substrate and of the A431 α1,4-fucosyltransferase with lacto-*N*-biose 1 as substrate.

N-acetyllactosamine as the acceptor substrate (Fig. 8a) but the α1,3-fucosyltransferase was carried down in the immunoprecipitate when an anti-rabbit globulin antibody was used to precipitate immune complexes (Fig. 8b); thus demonstrating the presence of antibodies that combined specifically with the enzyme protein without inhibiting its catalytic function. When the immune serum was replaced by a serum sample taken from the same rabbit before immunization no enzyme activity was detectable in the immunoprecipitates. The antibody directed towards the liver α1,3-fucosyltransferase failed to show any reactivity towards the myeloid α1,3-fucosyltransferase purified from granulocytes of a patient with CML (Fig. 8a and b) despite the fact that this enzyme also transfers fucose to the *O*-3 position of the *N*-acetylglucosamine units in Type 2 acceptor chains [36].

The antiserum was also tested for anti-α1,4-fucosyltransferase activity using an α1,3/1,4-fucosyltransferase purified from A431 cells as the enzyme source [33] and the Type 1 disaccharide, lacto-*N*-biose 1, as acceptor substrate. This enzyme preparation, believed to be the product of the Lewis blood-group-gene has α1,3-fucosyltransferase activity directed towards glucose residues in lactose-based structures but only minimal α1,3-fucosyl-

transferase activity with the Type 2 acceptors and is primarily an α1,4-fucosyltransferase acting on *N*-acetylglucosamine residues in Type 1 chain acceptors. Surprisingly, the polyclonal antibodies reacted more strongly with the purified A431 enzyme than with the homologous liver α1,3-fucosyltransferase. With the A431 enzyme preparation the undiluted antiserum neutralized some 40% of the α1,4-fucosyltransferase activity (Fig. 8a) and when a second anti-rabbit globulin serum was added the precipitated immune complexes displayed α1,4-fucosyltransferase activity (Fig. 8b) that was not detectable in the immunoprecipitates when pre-immunization serum was used.

These results showed that the polyclonal antibodies raised against an α1,3-fucosyltransferase, that transfers fucose only to *N*-acetylglucosamine residues in Type 2 chain acceptors, had also the capacity to react with an α1,4-fucosyltransferase that adds fucose to *N*-acetylglucosamine residues in Type 1 chain acceptors.

The antibody to the liver α1,3-fucosyltransferase had no capacity to neutralize, or combine with the blood group H-gene associated α1,2-fucosyltransferase in human plasma (data not shown).

REACTIVITY OF THE PURIFIED LIVER α1,3-FUCOSYLTRANSFERASE WITH A POLYCLONAL ANTIBODY RAISED AGAINST A MIXED α1,3/1,4-FUCOSYLTRANSFERASE/α1,3-FUCOSYLTRANSFERASE FROM HUMAN MILK

The purified liver α1,3-fucosyltransferase was tested with a second polyclonal antibody raised against a fucosyltransferase preparation purified from human milk to the stage described by Prieels *et al.* [50] that was subsequently shown to be a mixture of the Lewis blood group-gene associated α1,3/1,4-fucosyltransferase and a Type 2 chain specific α1,3-fucosyltransferase [34]. Larger quantities of the enzyme protein were available for immunization with milk α1,3/1,4-fucosyltransferase(s) than with the liver preparation and the antibodies produced were stronger than those engendered by the liver α1,3-fucosyltransferase. The antiserum raised against the milk enzyme(s) was previously shown to contain a mixture of neutralizing and combining antibodies that reacted with both the Lewis gene-associated α1,3/1,4-fucosyltransferase and the Lewis gene-independent α1,3-fucosyltransferase isolated from human milk and with the α1,3-fucosyltransferase purified from human plasma [8, 35].

