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Purification of the Blood Group *H* Gene Associated α-2-L-Fucosyltransferase from Human Plasma

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The α-2-L-fucosyltransferase in human plasma has been freed from α-3-L-fucosyltransferase activity and purified approximately 200,000-fold by a series of steps involving ammonium sulphate precipitation, hydrophobic chromatography on Phenyl Sepharose 4B and affinity chromatography first on GDP-adipate-Sepharose and then on GDP-hexanolamine-Sepharose. The purified α-2-L-fucosyltransferase had a M_r on gel filtration HPLC of 158,000 and showed optimal activity in the pH range 6.5-7.0. The enzyme transferred fucose equally well to Type 1 (Galβ1-3GlcNAc) and Type 2 (Galβ1-4GlcNAc) substrates but Type 3 (Galβ1-3GalNAc) structures were less efficient acceptors. Competition experiments indicated that a single enzyme species in the purified preparation was responsible for reactivity with the Type 1 and Type 2 structures. Thus the differences in conformation between the Type 1 and Type 2 disaccharides do not appear to influence the capacities of their terminal non-reducing β-D-galactosyl residues to function as acceptor substrates for the α-2-L-fucosyltransferase expressed by the blood group *H* gene in haemopoietic tissue.

The blood group H determinant, Fucα1-2Galβ-R, is the biosynthetic precursor of the blood group A and B antigenic structures. The biosynthesis of H-active structures is catalysed by an α-2-L-fucosyltransferase according to the reaction:

$$\text{GDP-Fuc} + \text{Gal}\beta\text{-R} \rightarrow \text{Fuc}\alpha\text{1-2Gal}\beta\text{-R} + \text{GDP}$$

where R is the carbohydrate chain of a glycoconjugate. The glycosyltransferases encoded by the blood group *A* and *B* genes have an absolute requirement for this terminal H-active structure on the oligosaccharide chains of the acceptor molecules, but within certain well

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defined limits (reviewed in [1]), these enzymes can function despite considerable diversity in the nature, positional linkage and anomeric configuration of the sugars comprising the remainder of the chains. The distribution of the diverse oligosaccharides carrying the determinants varies in different tissues and cells of the body with the predominance of Type 2 (Galβ1-4GlcNAcβ-) structures in the cell surface glycoproteins and glycolipids of erythrocytes [2] and a mixture of Type 1 (Galβ1-3GlcNAcβ-), Type 2 and Type 3 (Galβ1-3GalNAcα-) [3] chains in the glycoconjugates of epithelial tissues of the gut, lungs and submaxillary glands. Type 4 (Galβ1-3GalNAcβ-) blood group active structures, based on the glycolipids of the "globo" series form a substantial part of the glycolipid ABH antigens in human kidney [4] but represent only a minor component on erythrocytes [5].

In humans, with very rare exceptions, the A, B or H antigens are expressed on red cells in strict accordance with the *ABO* genes that are inherited by an individual. Expression of these antigens in epithelial tissues and secretions is, however, dependent on the inheritance of another gene, *Se*. This gene was formerly considered to regulate the expression of the *H* gene (see [1]). The dissimilarity in the conformations of the Type 1 and Type 2 disaccharides, especially in the very different environments of the 2-OH groups of the β-D-galactosyl units, led Lemieux [6] to question whether the same α-2-fucosyltransferase could utilise both these disaccharides as substrates. This query, together with the known tissue distribution of the Type 1 and Type 2 blood group active structures, suggested to Oriol *et al.* [7] that the *Se* gene does not have a regulatory function but itself encodes an α-2-fucosyltransferase that is distinct from the *H* gene-encoded α-2-fucosyltransferase found in haemopoietic tissues. It was proposed that the *H* gene-specified enzyme has a preference for Type 2 precursor structures whereas the *Se* gene-specified enzyme favours Type 1 structures [7]. Several studies have now confirmed the existence of more than one type of α-2-fucosyltransferase and shown that the enzyme in epithelial tissues of ABH secretors has a preference for Type 1 structures [8-11]. Although the demonstration of more than one form of α-2-fucosyltransferase is consistent with two structural genes encoding the transferases the evidence so far accumulated does not conclusively rule out other explanations such as post-transcriptional and post-translational modifications of a single *H*-gene product. In the present paper we report the purification of α-2-L-fucosyltransferase from human plasma and describe some of its properties in order to compare them with the properties of α-2-L-fucosyltransferases isolated from other sources. A preliminary report of this work has already appeared [12].

Materials and Methods

Materials

GDP-L-[^{14}C]fucose (210-292 mCi/mmol) was obtained from Amersham International, U.K. and unlabelled GDP-L-fucose was synthesised by the method of Nunez *et al.* [13]. GDP-Hexanolamine was prepared by the method of Beyer *et al.* [14]. *N*-Acetyllactosamine (Galβ1-4GlcNAc) was synthesised as described by Alais and Veyrieres [15] and lacto-*N*-biose I (Galβ1-3GlcNAc) by the method of Flowers [16]. 2'-Fucosyllactose (Fucα1-2Galβ1-4Glc), 3-fucosyllactose (Galβ1-4[Fucα1-3]Glc), lacto-*N*-tetraose (Galβ1-3GlcNAcβ1-3Galβ1-4Glc), lacto-*N*-neotetraose (Galβ1-4GlcNAcβ1-3Galβ1-4Glc), lacto-*N*-fucopentaose I (Fucα1-2Galβ1-3GlcNAcβ1-3Galβ1-4Glc), lacto-*N*-fucopentaose II (Galβ1-3[Fucα1-4]GlcNAcβ1-

3Galβ1-4Glc) and lacto-*N*-difucohexaose I (Fucα1-2Galβ1-3[Fucα1-4]GlcNAcβ1-3Galβ1-4Glc) were isolated from human milk by the method of Donald and Feeney [17]. 3'-Sialyllactose (NeuAcα2-3Galβ1-4Glc) and 6'-sialyllactose (NeuAcα2-6Galβ1-4Glc) were isolated from human milk by means of the chromatography system described by Veh *et al.* [18].

