Glycoconjugate J (1985) 2:61–78

Variant Forms of α-2-L-Fucosyltransferase in Human Submaxillary Glands from Blood Group ABH "Secretor" and "Non-secretor" Individuals

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Received November 12, 1984.

Key words: α-2-L-fucosyltransferase, blood group H gene, secretor gene, submaxillary glands

The properties of the α-2-L-fucosyltransferases in submaxillary gland preparations from blood group ABH "secretors" and "non-secretors" were compared. The level of activity in the "non-secretor" gland homogenates amounted to about 5% only of that found in the "secretor" gland preparations. The enzymes from the two sources differed in solubility properties, charge and affinities for donor and acceptor substrates. The enzyme from "secretor" glands showed a preference for acceptors with Type 1 [D-galactosylβ(1-3)-*N*-acetyl-D-glucosamine] structures whereas the enzyme from "non-secretor" glands had a preference for Type 2 [D-galactosylβ(1-4)-N-acetyl-D-glucosamine] structures.

These results demonstrate that expression of the secretor gene (*Se*) is associated with a molecular form of the α-2-L-fucosyltransferase that is different from the species present in the same tissue when the *Se* gene is not expressed.

The antigens that constitute the ABO blood group system are glycoconjugates and the specificity resides in the carbohydrate structures at the non-reducing ends of the oligosaccharide chains [1-3]. Both A and B structures are derived from a common precursor, the blood group H structure, which contains a terminal L-fucosylα(1-2)-D-galactosyl β(1- disaccaride grouping [4]. With very rare exceptions H determinants are present on all human red cells but they are absent or expressed only very weakly in secretions, such as saliva, unless an individual also carries a secretor gene, *Se* [5]. Thus, those homozygous or heterozygous for *Se* are ABH "secretors" whereas those homozygous for the allele *se* are "non-secretors".

The mode of action of *Se* is not yet conclusively established. Before the structures and biosynthesis of the ABH determinants were known the function usually attributed to the gene *Se* was that of allowing the ABH substances to be secreted in a water-soluble

form. When it became apparent that the formation of H structures was the crucial stage at which the biosynthetic pathways of the blood group specific glycoproteins diverged in "secretors" and "non-secretors" it was proposed that the *Se* gene is a structural gene (called at the time a transforming gene) responsible for the addition of fucosyl units to form H determinants [6]. Subsequently this proposal was modified in order to have a single hypothesis which explained the formation of ABH structures on the red cells as well as in secretions [7]; an *H* gene was invoked which codes for the α-2-L-fucosyltransferase (EC 2.4.1.-) synthesising H structures and the secretor gene, *Se*, was seen as a regulator gene controlling the expression of the *H* gene in epithelial tissues. Recently the idea that *Se* is a structural gene has been revived and a model proposed in which *H* and *Se* are both structural genes encoding α-2-L-fucosyltransferases. According to this hypothesis one of these genes (*H*) is expressed in tissues of mesodermal origin and the other (*Se*) in epithelial tissues [8].

ABH determinants can be built on two types of precursor chain; Type 1 chains have terminal non-reducing Galβ1-3GlcNAc disaccharide structures whereas Type 2 chains have terminal non-reducing *N*-acetyllactosamine (Galβ1-4GlcNAc) structures [9]. The glycosphingolipids and glycoproteins with ABH activity isolated from red cell membranes [10-12] have ABH structures derived from Type 2 chains whereas the ABH active glycoproteins found in secretions have both Type 1 and Type 2 structures [9, 13]. Conformational analysis of the Type 1 and Type 2 disaccharides led Lemieux [14] to comment that since these compounds probably have very different favoured conformations they must present very dissimilar profiles to antibodies and enzymes. In the light of this observation, and the known distribution of Type 1 and Type 2 H structures, the proposal [8] that *H* and *Se* are both structural genes also incorporated the idea that the *H* gene expressed in tissues of mesodermal origin, such as vascular endothelia and red blood cells, encodes an α-2-L-fucosyltransferase acting preferentially on Type 2 chains whereas the *Se* gene expressed in epithelial tissues encodes a different α-2-L-fucosyltransferase which acts preferentially on Type 1 chains.

It has long been known that salivas belonging to the "non-secretor" group are not entirely devoid of ABH serological activity [15] and low levels of α-2-L-fucosyltransferase activity are detectable in submaxillary gland preparations from "non-secretor" individuals [16, 17]. In the present paper various properties of the α-2-L-fucosyltransferases in submaxillary glands from "secretor" and "non-secretor" individuals are examined and a comparison made of the relative affinities of partially purified preparations for Type 1 and Type 2 acceptor substrates. A brief report of some of this work was given earlier [17].