When tested with the purified liver α1,3-fucosyltransferase the undiluted antiserum neutralized 60% of the enzyme activity measured with *N*-acetyllactosamine as substrate (Fig. 8c). Immunoprecipitation of the antigen-antibody mixture with a rabbit anti-globulin serum revealed antibodies that combined with the enzyme protein to give α1,3-fucosyltransferase activity in the immunoprecipitate (Fig. 8d). Similar profiles of antibody neutralizing and combining activities were obtained when this antiserum was tested with the α1,3/1,4-fucosyltransferase purified from human A431 cells [33] which was free from the Type 2 chain-specific α1,3-fucosyltransferase contaminant present in the milk preparation used for immunization (Fig. 8c and d). When tested with Type 2 chain-specific α1,3-fucosyltransferases purified from normal human granulocytes (data not shown) or from patients with CML (Fig. 8c and d), this polyclonal antibody preparation failed to show either neutralizing or combining activity. No cross reactivity was found with the blood-group *H*-gene associated α1,2-fucosyltransferase in human plasma (data not shown) and negative results were obtained in control tests carried out for each of the fucosyltransferase preparations with a pre-immunization serum sample from the same rabbit.

The specificity of the antibodies raised against the liver α1,3-fucosyltransferase and the mixed α1,3/1,4-fucosyltransferase/α1,3-fucosyltransferase preparation from human milk were thus closely similar inasmuch as each immune serum reacted with both the Type 2 chain-specific liver α1,3-fucosyltransferase and the Type 1 chain-specific A431 α1,4-fucosyltransferase and neither antibody preparation reacted with the Type 2 chain-specific α1,3-fucosyltransferase(s) from myeloid cells.

mRNA EXPRESSION IN HUMAN LIVER AND IN THE HUMAN HEPATIC CELL LINE HepG2

Northern blot analyses were carried out with mRNA from normal human liver and the hepatic carcinoma cell line HepG2 with [^{32}P]labelled *Fuc-TIV*, *Fuc-TV*, *Fuc-TVI* and *Fuc-TVII* cDNA probes. Both the normal liver mRNA (Fig. 9) and the mRNA from the hepatic cell line gave two transcripts (~ 2.3 kb and 3.5 kb) with the *Fuc-TV* and *Fuc-TVI* probes (Fig. 9) but did not show significant transcripts with the *Fuc-TIV* or *Fuc-TVII* probes. mRNA isolated from the myeloid cell line, HL-60, which expresses Fuc-TIV and Fuc-TVII transcripts [11, 17], was used as a positive control for these two gene probes (data not shown).

Discussion

The α1,3-fucosyltransferase solubilized from human liver has been purified to apparent homogeneity as judged by HPLC gel exclusion chromatography. Care was taken to remove the gall bladder from the liver as this is a source of contamination with the Lewis-gene associated α1,3/1,4-fucosyltransferase [44]. Purification of ~ 44 000-fold was achieved primarily through GDP-hexanolamine Sepharose 4B affinity chromatography [51] but the earlier purification steps allowed concentration of the enzyme and a more efficient recovery from the affinity

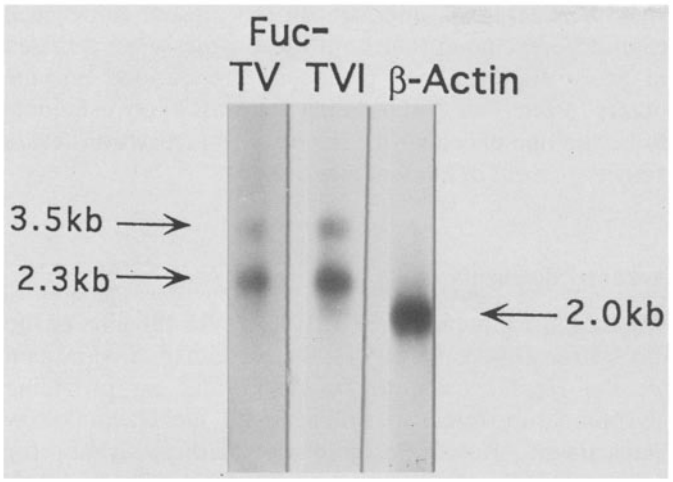

Figure 9. Northern blot analysis of mRNA from normal human liver with *Fuc-TV* and *Fuc-TVI* cDNA probes. Poly (A)$^+$RNA (~ 2 μg human liver mRNA) was probed sequentially with [^{32}P] labelled *Fuc-TV* and *Fuc-TVI* cDNA probes as described in Materials and methods section. The same mRNA was subsequently probed with a [^{32}P] labelled β-actin cDNA.