Galβ1-3GalNAc-OMe was purchased from BioCarb Chemicals, Lund, Sweden and Galβ1-3GalNAc was synthesised by a modification of the method described [16] for the synthesis of lacto-*N*-biose I. Phenyl β-D-galactoside, *o*-nitrophenyl α- and β-D-galactosides, Galβ1-6GlcNAc and Galβ1-3Ara were purchased from Sigma, U.K. The molecular weight standards, ferritin, aldolase, bovine serum albumin and ovalbumin were purchased from Pharmacia Fine Chemicals AB, Uppsala, Sweden.

Fucosyltransferase Assays

α-2-L-Fucosyltransferase was routinely assayed by the method of Chester *et al.* [19]. The enzyme source (5-20 μl) was added to a solution containing phenyl β-D-galactoside (20 mM), GDP-[^{14}C]fucose (2.9 μM), manganese chloride (10 mM), neutral ATP (5 mM), sodium azide (0.055%, w/v) and sodium cacodylate-HCl buffer pH 7.0 (55 mM) in a final volume of 100 μl. When purified enzyme was assayed the incubations were performed in siliconised glass tubes and ATP was replaced by bovine serum albumin (0.25% final concentration, w/v). Following incubation at 37°C for between 1 and 16 h, the reaction products were separated by descending paper chromatography on Whatman No. 40 paper in ethyl acetate/pyridine/water, 10/4/3 by vol; solvent *a*) for 4 h. The chromatograms were scanned on a radiochromatogram scanner and the radioactive peaks were cut out and counted in a liquid scintillation counter.

α-3-L-Fucosyltransferase was assayed as above except that *N*-acetyllactosamine (final concentration 5 mM) was used as an acceptor in place of phenyl β-D-galactoside. Separation of 2'- and 3-fucosylated products of *N*-acetyllactosamine (Fucα1-2Galβ1-4GlcNAc, $R_{Lactose}$ 0.98; Galβ1-4[Fucα1-3]GlcNAc, $R_{Lactose}$ 0.74) was carried out on Whatman DE81 paper for 48 h in solvent *a*.

Acceptor Substrate Specificity

The assay mixtures for testing the acceptor substrate specificity of the purified enzyme contained acceptor (5 mM), GDP-[^{14}C]fucose (56 μM), manganese chloride (10 mM), bovine serum albumin (0.25%, w/v), sodium azide (0.055%, w/v), sodium cacodylate-HCl buffer pH 7.0 (55 mM) and 10 μl enzyme in a final volume of 100 μl. Enzyme products were separated by descending paper chromatography. Phenyl β-D-galactoside, *o*-nitrophenyl β-D-galactoside and *o*-nitrophenyl α-D-galactoside products were separated on Whatman No. 40 paper in solvent *a* for 4 h. The product with D-galactose was separated by chromatography on No. 40 paper for 16 h, in solvent *a*. *N*-Acetyllactosamine, lacto-*N*-biose I, Galβ1-3GalNAc, Galβ1-3GalNAcOMe, Galβ1-3Ara, Galβ1-6GlcNAc lactose, 2'-fucosyllactose and 3-fucosyllactose products were separated on Whatman DE81 paper for 48 h in the same solvent system. Lacto-*N*-tetraose and lacto-*N*-neotetraose products were separated on Whatman DE81 paper in propan-1-ol/ethyl acetate/pyridine/water, 5/1/1/3 by vol, for 24 h. The products

formed with lacto-*N*-fucopentaoses I and II and lactodifucohexaose I were separated for 72 h in ethyl acetate/pyridine/water, 2/1/1 by vol. The products of 3'-sialyllactose and 6'-sialyllactose were separated by high voltage paper electrophoresis at 40 mA for 40 min on Whatman 3MM paper in 40 mM pyridine-acetate buffer pH 5.4. The products had a mobility relative to picric acid of 0.6. The chromatograms were scanned for radioactivity and counted as described above.

The linkage and anomeric specificity of the transferred fucose was confirmed by the susceptibility of the product to a linkage specific α-2-L-fucosidase isolated from *Trichomonas foetus* [20]. The products on chromatography paper were washed thoroughly in toluene after scintillation counting in order to remove scintillation fluid, and then the papers were dried and eluted with water. The eluates were dried under vacuum, dissolved in 50 µl water and added to 10 µl *Trichomonas foetus* α-2-L-fucosidase in 0.1 M sodium phosphate buffer pH 7.0. Following incubation at 37°C for 2 h the products were separated by descending chromatography on Whatman No. 40 paper for 6 h in solvent *a* and areas of radioactivity were counted as described.