Materials and Methods

Materials

GDP-L-[U-^{14}C]fucose (210-292 mCi/mmol), UDP-*N*-acetyl-D-[1-^{14}C]galactosamine (54 mCi/mmol) and UDP-D-[U-^{14}C]galactose (300 mCi/mmol) were obtained from Amersham International Ltd., UK. *N*-Acetyllactosamine (Galβ1-4GlcNAc) prepared by a modification of the method of Kuhn and Kirshenlohr [18], and lacto-*N*-biose I (Galβ1-3GlcNAc)

synthesised by a modification of the method of Flowers [19] were kindly supplied by Dr A.S.R. Donald, Clinical Research Centre, Harrow, UK. 2′ -Fucosyllactose (Fucα1-2Galβ1-4 Glc), lacto-*N*-tetraose (Galβ1-3GlcNAcβ1-3Galβ1-4Glc) and lacto-*N-neo*tetraose (Galβ1-4 GlcNAcβ1-3Galβ1-4Glc) were isolated from human milk as described [20]. Phenyl-β-D-galactoside was obtained from Koch-Light, Colnbrook, UK. Sodium periodate and Triton X-100 were purchased from BDH Chemicals, UK. Diethylaminoethyl cellulose (DE 52) and other chromatography papers were obtained from Whatman, UK. Sephacryl S-300 and CNBr-activated Sepharose 4B were purchased from Pharmacia, Hounslow, UK. Adipic acid dihydrazide, bovine pancreas chymotrypsinogen, human serum albumin, bovine liver catalase and Jack bean urease were obtained from Sigma Chemical Co., Poole, UK.

Submaxillary Gland Preparations

Human submaxillary glands were collected *post mortem* and frozen at -40°C until required. The ABO blood groups of the donors were determined on cadaver blood samples and the ABH secretor and Lewis (Le^a and Le^b) status was established by haemagglutination-inhibition tests [21] on the supernatants obtained after homogenisation and centrifugation of small samples of the glands.

For the enzyme preparations the glands were freed of fatty and connective tissue then sliced thinly while still semi-frozen. The slices were rinsed in homogenisation medium (1 mM $NaHCO_3$ containing 1 mM dithiothreitol) to remove much of the trapped blood and were then homogenised in fresh medium (5 ml per gram of tissue) with an Ultra-Turrax blender at setting 5 for five bursts of 10 sec at 4°C. The homogenate was filtered through a coarse metal grid and the filtrate was further homogenised with a Dounce-type homogeniser (5-10 strokes at 4°C). The homogenate was centrifuged in an MSE Chillspin at 1000 rpm for 5 min and the sedimented material was resuspended in a volume of homogenisation medium equal to the starting volume ("low-speed pellet"). The supernatant was transferred to fresh centrifuge tubes and centrifuged at 200 000 × *g* for 30 min using a 75 Ti rotor in a Beckman L2-65B ultracentrifuge. The pellets were resuspended in a volume of homogenisation medium equal to the volume of the supernatant and the fraction was designated "high-speed pellet".

Extraction with Triton X-100

To extract the Triton-soluble material, detergent was added to a concentration of 1% (w/v) (unless otherwise stated) to the low-speed supernatant fraction and this was then centrifuged at 200 000 × *g* as described above. The pellet was resuspended in the original volume of homogenisation medium.

Chromatography of Triton X-100-solubilised Fucosyltransferase on GDP-adipate-Sepharose

GDP-adipate-Sepharose 4B was prepared as described by Lamed *et al.* [22]. Adipic acid dihydrazide was allowed to react with CNBr-activated Sepharose 4B and the resultant

Sepharose-adipic-dihydrazide was coupled with periodate-oxidised GDP. The degree of coupling was estimated from the phosphate content of the gel assayed by the method of Bartlett [23]; in different preparations, amounts ranging from 1.5 to 4 μmol of GDP were bound per ml of wet gel.

Chromatography of the Triton X-100-soluble material from the submaxillary glands was carried out on a column (4 × 1.2 cm) of GDP-adipate-Sepharose equilibrated with 20 mM Tris-maleate buffer pH 6.8. The column was eluted sequentially with Tris-maleate buffer pH 6.8 at concentrations of 20 mM and 200 mM and finally with 50 mM Tris-maleate buffer pH 6.8 containing 1 M NaCl. Fractions of approximately 1 ml were collected.

Ion Exchange Chromatography of Triton X-100-solubilised Fucosyltransferases

The Triton-soluble material was loaded onto a column (30 × 1.5 cm) of DEAE-cellulose (DE 52) equilibrated with 10 mM Tris-maleate buffer pH 7.6. The column was eluted with one column volume of 10 mM Tris-maleate buffer pH 7.6 followed by a linear gradient of this buffer at concentrations from 40 mM to 200 mM. Fractions of approximately 2 ml were collected.

Gel Filtration Chromatography of Triton X-100-solubilised Fucosyltransferase Preparations for Molecular Size Determinations

Samples (2.5 ml) of Triton X-100-solubilised fucosyltransferase preparations from submaxillary glands were freed from unbound Triton by passage through columns (1.5 × 5.5 cm) of Sephadex G-25 equilibrated with 10 mM Tris-maleate buffer pH 7.6. Samples of the eluates containing approximately the same amount of enzyme activity were loaded on a column (40 cm × 1.6 cm) of Sephacryl S-300, also equilibrated with 10 mM Tris-maleate buffer pH 7.6, and the column was then eluted with the same buffer. Fractions of approximately 1 ml were collected. The column was calibrated with bovine pancreas chymotrypsinogen (Mol wt 23 000), human serum albumin (Mol wt 68 500), bovine liver catalase (Mol wt 221 600) and Jack bean urease (Mol wt 440 000). The proteins were detected by the Coomassie brilliant blue (G250) method of Read and Northcote [24].