column. The stability of the enzyme was preserved by carrying out all operations in the presence of 25% glycerol once the protein concentration had been reduced to a low level. Gel exclusion chromatography of the purified enzyme, combined with the results of SDS-PAGE, indicated a M_r of ~ 44000. Specificity studies demonstrated that the purified liver enzyme reacts only with Type 2 acceptors and has a preference for such acceptors substituted either with α2,3-linked sialic acid or α1,2-linked fucose on the terminal β-galactosyl residue (Table 3). The Type 2 tetrasaccharide, lacto-*N*-*neo*tetraose also appeared to be preferred to the unsubstituted disaccharide acceptor but the lower K_m could be related to other interactions occurring with the longer oligosaccharide chain. The results with glycoprotein acceptors (Table 4) supported the inferences deduced from the assays with the low-molecular-weight substrates concerning the preferential utilization of sialylated acceptors by the purified enzyme. The hepatic cell line, HepG2 displayed a similar specificity pattern towards sialylated and unsialylated acceptors.

The specificity of the α1,3-fucosyltransferase in whole liver or isolated hepatocytes has been investigated in two other studies. Jezequel-Cuer *et al.* [52] described a partially purified preparation (26-fold enrichment) of an α1,3-fucosyltransferase from the Golgi fraction of hepatocytes isolated from normal human liver that was free from α1,2- and α1,3/1,4-fucosyltransferase activity and transferred fucose to *N*-acetyllactosamine and to its α2,3-sialylated derivative. This enzyme preparation also had low activity with lactose and 2′-fucosyllactose. The apparent K_m values for α2,3-sialyl-*N*-acetyllactosamine and *N*-acetyllactosamine (0.5 mM and 2.6 mM, respectively) are closely similar to those recorded for these substrates with the purified liver enzyme (0.9 mM and 2.9 mM, respectively) [18, and this paper]. A comparative study on the α1,3-fucosyltransferase specificities in a number of different tissues [44] reported the use of fucosylated and sialylated Type 2 substrates by both a whole liver extract and by isolated hepatocytes but in this study, which utilized substrates with a hydrophobic arm attached at the reducing end of the molecule, the fucosylated substrate appeared to be more than twice as effective an acceptor as the sialylated compound.

The α1,3-fucosyltransferase genes that have been cloned fall into three groups. *Fuc-TIII*, *Fuc-TV* and *Fuc-TVI* map to chromosome 19 and the deduced protein sequences share approximately 85–90% amino acid identity [9, 13, 14]. *Fuc-TIV* maps to chromosome 11 and shares about 60% amino acid identity with the first three genes [10–14] whereas the fifth gene, *Fuc-TVII*, maps to chromosome 9 and the deduced protein sequence shares only some 40% amino acid identity with the other four gene encoded proteins [16, 17]. Analysis of mRNA from normal liver with [^{32}P]labelled probes for *Fuc-TIV*, *Fuc-TV*, *Fuc-TVI* and *Fuc-TVII* revealed transcripts for *Fuc-TV* and *Fuc-TVI* (Fig. 9) but no significant transcripts for the other two genes. These findings were repeated for the mRNA from the hepatic carcinoma cell line HepG2 and confirm results reported earlier for this cell line [53].

In agreement with the assignment of the purified liver α1,3-fucosyltransferase to the group comprising enzymes encoded by *Fuc-TIII*, *Fuc-TV* and *Fuc-TVI* genes, the polyclonal antibody raised against the purified enzyme cross-reacted with the Lewis blood group-gene associated α1,3/1,4-fucosyltransferase isolated from A431 cells [33] that is believed to be encoded by *Fuc-TIII* [9], but failed to react with the α1,3-fucosyltransferase from immature myeloid cells (CML cells) [36] that is thought to be the product of either *Fuc-TIV* or *Fuc-TVII* genes [16, 17]. The purified liver enzyme also combined with the polyclonal antibody raised against the mixed α1,3/1,4- and α1,3-fucosyltransferase preparation from human milk; this polyclonal antibody similarly fails to react with the α1,3-fucosyltransferase(s) from normal granulocytes or immature myeloid cells but cross reacts with the Type 2 chain-specific α1,3-fucosyltransferases purified from human milk and plasma [8, 35]. The liver enzyme is clearly not the product of *Fuc-TIII* since it does not have the α1,4-fucosyltransferase activity associated with expression of this gene. The cross reactivity of the antibody directed to the liver α1,3-fucosyltransferase with enzyme preparations having α1,4-fucosyltransferase activity is consistent with the interpretation that the liver enzyme is the product of *Fuc-TV* or *Fuc-TVI* genes which share considerable sequence homology with *Fuc-TIII*.