Protein Assay

Protein was estimated by a modification of the Bradford method [21].

Enzyme Purification

All purification steps were performed at 4°C. Pooled, human blood group O plasma which had been stored at -40°C was thawed and filtered through nylon mesh. The following is a typical purification schedule:

Step 1. Ammonium sulphate precipitation. Ammonium sulphate was added to 444 ml plasma to give 60% saturation. The precipitate was collected by centrifugation of the suspension at 8000 x *g* for 30 min and then dissolved in 10 mM Tris-HCl buffer pH 7.6, containing 0.5 M sodium chloride to give a final volume of 197 ml.

Step 2. Batch chromatography on Phenyl-Sepharose CL-4B. The dissolved pellet was added to 300 ml Phenyl-Sepharose CL-4B, equilibrated with 10 mM Tris-HCl buffer pH 7.6 containing 0.5 M sodium chloride, in a sintered-glass funnel. The solution was allowed to flow through the adsorbent which was then washed with 1.2 l equilibration buffer and eluted with 10 mM Tris-HCl buffer pH 7.6.

Step 3. Chromatography on GDP-adipate-Sepharose. GDP-adipate-Sepharose 4B (2 µmol GDP/ml gel) was prepared by the method of Lamed *et al.* [22]. The degree of ligand coupling was estimated from the phosphate content of the gel by the method of Bartlett [23]. The eluate from the previous step was adjusted to pH 7.4 with 0.5 M sodium cacodylate-HCl buffer pH 5.5 and loaded onto the GDP-adipate-Sepharose column (1.1 cm x 25.0 cm) equilibrated in 20 mM Tris-HCl buffer pH 7.4, containing 0.1 M NaCl, and 10% (w/v) glycerol. The column was washed in the equilibration buffer and finally eluted with 33 µM GDP and 1 mM $MnCl_2$ in the same buffer. The column flow rate was 19.2 ml per hour and fractions of 9.6 ml were collected.

Table 1. Purification of α-2-L-fucosyltransferase from human plasma.

Step	Activity[a] (μ units)	Protein (mg)	Specific activity	Yield (%)	Purification (-fold)
1. Plasma	1,151	74,899	0.015	100	1
2. $(NH_4)_2SO_4$/Phenyl Sepharose	465	313	1.49	40	97
3. GDP-Adipate-Sepharose	104	201	0.52	9	34
4. GDP-Hexanolamine-Sepharose	49	0.017	2,882	4.2	187,143

[a] One unit of enzyme transfers 1 μmol of fucose per minute at 37°C.

Step 4. Chromatography on GDP-hexanolamine-Sepharose. GDP-Hexanolamine-Sepharose was synthesised by coupling GDP-hexanolamine to CNBr-activated Sepharose by the procedure of Cuatrecasas [24]. The active fractions from the GDP-adipate-Sepharose column were pooled, adjusted to pH 6.0 with 0.5 M sodium cacodylate-HCl buffer pH 5.5 and loaded onto a GDP-hexanolamine-Sepharose column (0.7 x 3.9 cm) equilibrated in 25 mM sodium cacodylate-HCl, pH 6.0, containing 0.1 M sodium chloride, and 25% (w/v) glycerol. The column was washed with 28 ml of equilibration buffer and eluted with 50 μM GDP in the same buffer. The column flow rate was 24 ml per hour.

Polyacrylamide Gel Electrophoresis

SDS polyacrylamide gel electrophoresis was performed with 12% acrylamide gels by the method of Laemmli [25] and non-denaturing gel electrophoresis with 10% acrylamide slab gels at pH 8.9 overlaid with a 4.5% stacking gel at pH 6.7. Gels were stained for protein with a commercial silver stain ('Quick Silver' supplied by Amersham, U.K.).

Determination of Molecular Weight

Gel exclusion high performance liquid chromatography was carried out on a TSK G4000 SW column (7.5 x 600 mm) and the buffer system was 0.1 M sodium cacodylate-HCl pH 6.0 containing 10% (w/v) glycerol. The flow rate was 0.4 ml/min and 0.5 ml fractions were collected. Ferritin (M_r 440,000), aldolase (M_r 158,000), bovine serum albumin (M_r 67,000) and ovalbumin (M_r 43,000) were used as molecular weight standards.

Results

Purification Scheme

A typical purification scheme is summarised in Table 1. Human plasma contains both α-2- and α-3-fucosyltransferase activities [26]. Previous studies on the solubilised fucosyltransferases from human submaxillary glands had shown that α-3-fucosyltransferase