Glycosyltransferase Assays

For the assays of α-2-L-fucosyltransferase, 20-50 μl of the enzyme source were added to GDP-L-[^{14}C]fucose (normally 0.18 - 0.24 nmol, 75 000 cpm), ATP (2 mM), $MnCl_2$ (5 mM), acceptor (10 mM unless otherwise stated) and Tris-maleate buffer pH 6.8 (10 mM) in a total volume of 100 μl. The mixtures were incubated at 37° C for either 4 or 16 h. When phenyl-β-D-galactoside was used as the acceptor substrate the mixture was spotted on Whatman No. 40 paper and developed with ethyl acetate/pyridine/water, 10/4/3 by vol (solvent 1), for 4 h. With disaccharide or tetrasaccharide acceptors the incubation mixtures were chromatographed on Whatman DE 81 paper. The mixtures containing the products obtained with disaccharides were chromatographed in solvent 1 for 40 h and those containing the tetrasaccharide products were separated in ethyl acetate/pyridine/water, 2/1/1 by vol (solvent 2), for 64 h. The papers were dried and scanned for radioactivity in

a Packard radiochromatogram scanner. The mobilities of the radioactive peaks were measured relative to known compounds and these areas were then cut out and counted by liquid scintillation spectrometry.

Two distinct α-3-L-fucosyltransferase activities have been demonstrated in human saliva and milk [25, 26]. One is present in the secretions of virtually all individuals and transfers L-fucose only to the C-3 position of subterminal *N*-acetyl-D-glucosamine units. The other is expressed only when an individual carries an *Le* gene and is an α-3/4-fucosyltransferase which catalyses the transfer of L-fucose to the C-3 and C-4 position of subterminal *N*-acetyl-D-glucosamine and D-glucose residues. In the present experiments these activities were assayed under the same conditions as the α-2-L-fucosyltransferase except that *N*-acetyllactosamine (2.5 mM) was used as the acceptor substrate for the combined activities of the two enzymes and 2′-fucosyllactose (2.5 mM) as a specific acceptor substrate for the α-3/4-fucosyltransferase activity. After incubation the mixtures were spotted on Whatman DE 81 paper and chromatographed in solvent 1 for either 16 h (2′-fucosyllactose product) or 40 h (*N*-acetyllactosamine product). When both α-2- and α-3-L-fucosyltransferase activities occur together in an enzyme source the substrate *N*-acetyllactosamine functions as an acceptor for both these enzymes. Difucosyl products are seldom observed and the product of transfer to the C-2 position of the terminal galactose residue (2′-fucosyllactosamine, R_{Lac} 1.0 in solvent 1) is separable from the product of transfer to the C-3 position of the subterminal *N*-acetylglucosamine unit (3-fucosyllactosamine, R_{Lac} 0.75 in solvent 1).

β-D-Galactosyltransferase (UDP-galactose:*N*-acetylglucosamine β-galactosyltransferase, EC 2.4.1.90) was measured with incubation mixtures consisting of: enzyme source (20 μl), UDP-D-[^{14}C]galactose (0.12 nmol, 120 000 cpm), ATP (5 mM), $MnCl_2$ (20 mM), *N*-acetyl-D-glucosamine (1 mM) and Tris-maleate buffer pH 6.8 in a total volume of 100 μl. The products were separated on Whatman DE 81 paper in solvent 1. α-3-*N*-Acetyl-D-galactosaminyltransferase (UDP-*N*-acetylgalactosamine:2′-fucosylgalactose α-3-*N*-acetylgalactosaminyltransferase, EC 2.4.1.40) was assayed under the same conditions except that UDP-*N*-acetyl-D-[^{14}C]galactosamine (1.4 nmol, 128 000 cpm) was used as the donor substrate and 2′-fucosyllactose (2.5 mM) as the acceptor substrate. The products were spotted on DE 81 paper and separated in propan-1-ol/ethyl acetate/pyridine/water, 5/1/1/3 by vol (solvent 3).

Results

Relative Levels of Glycosyltransferases in Submaxillary Glands from "Secretors" and "Non-secretors"

Supernatants from homogenates of submaxillary glands from blood group A donors were tested in haemagglutination inhibition tests for A, H, Le^a and Le^b activity. Those containing A, H and Le^b serological activities were grouped as "secretors" and those containing only Le^a activity as "non-secretors". Homogenates of three individual preparations from "secretor" glands and three from "non-secretor" glands were examined for α-2-L-fucosyltransferase, α-3-L-fucosyltransferase, α-3/4-L-fucosyltransferase, β-D-galac-

Table 1. Relative activities of some glycosyltransferases in "secretor" and "non-secretor" submaxillary glands.

Enzyme	Acceptor	Activity (nmol/h/g)	
		"secretor"	"non-secretor"
α-2-L-fucosyltransferase	phenyl-β-D-galactoside	3.74	0.15
α-3-L-fucosyltransferase	*N*-acetyllactosamine	12.22	11.40
α-3/4-L-fucosyltransferase	2'-fucosyllactose	3.30	3.32
α-*N*-acetyl-D-galactosaminyl-transferase	2'-fucosyllactose	4.48	6.04
β-D-galactosyltransferase	*N*-acetylglucosamine	21.06	22.41

The assay conditions are given in Materials and Methods. The submaxillary gland homogenate (20 μl) was incubated in a final volume of 100 μl for 4 h at 37°C with acceptor concentrations of 2.5 mM. Enzyme activity is expressed in terms of nmol [^{14}C]sugar incorporated/h/g tissue and the values given are the means of those obtained for three individual glands.

tosyltransferase and α-3-*N*-acetyl-D-galactosaminyltransferase activities (Table 1). The only significant difference observed between the two groups was the amount of α-2-L-fucosyltransferase activity; the "non-secretor" tissues contained only about 5% of the level of activity measured in the "secretor" tissues.