A deficiency of α1,3-fucosyltransferase occurs in the plasma of certain rare individuals [54–56] and by cloning the *Fuc-TV* and *Fuc-TVI* genes from a person with this phenotype, Mollicone *et al.* [56] established that the deficiency arose from codon changes in the *Fuc-TVI* gene leading to loss of enzyme activity. They were thus able to infer that the α1,3-fucosyltransferase normally found in plasma is encoded by *Fuc-TVI* and not *Fuc-TV* as had earlier been proposed [13]. The origin of α1,3-fucosyltransferase in plasma is not known and may result from solubilization of enzyme from more than one tissue. However the liver is a probable major source and the acceptor specificity profile of the α1,3-fucosyltransferase earlier purified 600 000-fold from plasma [29] shows considerable similarities with that of the liver enzyme (Table 5). Moreover, comparison of the specificity of the purified liver α1,3-fucosyltransferase with that recorded for the enzymes transiently expressed in mammalian COS cells by the cloned *Fuc-TIII* [9], *Fuc-TIV* [11], *Fuc-TV* [13] and *Fuc-TVI* [14] cDNAs suggest a closer relationship to the specificity of the *Fuc-TVI* encoded enzyme than to the other three (Table 5). The *Fuc-TV* gene product was reported to have a slight preference for sialylated acceptors but also displayed a broader specificity than *Fuc-TVI* in that it had appreciable activity with 2′-fucosylactose and, to a lesser extent, with lacto-*N*-biose I: activities that were marginal for the enzyme expressed by the *Fuc-TVI* gene or the purified liver enzyme (Table 5). The specificity differed from that of the expressed *Fuc-TIV* cDNA in its ability to utilize sialylated acceptors [11] and from that of the *Fuc-TVII* gene in its utilization of unsialylated substrates [16, 17]. Both *Fuc-TV* and *Fuc-TVI* cDNA probes hybridized with liver mRNA but the close sequence homology between *Fuc-TV* and *Fuc-TVI* means that there is considerable overlap in detection of the two mRNAs [13, 14]. Therefore, although on the present evidence it cannot be excluded that both these genes are expressed in liver, the specificity profile of the purified enzyme points to *Fuc-TVI* [14] as being the most likely candidate to be the one encoding the major α1,3-fucosyltransferase activity present in human liver cells.

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Table 5. Comparison of acceptor specificities of cloned α1,3-fucosyltransferase genes transfected into mammalian cells with the specificities of purified liver and plasma α1,3-fucosyltransferases

Acceptor	*Cloned genes*[a] Fuc-T:					*Purified enzymes*	
	III	IV	V	VI	VII	*Plasma*[b]	*Liver*[c]
Galβ1-4GlcNAc	100	100	100	100	< 2	100	100
Galβ1-3GlcNAc	420	< 1	10	0	–	0	1
Galβ1-4Glc	145	3	11	0	–	3	–
NeuAcα2-3Galβ1-4GlcNAc	56	< 1	115	100	100	147	133
NeuAcα2-6Galβ1-4GlcNAc	–	–	–	–	–	0	0
Fucα1-2Galβ1-4GlcNAc	–	–	–	–	–	162	120
Fucα1-2Galβ1-4Glc	254	6	42	0	–	17	1

[a]Data from Weston *et al.* [14] and Natsuka *et al.* [17].
[b]Data from Johnson and Watkins [29].
[c]Data from this paper.
'–' Results not recorded.
Results are expressed relative to the incorporation into *N*-acetyllactosamine (Galβ1-4GlcNAc; 100%) except for *Fuc-TVII* where incorporation into NeuAcα2-3Galβ1-4GlcNAc is taken as 100%.

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