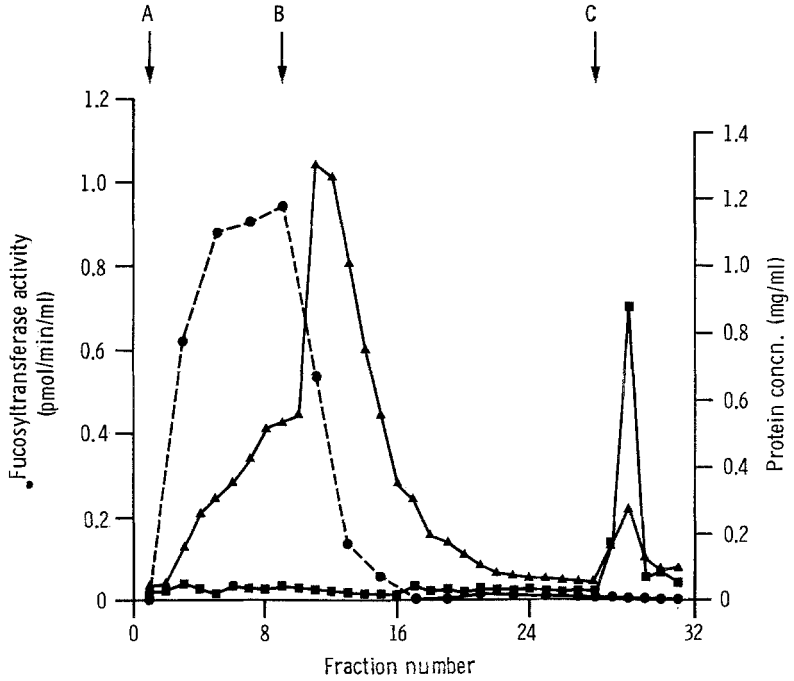

Figure 1. Chromatography of α-2- and α-3-fucosyltransferases on GDP-adipate-Sepharose.
The column was loaded at A, washed with equilibration buffer at B and eluted with 33 μM GDP and 1 mM $MnCl_2$ at C as described in the text. ▲, α-2-L-fucosyltransferase activity (Acceptor: phenyl β-D-galactoside). ■, α-3-L-fucosyltransferase activity (Acceptor: *N*-acetyllactosamine). ●, Protein concentration.

activity could be bound to GDP-adipate-Sepharose whereas the α-2-enzyme was not retarded [27]. The plasma enzymes were found to behave similarly and therefore chromatography on GDP-adipate-Sepharose was carried out before the GDP-hexanolamine-Sepharose step. The two enzymes were separated successfully (Fig. 1) but a considerable loss of activity occurred at this stage. Affinity binding to GDP-hexanolamine-Sepharose and elution with GDP gave an overall purification factor of 187,000 for the α-2-L-fucosyltransferase and a specific activity of 0.003 μmol/min/mg.

Polyacrylamide Gel Electrophoresis

Several protein bands, with no one predominant band, were detected on silver staining a PAGE gel of the most active α-2-fucosyltransferase preparation indicating that the enzyme had not been purified to homogeneity.

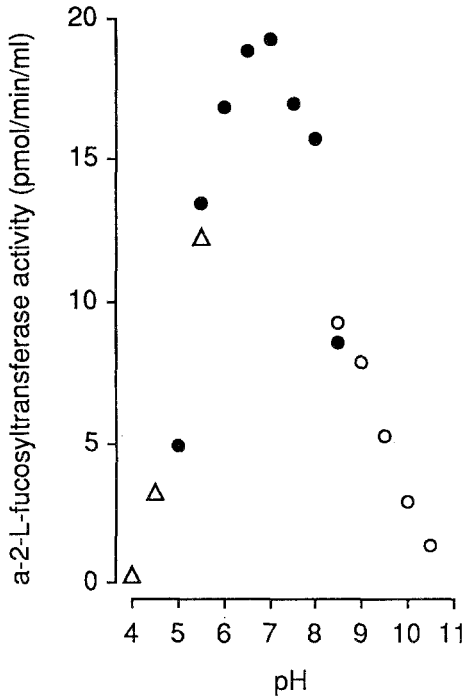

Figure 2. Effect of pH on α-2-L-fucosyltransferase activity.
The incubation mixtures contained in a final volume of 100 µl; 5 mM phenyl β-D-galactoside, 1.25 µM GDP-[^{14}C]fucose, 10 mM manganese chloride, 0.25% (w/v) bovine serum albumin and 50 mM buffer solutions: △, Mixtures of sodium acetate and sodium cacodylate; ●, Mixtures of sodium cacodylate and Tris; ■, Mixtures of Tris and glycine.

Properties of the Purified α-2-L-Fucosyltransferase

pH Activity Profile. The enzyme had optimal activity in the pH range 6.5-7.0 (Fig. 2).

Divalent Cation Requirements. The purified enzyme had some activity in the absence of added cations but Mn^{2+}, Ca^{2+} and Ba^{2+} ions enhanced the incorporation of [^{14}C]fucose (Fig. 3). Mn^{2+} ions were the most effective activators of those tested and maximal activation occurred at a concentration of 10 mM. When examined over the same concentration range Zn^{2+} and Cu^{2+} completely inhibited α-2-L-fucosyltransferase activity.

Molecular Weight Determination. HPLC on a TSK G4000 SW gel exclusion column gave a single peak of α-2-fucosyltransferase activity (Fig. 4) corresponding to a molecular weight of 158,000. Active fractions were dialysed against 0.01% SDS, freeze-dried and subjected to SDS PAGE electrophoresis but after silver staining no protein bands were observed.