Subcellular Distribution of Glycosyltransferases in Submaxillary Gland Preparations

The homogenates from four "secretor" and four "non-secretor" submaxillary glands were separated into the low-speed pellet, high-speed pellet and supernatant fractions as described. Samples from each fraction were assayed for α-2- and α-3-L-fucosyltransferase and β-D-galactosyltransferase activities, and the results were expressed as the percentage of the total of each enzyme activity recovered in the separate fractions. Typical distribution patterns are shown in Fig. 1. No significant differences were observed for the distribution of the α-3-L-fucosyltransferases or the β-D-galactosyltransferases in preparations from "secretor" or "non-secretor" glands. In both groups the major part of the β-D-galactosyltransferase activity was found in the soluble fraction and about 20% of the α-3/4-L-fucosyltransferase activity measured with 2'-fucosyllactose, and about 30% of α-3-L-fucosyltransferase activity measured with *N*-acetyllactosamine, were released into the soluble fraction. The distribution of the α-2-L-fucosyltransferase activity, however, differed in the preparations from "secretor" and "non-secretor" glands. In the "secretor" glands the enzyme was largely membrane-bound and only 2.5% of the activity was present in the soluble fraction whereas 25% of the activity occurred in the soluble fraction in preparations from the "non-secretor" glands.

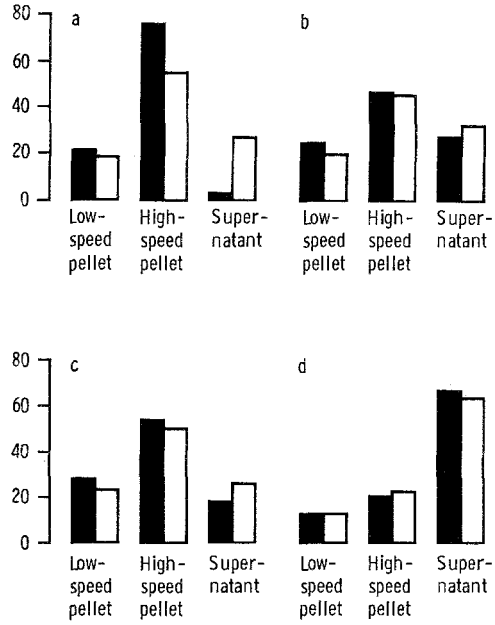

Figure 1. Subcellular distribution of glycosyltransferases. Submaxillary glands from "secretors" and "non-secretors" were homogenised and fractionated as described in the text. Aliquots of the low-speed pellet, high-speed pellet and supernatant were assayed for a) α-2-L-fucosyltransferase activity with phenyl-β-D-galactoside as acceptor, b) α-3-L-fucosyltransferase activity with *N*-acetyllactosamine as acceptor, c) α-3/4-L-fucosyltransferase activity with 2' -fucosyllactose as acceptor, and d) β-D-galactosyltransferase activity with *N*-acetylglucosamine as acceptor. The reaction mixtures and assay procedures are given in the Materials and Methods section. The enzyme activity of each fraction is expressed as a percentage of the total activity recovered. Closed columns; "secretor" submaxillary glands: Open columns; "non-secretor" submaxillary glands.

Effect of pH and Manganese Concentration on the Activity of α-2-L-Fucosyltransferases from "Secretor" and "Non-secretor" Submaxillary Glands

The resuspended high-speed pellet fractions from "secretor" and "non-secretor" submaxillary glands were assayed for α-2-L-fucosyltransferase activity at a series of pH values from 5.5 to 8.5 with phenyl β-D-galactoside as acceptor substrate. Both preparations had broad optima (Fig. 2). Little difference in activity occurred between pH 6.0 and 7.2 for the "secretor" tissue or between pH 6.4 and 7.6 for the "non-secretor" preparations. All subsequent assays were carried out at pH 6.8.

The high-speed pellet fractions were also tested for α-2-L-fucosyltransferase activity at a range of manganese chloride concentrations (Fig. 3). With phenyl β-D-galactoside as acceptor substrate the "secretor" preparations had maximum activity in the presence of 5 mM $MnCl_2$ and higher concentrations were inhibitory. The "non-secretor" fraction required 10-20 mM $MnCl_2$ for maximum activity and concentrations of 50 mM and above were inhibitory.