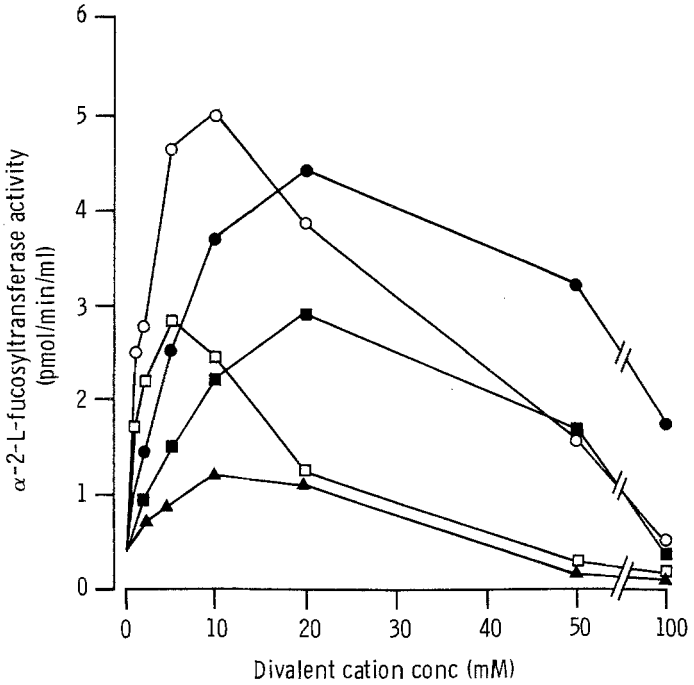

Figure 3. Effect of divalent cations on α-2-L-fucosyltransferase activity.
The incubation mixtures contained in a final volume of 100 µl; 20 mM phenyl β-D-galactoside, 2.8 µM GDP-[^{14}C]fucose, 0.25% bovine serum albumin, 0.045% (w/v) sodium azide, 45 mM sodium cacodylate-HCl buffer pH 7.0 and varying concentrations of the following: ○, $MnCl_2$; ●, $MgCl_2$; □, $CoCl_2$; ■, $CaCl_2$; ▲, $BaCl_2$.

Acceptor Substrate Specificity. The comparative efficiency of various oligosaccharides to function as acceptor substrates for the α-2-L-fucosyltransferase is shown in Table 2. At 5 mM concentration the enzyme was equally active with the Type 1 disaccharide lacto-*N*-biose I and the Type 2 disaccharide *N*-acetyllactosamine. With the tetrasaccharides, lacto-*N*-tetraose and lacto-*N*-neotetraose, a slight preference was apparent for lacto-*N*-tetraose which has a Type 1 structure at the non-reducing terminal. The methyl glycoside of the Type 3 disaccharide, Galβ1-3GalNAc-α-1-OMe was a poorer acceptor than either the Type 1 or Type 2 chain structures. D-Galactose and all other disaccharides tested with terminal non-reducing β-D-galactosyl units functioned as acceptors for the α-2-fucosyltransferase but they were less efficient than the Type 1 or Type 2 structures. In confirmation of the earlier findings with untreated serum the best acceptor was the arylglycoside *o*-nitrophenyl β-D-galactoside [19]. [^{14}C]-Fucose was released from all the products when treated with α-2-L-fucosidase; demonstrating that the fucose is coupled through an α(1-2)-linkage. Compounds which are

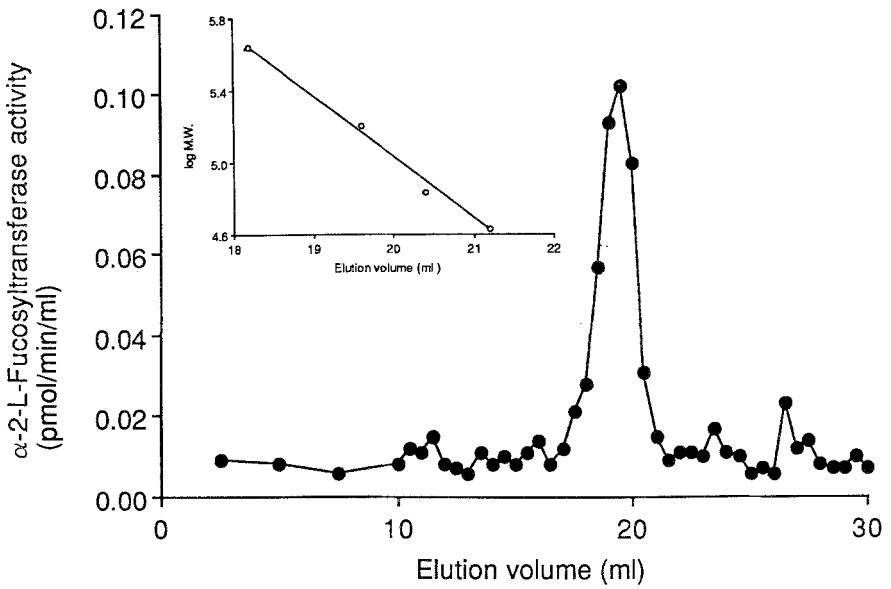

Figure 4. HPLC profile of α-2-L-fucosyltransferase activity eluted from a TSK G4000 SW column. The insert shows the elution volumes of the molecular weight markers; Ferritin (M_r 440,000), aldolase (M_r 158,000), bovine serum albumin (M_r 67,000) and ovalbumin (M_r 43,000).

fucosylated on the sugar subterminal to the β-galactosyl residue, such as 3-fucosyllactose and lacto-*N*-fucopentaose II, are not acceptors for the α-2-fucosyltranferase. Substitution of the β-galactosyl unit of lactose with sialic acid in either the O-3 or O-6 positions prevents the addition of L-fucose to the O-2 position.

Michaelis-Menton Constants for Acceptor and Donor Substrates

Apparent K_M and V_{max} values, determined from Lineweaver-Burk plots, were calculated for phenyl β-D-galactoside, *N*-acetyllactosamine, lacto-*N*-biose I, lacto-*N*-neotetraose, lacto-*N*-tetraose and the Type 3 disaccharide Galβ1-3GalNAc (Table 3). The Type 1 and Type 2 disaccharides had similar apparent K_M values and these were lower than that obtained for the Type 3 disaccharide. The Type 1 tetrasaccharide lacto-*N*-tetraose has a slightly lower K_M than its Type 2 analogue lacto-*N*-neotetraose. The V_{max} values were similar for all five compounds. With phenyl β-D-galactoside as the acceptor substrate an apparent K_M of 11 μM was calculated for the donor substrate GDP-fucose.

Competition Studies. The purified enzyme preparation utilises both Type 1 and Type 2 acceptors and therefore competition studies were performed to determine whether these activities were associated with a single enzyme or whether two enzymes were present:

Table 2. Substrate specificity of α-2-fucosyltransferase with low molecular weight acceptors.

Acceptor (5 mM)	Chain Type	% Incorporation [^{14}C]fucose[a]
o-Nitrophenyl β-D-Gal	-	137
Lacto-*N*-tetraose	Type 1	131
Lacto-*N*-neotetraose	Type 2	113
Phenyl β-D-galactoside	-	100
Lacto-*N*-biose I	Type 1	82
N-Acetyllactosamine	Type 2	80
Lactose	-	75
Galβ1-3GalNAcOMe	Type 3	58
Galβ1-3Ara	-	36
Galβ1-6GlcNAc	-	28
D-Galactose	-	22
3'-Sialyllactose	-	1
6'-Sialyllactose	-	1
2'-Fucosyllactose	-	1
o-Nitrophenyl α-D-Gal	-	0
3-Fucosyllactose	-	0
Lacto-*N*-fucopentaose I	Type 1	0
Lacto-*N*-fucopentaose II	Type 1	0
Lacto-*N*-difucohexaose I	Type 1	0

[a] Relative to the incorporation into phenyl β-D-galactoside.

(i) With the chromatographic techniques used in this study the products of transfer of L-fucose to the Type 1 (lacto-*N*-biose I) and Type 2 (*N*-acetyllactosamine) disaccharides are not separable and neither are the products of transfer to the Type 1 (lacto-*N*-tetraose) and Type 2 (lacto-*N*-neotetraose) tetrasaccharides. Therefore the Type 1 tetrasaccharide was tested as a potential inhibitor of the Type 2 disaccharide and the Type 2 tetrasaccharide as a potential inhibitor of the Type 1 disaccharide.

Figs. 5 and 6 show that lacto-*N*-tetraose (Type 1) is a competitive inhibitor of α-2-L-fucosyltransferase with respect to *N*-acetyllactosamine (Type 2) and lacto-*N*-neotetraose (Type 2) a competitive inhibitor with respect to lacto-*N*-biose I (Type 1). These results demonstrate that both Type 1 and Type 2 structures are recognised at the same substrate binding site of the α-2-L-fucosyltransferase although they do not eliminate the possibility that two enzymes are present.

(ii) *N*-Acetyllactosamine and lacto-*N*-biose I were used as acceptors for the enzyme, individually and together, at concentrations at which the enzyme reaction rate approached the V_{max}. If two separate enzyme activities are present the total amount of product expected from combining the acceptors should be the sum of the products obtained when the

Table 3. K_M and V_{max} values for α-2-fucosyltransferase with Type 1, Type 2 and Type 3 substrates.
Values were obtained with varying substrate concentrations under standard conditions except that the GDP-fucose concentration was increased to 27.8 μM.

Acceptor	Chain Type	K_M (mM)	V_{max} [a]
Phenyl β-D-galactoside		4.0	1.00
Lacto-*N*-biose I	Type 1	4.5	0.75
N-Acetyllactosamine	Type 2	5.0	0.75
Galβ1-3GalNAc	Type 3	9.2	0.80
Lacto-*N*-tetraose	Type 1	3.4	0.86
Lacto-*N*-neotetraose	Type 2	5.0	0.75

[a] V_{max} relative to value obtained for phenyl β-D-galactoside.

acceptors are incubated individually. The amount of product obtained in the presence of the two acceptors was not significantly greater than the amount of product obtained with the individual acceptors (Table 4). Similar results were obtained with lacto-*N*-tetraose and lacto-*N*-neotetraose used singly and in combination. These results strongly indicate that the same enzyme utilises both Type 1 and Type 2 acceptors.

(iii) The total amount of product expected under steady-state conditions when an enzyme is incubated with two competing substrates, A and B, can be calculated from the equation

$$v_t = v_a + v_b = \frac{V_a \cdot [A]K_a + V_b \cdot [B]/K_b}{1 + [A]/K_a + [B]/K_b}$$

where v_t is the total rate of product formation, v_a and v_b are the rates of product formation from substrates A and B respectively. V_a and V_b are V_{max} values for A and B respectively and K_a and K_b are K_M constants for A and B respectively [28].

When this equation was applied the observed velocity for the reaction in the presence of both *N*-acetyllactosamine and lacto-*N*-biose I was closer to that expected for a single enzyme than for two separate enzymes, as was also the observed velocity for the reaction in the presence of both lacto-*N*-tetraose and lacto-*N*-neotetraose (Table 5).