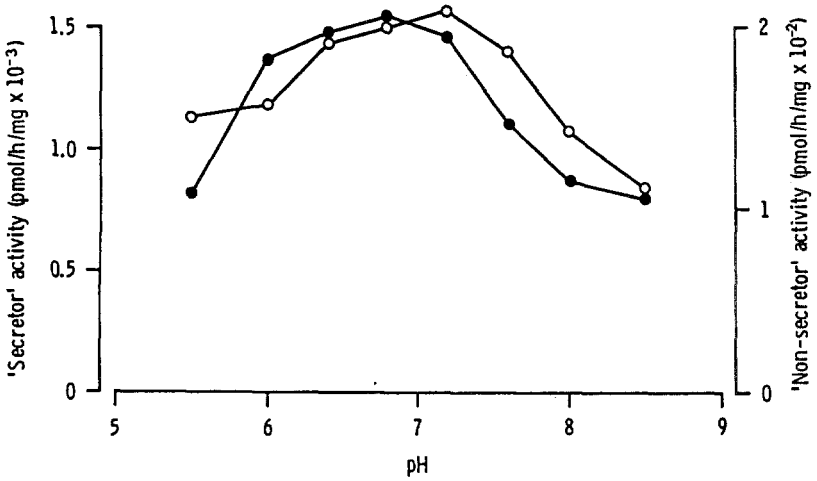

Figure 2. Effect of pH on the α-2-L-fucosyltransferase activity in preparations from "secretor" and "non-secretor" submaxillary glands. The high-speed pellet fractions from "secretor" and "non-secretor" submaxillary glands were assayed for α-2-L-fucosyltransferase activity with phenyl-β-D-galactoside as acceptor substrate. The reaction mixture was the same as that given in Materials and Methods except that Tris-maleate buffer was used at various pH values between 5.5 and 8.5. The mixtures were incubated for 4 h at 37°C. ●, "secretor" submaxillary gland preparation. ○, "non-secretor" submaxillary gland preparation.

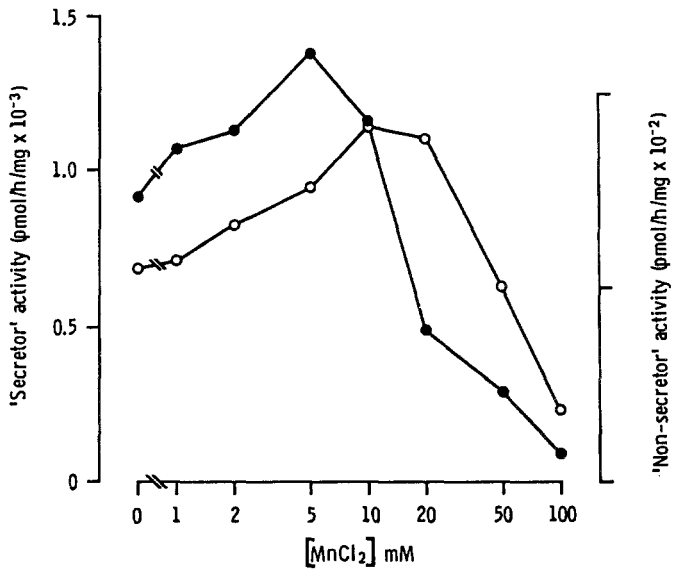

Figure 3. Effect of $MnCl_2$ concentration on α-2-L-fucosyltransferase activity in preparations from "secretor" and "non-secretor" submaxillary glands. The high-speed pellet fractions from "secretor" and "non-secretor" submaxillary glands were assayed for α-2-L-fucosyltransferase activity with phenyl-β-D-galactoside as acceptor substrate. The reaction mixture was the same as that given in Materials and Methods except that $MnCl_2$ was used at concentrations ranging from 1.0 to 100 mM. The mixtures were incubated for 4 h at 37°C. ●, "secretor" submaxillary gland preparation; ○, "non-secretor" submaxillary gland preparation.

Table 2. Solubility in Triton X-100 of α-2-L-fucosyltransferases in "secretor" and "non-secretor" submaxillary glands.

Triton X-100 concentration % (w/v)	Percentage of soluble α-2-L-fucosyltransferase	
	"Secretor" tissue	"Non-secretor" tissue
0	2.9	25.6
0.1	7.5	24.8
0.2	13.9	26.7
0.5	21.2	41.3
1.0	24.4	69.3
2.0	24.7	69.7

The soluble and insoluble fractions were assayed for α-2-L-fucosyltransferase activity as described in Materials and Methods. The results are expressed as the amount of activity recovered in the soluble fraction relative to the total activity recovered for each Triton X-100 concentration.

Solubilities of the α-2-L-Fucosyltransferases from "Secretor" and "Non-secretor" Glands in Triton X-100

Homogenates of "secretor" and "non-secretor" submaxillary glands were centrifuged at 1 000 rpm (120 × *g*) and aliquots of the supernatants were mixed with various concentrations of Triton X-100 and then centrifuged at 100 000 × *g* for 1 h. The resultant pellet and supernatant fractions were assayed for α-2-L-fucosyltransferase activity with phenyl β-D-galactoside as acceptor substrate. The percentage of α-2-L-fucosyltransferase activity in the supernatant fraction from the "secretor" tissue increased up to about 25% in 1% Triton X-100 whereas at the same Triton concentration approximately 70% of the α-2-L-fucosyltransferase was released into the soluble fraction from "non-secretor" glands (Table 2).

No significant differences were detected in the detergent solubility of the α-3-*N*-acetylgalactosaminyltransferase or the α-3-L-fucosyltransferase in preparations from "secretor" and "non-secretor" submaxillary glands. Up to 70% of the α-3-*N*-acetyl-D-galactosaminyltransferase activity could be solubilised with 1% Triton X-100 and about 45% of the α-3-L-fucosyltransferases were soluble in the presence of 2% Triton X-100 (data not shown).