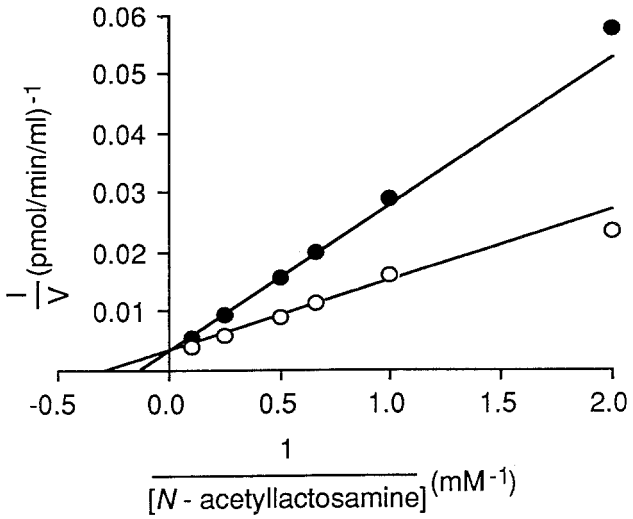

Figure 5. Lineweaver-Burk plot of α-2-L-fucosyltransferase activity with varying concentrations of *N*-acetyllactosamine in the presence (●) and absence (○) of 3 mM lacto-*N*-tetraose. The conditions were the same as in Table 3.

Discussion

All the available evidence suggests that the α-2-fucosyltransferase activity in serum derives largely from haemopoietic sources (reviewed in [29]). The enzyme levels show a relationship with ABO blood group [19, 30] but do not correlate with ABH secretor status [26]. Rare individuals who lack ABH antigens on their erythrocytes but secrete ABH active substances in saliva, that is, those belonging to the so-called "para-Bombay" phenotypes [3], have a deficiency of α-2-fucosyltransferase activity in their sera [26, 30]; a result which indicates that little of the enzyme found in those with normal ABO phenotypes permeates into the serum from secretory tissue. By means of a sensitive detection technique Le Pendu *et al.* [32] demonstrated α-2-fucosyltransferase activity in the sera of some individuals with the para-Bombay phenotype but it amounted to only about 5-10% of that present in the sera of those with normal ABO phenotypes. Hence at least 90-95% of α-2-fucosyltransferase in normal serum appears to be of haemopoietic origin and therefore may be considered to be the product of the *H* gene. Recent studies point to the haemopoietic activity of the bone marrow and to circulating erythrocytes and platelets as a major source of the serum α-2-fucosyltransferase [33].

Although polyacrylamide gel electrophoresis has shown that the enzyme preparation described in this paper is not homogeneous the overall purification factor of about 200,000 and final specific activity of 0.003 μmol/min/mg represent the highest values yet recorded

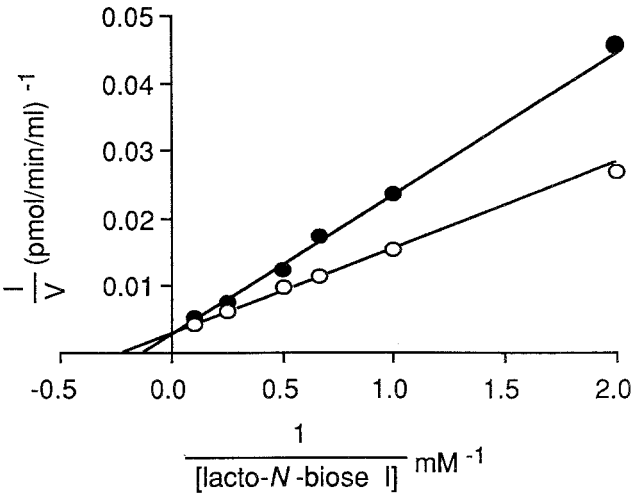

Figure 6. Lineweaver-Burk plot of α-2-L-fucosyltransferase activity with varying concentrations of lacto-*N*-biose I in the presence (●) and absence (○) of 3 mM lacto-*N*-neotetraose. The conditions were the same as in Table 3.

for the human α-2-fucosyltransferase. Yazawa and Furukawa [34] described a purification procedure for the plasma enzyme involving DEAE-Sepharose, GDP-Sepharose and SP-Sepharose chromatography which yielded an enzyme purified 20,000-fold. Although a major protein band corresponding in position to α-2-fucosyltransferase activity was found on polyacrylamide gel electrophoresis other minor bands were apparent and the low specific activity suggested either that this preparation contained a high proportion of inactivated enzyme or that the enzyme co-migrated on the gel with an impurity. A molecular weight in the range 120,000-180,000 was earlier reported from our laboratory for a partially purified α-2-fucosyltransferase from plasma [35] and Yazawa and Furukawa [34] obtained a molecular weight of about 150,000 on Sephadex G-200 gel filtration of their enzyme preparation. HPLC on a TSK 4000 gel filtration column of the purified plasma enzyme described in this paper indicated a molecular weight of about 158,000.

Studies on the acceptor substrate specificity of α-2-fucosyltransferase in partially purified serum or plasma preparations from which care had been taken to ensure the removal of the α-3-fucosyltransferase activity [35], or where specifically modified substrates were used that would not be acceptors for the α-3-enzyme [10], showed high transfer efficiency to Type 1 and Type 2 acceptors with no marked preference for either substrate. A 65-fold purified plasma enzyme described by Kamazaki and Yoshida [8] had an apparent K_M for the Type 2 structure (25 mM) that was four to five times higher than that found for the Type 1 structure (5.8 mM) but it is not entirely clear that this preparation was free of α-3-fucosyltransferase

Table 4. Competition experiments at saturating concentrations of the acceptors; (a) lacto-*N*-biose I and *N*-acetyllactosamine and (b) lacto-*N*-tetraose and lacto-*N*-neotetraose. Assay conditions as in Table 3 except that all acceptor substrates were at concentrations of 30 mM.

Acceptor substrates		Fucose transferred (pmol/min/ml)
(a) Lacto-*N*-biose I	*N*-Acetyllactosamine	
+	-	106
-	+	109
+	+	115
(b) Lacto-*N*-tetraose	Lacto-*N*-neotetraose	
+	-	312
-	+	251
+	+	302

+, Substrate present.
-, Substrate absent.

activity because the compound used to monitor for this activity, namely 2'-fucosyllactose, is a very poor substrate for the serum *N*-acetylglucosamine : α-3-fucosyltransferase [26]. The purified α-2-fucosyltransferase described in the present paper utilised as acceptor substrates a variety of disaccharides containing non-reducing β-galactosyl units (Table 2); the best were the Type 1 and Type 2 disaccharides and the enzyme did not show a preference for either one when the activities were compared at a fixed substrate concentration or when apparent K_M and V_{max} values were determined. The Type 3 disaccharide was a less good acceptor at a fixed concentration and kinetic analysis revealed that the apparent K_M value was higher although the V_{max} was approximately the same as for the Type 1 and Type 2 disaccharides. This behaviour is in contrast to the specificity pattern exhibited by the α-2-fucosyltransferases from submaxillary glands [9] or milk [8] from ABH secretors which showed a marked preference for Type 1 acceptors. A different pattern again was exhibited by the highly purified α-2-fucosyltransferase from porcine submaxillary glands; this enzyme had a preference for the Type 3 disaccharide Galβ1-3GalNAc and although the Type 1 structure Galβ1-3GlcNAc was also a reasonably good acceptor the Type 2 disaccharide, Galβ1-4GlcNAc, was a much poorer substrate than either of the other disaccharides [36]. The enzyme purified from plasma did show a slight preference for the Type 1 tetrasaccharide lacto-*N*-tetraose relative to the Type 2 structure lacto-*N*-neotetraose and this difference may indicate that greater specificity preferences might be shown for larger acceptor substrates where other molecular interactions could affect the binding of the transferase. Competition experiments strongly suggested, however, that the Type 1 and Type 2 oligosaccharides were competing for the same α-2-fucosyltransferase and hence that, although the enzyme had not

Table 5. Competition experiments under steady state conditions; (a) lacto-*N*-biose I and *N*-acetyllactosamine and (b) lacto-*N*-tetraose and lacto-*N*-neotetraose.
Assay conditions as in Table 3 except that all acceptor substrates were at concentrations of 3 mM.

Acceptor substrates		Fucose transferred (pmol/min/ml)	Product expected:	
(a) Lacto-*N*-biose I	*N*-Acetyllactosamine		One enzyme	Two enzymes
+	-	50		
-	+	50		
+	+	72	81	100
(b) Lacto-*N*-tetraose	Lacto-*N*-neotetraose			
+	-	79		
-	+	56		
+	+	103	93	134

+, Substrate present.
- , Substrate absent.

been purified to homogeneity, the preparation was not a mixture of two α-2-fucosyltransferases with differing substrate specificities that had co-purified. Thus, despite the dissimilarities in the conformations of the Type 1 and Type 2 disaccharides that initially raised doubts about whether a single enzyme could utilise both structures as substrates [8] it appears that the α-2-fucosyltransferase encoded by the *H* gene expressed in haemopoietic tissue is able to transfer fucose equally well to the β-galactosyl residue in both Type 1 and Type 2 structures. The predominance on the erythrocyte surface of ABH determinants based on Type 2 oligosaccharide chains [3] must therefore be attributed to the availability of the requisite precursor structures rather than to a preference of the *H* gene product for the Type 2 structures.

Recently Ernst *et al.* [37] described a gene transfer system for the isolation of human DNA sequences controlling the expression of an α-2-L-fucosyltransferase. Mouse L cells were transfected with DNA from the human A431 cell line and tested for expression of the enzyme [38]. Phenyl β-D-galactoside was the only substrate used for characterisation of the transferase but on the basis of pH optimum and apparent K_M values for donor and acceptor substrates the authors concluded that the enzyme resembled the *H* transferase found in haemopoietic tissue more closely than the enzyme occurring in tissues where the *Se* gene is functional. It is noteworthy that the apparent K_M (3-4 mM) for phenyl β-D-galactoside found for the enzyme expressed in the transfectants is practically identical with the value recorded in this paper (4 mM) for the α-2-fucosyltransferase purified from human plasma. The apparent K_M for GDP-fucose (12.4 μM) was also closely similar to the value (11 μM) found for the purified plasma transferase and differed from the much higher apparent K_M values found for the enzymes in tissues where the *Se* gene is operative [8-10].

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