Ion Exchange Chromatography of Triton X-100-solubilised α-2-L-Fucosyltransferases from "Secretor" and "Non-secretor" Glands

The Triton X-100-solubilised fractions from "secretor" and "non-secretor" submaxillary glands were chromatographed on columns of DEAE-cellulose (DE 52) eluted with Tris-maleate buffer. The proportion of α-2-L-fucosyltransferase activity bound to the ion exchange cellulose varied from 5 to 25% of the total recovered activity when samples from six different "secretor" glands were examined. No further material was bound when the

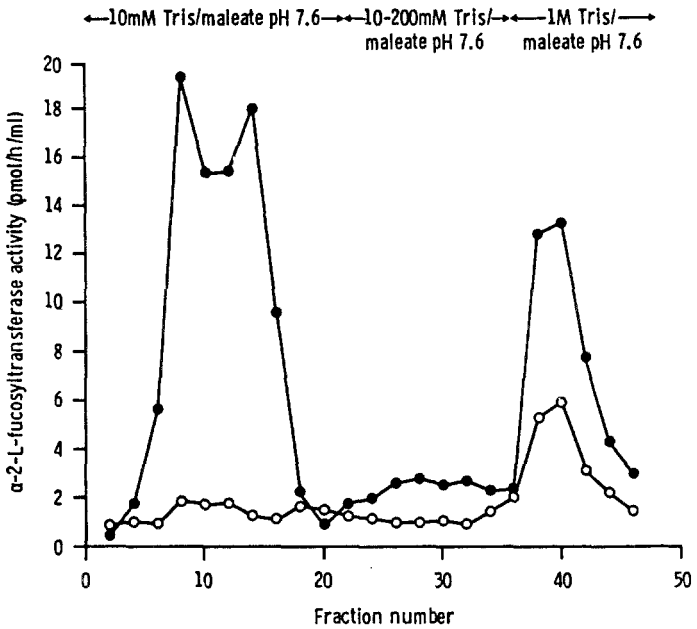

Figure 4. Chromatography of α-2-L-fucosyltransferases from "secretor" and "non-secretor" submaxillary glands on DEAE-cellulose. Samples of Triton X-100-solubilised material from "secretor" and "non-secretor" submaxillary glands were chromatographed on DEAE-cellulose (DE 52) as described in the text. Alternate fractions (20 μl) were assayed for α-2-L-fucosyltransferase activity for 16 h at 37°C with phenyl-β-D-galactoside as substrate. ●, "secretor" submaxillary gland preparation; ○, "non-secretor" submaxillary gland preparation.

unretarded enzyme was recycled through a freshly prepared DEAE-cellulose column indicating that the initial failure to bind did not result from overloading the column. In contrast, nearly all the α-2-L-fucosyltransferase activity in the samples from "non-secretors", which contained approximately the same amount of total protein as the "secretor" preparations, bound to the ion exchange material and was eluted at approximately the same position as the retained portion of the enzyme in the "secretor" samples (Fig. 4).

Molecular Sizes of the Triton X-100-solubilised α-2-L-Fucosyltransferases from "Secretor" and "Non-secretor" Submaxillary Glands

Triton X-100 extracts from "secretor" and "non-secretor" submaxillary glands were chromatographed on columns of Sephadex G-25 to remove unbound Triton and the resultant α-2-L-fucosyltransferase preparations were then loaded on a column of Sephacryl S-300 and eluted with Tris-maleate buffer pH 7.6. All the α-2-L-fucosyltransferase activity from the "secretor" glands emerged close to the void volume indicating that the material had an approximate molecular weight of 500 000 (Fig. 5). With the preparations from the "non-secretor" glands two peaks of activity were detected; one coincided with the activity found in the "secretor" preparations and the second had an approximate molecular weight of 210 000.

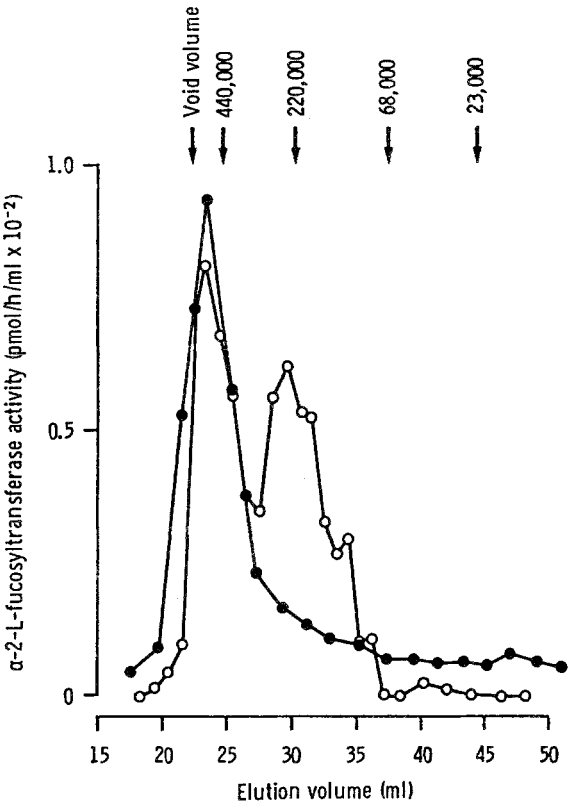

Figure 5. Chromatography of α-2-L-fucosyltransferase from "secretor" and "non-secretor" submaxillary glands on Sephacryl S-300. Samples of Triton X-100-soluble extracts from "secretor" and "non-secretor" submaxillary glands were freed from unbound Triton and chromatographed on Sephacryl S-300 as described in the text. Alternate fractions (20 μl) were assayed for α-2-L-fucosyltransferase activity for 16 h at 37°C with phenyl-β-D-galactoside as substrate. ●, "secretor" submaxillary gland preparation; ○, "non-secretor" submaxillary gland preparation.

Separation of the α-2- and α-3-L-Fucosyltransferases on GDP-Adipate-Sepharose 4B

The low-speed supernatant fractions from the submaxillary gland homogenates were treated with Triton X-100 and centrifuged at 100 000 × *g* for 1 h. The clear supernatant fractions were freed from unbound Triton X-100 by passage through columns of Sephadex G-25 equilibrated with 20 mM Tris-maleate buffer pH 7.6 and then were loaded on a column of GDP-adipate-Sepharose 4B equilibrated with the same buffer (Fig. 6). The greater part of the α-3-L-fucosyltransferase activity (measured with *N*-acetyllactosamine as acceptor) bound to the GDP-Sepharose whereas the α-2-L-fucosyltransferase was eluted unretarded. Similar results were obtained irrespective of whether the preparations came from "secretor" or "non-secretor" submaxillary glands. The bound α-3-L-fucosyltransferase could be released from the GDP-Sepharose with buffer containing 1 M NaCl.

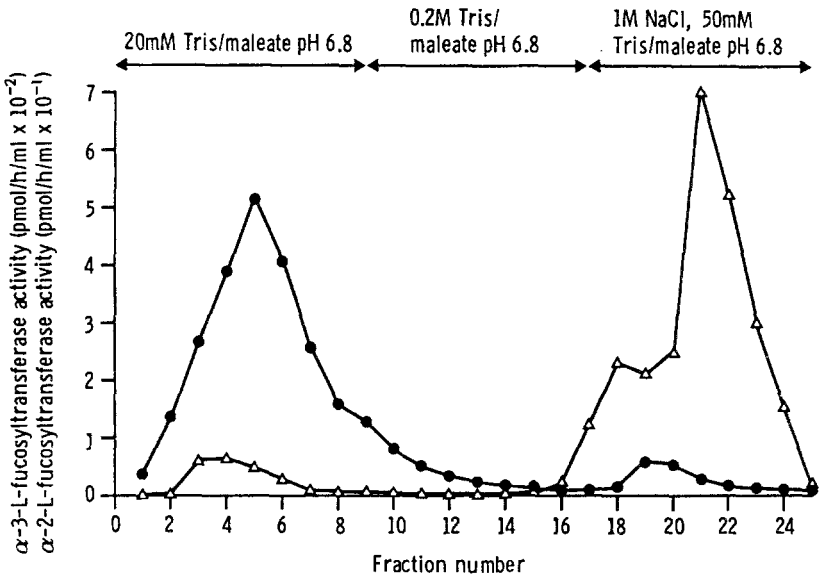

Figure 6. Chromatography of α-2- and α-3-L-fucosyltransferases on GDP-Sepharose. Triton X-100-soluble material from a "secretor" submaxillary gland was freed from unbound Triton and chromatographed on a column of GDP-adipate-Sepharose as described in the text. Aliquots (20 μl) of each fraction were assayed for α-2-L-fucosyltransferase activity with phenyl β-D-galactoside as acceptor substrate, and α-3-L-fucosyltransferase with *N*-acetyllactosamine as acceptor substrate. The reaction mixtures were incubated for 16 h at 37°C. ●, α-2-L-fucosyltransferase activity; △, α-3-L-fucosyltransferase activity.

Substrate Specificity and Kinetic Parameters of α-L-Fucosyltransferases from "Secretor" and "Non-secretor" Submaxillary Glands

α-2-L-Fucosyltransferase preparations were made by Triton X-100 extraction of submaxillary gland homogenates, followed by chromatography on GDP-Sepharose as described above. The final preparations were tested for fucosyltransferase activity with *N*-acetyllactosamine as acceptor substrate. On chromatography of the incubation mixtures on Whatman DE 81 paper in solvent 1 for 40 h the radioactivity in the product with R_{Lac} value 0.75 (3-fucosyllactosamine) was always less than 10% of the radioactivity in the product with R_{Lac} value 1.0 (2′-fucosyllactosamine). Thus there was little or no α-3-L-fucosyltransferase in these preparations to compete with the α-2-L-fucosyltransferase for donor and acceptor substrates. Accurate K_M and V_{max} values could not be determined because of the lack of availability of unlabelled GDP-L-fucose and the impracticability of using sufficient quantities of GDP-L-[^{14}C]fucose to ensure saturating concentrations of the donor substrate. Nevertheless the preparations were considered suitable sources of α-2-L-fucosyltransferase to determine the relative affinities of the enzymes from "secretor" and "non-secretor" tissues for Type 1 and Type 2 oligosaccharide acceptor substrates and for the donor substrate GDP-L-fucose.

Table 3. Apparent K_M and relative V_{max} values for α-2-L-fucosyltransferases from "secretor" and "non-secretor" submaxillary glands with Type 1 and Type 2 acceptor substrates.

Acceptor substrate	Relative mobility of product	α-2-L-Fucosyltransferase					
		"Secretor" glands			"Non-secretor" glands		
		Apparent K_M (mM)		Relative[d]	Apparent K_M (mM)		Relative[d]
		Mean[c]	Range	V_{max}	Mean[c]	Range	V_{max}
Phenyl β-D-galactoside	1.5[a]	2.1	(1.8-2.5)	100	1.6	(1.4-1.8)	100
Lacto-*N*-biose I [Galβ1-3GlcNAc]	0.94[a]	1.0	(0.6-1.4)	100	3.7	(2.2-5.2)	120
Lacto-*N*-tetraose [Galβ1-3GlcNAcβ1-3Galβ1-4Glc]	0.74[b]	0.7	(0.4-0.9)	130	8.9	(5.2-12.0)	120
N-Acetyllactosamine [Galβ1-4GlcNAc]	1.0[a]	1.1	(1.0-1.3)	40	0.7	(0.4-1.0)	890
Lacto-*N*-*neo*tetraose [Galβ1-4GlcNAcβ1-3Galβ1-4Glc]	0.73[b]	1.1	(0.8-1.3)	20	1.3	(1.2-1.5)	250

Details of the incubation mixtures and assay procedures are given in the text.
[a] Mobility relative to lactose in solvent 1.
[b] Mobility relative to lacto-*N*-tetraose in solvent 2.
[c] Mean values for three separate submaxillary gland preparations.
[d] V_{max} relative to that obtained for phenyl β-D-galactoside.

Preliminary experiments showed that both the Type 1 oligosaccharides (lacto-*N*-biose I and lacto-*N*-tetraose) and the Type 2 oligosaccharides (*N*-acetyllactosamine and lacto-*N*-*neo*tetraose) were acceptor substrates for the α-2-L-fucosyltransferases in preparations from either "secretor" or "non-secretor" glands. In order to determine the apparent K_M and V_{max} values, varying concentrations (0.1 mM to 10 mM) of these four compounds were incubated with the enzyme preparations in the presence of 2.24 μM GDP-L-[^{14}C]fucose. The amounts of [^{14}C]fucose incorporated into the various products at different concentrations were used to derive Lineweaver-Burk plots from which the apparent K_M and V_{max} values were calculated. The values obtained for three separate preparations from "secretor" glands and three preparations from "non-secretor" glands are given in Table 3. The mean of the V_{max} values for the three preparations in each group are expressed relative to the values obtained with phenyl β-D-galactoside as acceptor substrate.

With the α-2-L-fucosyltransferase from "secretor" tissue the apparent K_M values for the Type 1 (lacto-*N*-biose I and lacto-*N*-tetraose) and Type 2 (*N*-acetyllactosamine and lacto-*N*-*neo*tetraose) acceptor substrates were not very different, but the rate of transfer of fucose to the Type 1 acceptors was markedly higher than the rate of transfer to the Type 2 acceptors. In contrast, the α-2-L-fucosyltransferases in the preparations from the "non-secretor" glands had lower apparent K_M values and higher relative V_{max} values with the Type 2 than with the Type 1 acceptor substrates (Table 3).

Phenyl-β-D-galactoside is the low-molecular-weight compound frequently used as acceptor substrate in assays of the α-2-L-fucosyltransferase [16] because it allows the specific measurement of this enzyme in tissues and fluids containing α-3- and/or α-3/4-L-fucosyltransferase activities. This compound was a less efficient acceptor for the partially purified "secretor" gland-derived α-2-L-fucosyltransferase than were the Type 1 oligosaccharides. With the "non-secretor" gland-derived enzyme phenyl β-D-galactoside was much less effective than the Type 2 oligosaccharide substrates (Table 3).

To obtain the apparent K_M values for GDP-fucose the three separate preparations of α-2-L-fucosyltransferases from "secretor" and "non-secretor" glands were incubated with concentrations of GDP-L-[^{14}C]fucose ranging from 2.4 to 35 μM in the presence of saturating concentrations of phenyl-β-D-galactoside. From the derived Lineweaver-Burk plots a mean apparent K_M value for GDP-L-fucose of 86 μM (range 71 - 100 μM) was calculated for the three "secretor" preparations and a mean value of 37 μM (range 23 - 50 μM) for the "non-secretor" preparations.

The low yields of soluble α-2-L-fucosyltransferase activity obtained after homogenisation of the submaxillary glands in the absence of detergent (Fig. 1) precluded any detailed studies being carried out on these enzyme fractions. However, in order to determine whether the buffer-soluble α-2-L-fucosyltransferase differed in K_M for GDP-fucose from the Triton X-100-solubilised material, the high-speed supernatant fractions from three "secretor" submaxillary glands were pooled and subjected to GDP-Sepharose chromatography to separate the α-2- from the α-3-L-fucosyltransferase. The partially purified enzyme which had not been exposed to Triton had a K_M for GDP-fucose of 71 μM.

Discussion

Human α-2-L-fucosyltransferases that transfer L-fucose from GDP-L-fucose to the C-2 position of terminal non-reducing β-D-galactosyl residues have been identified in haemopoietic [27-31] and epithelial [32, 33] tissues. The failure to detect this enzyme activity in milk [32], submaxillary glands or stomach mucosa [33] of "non-secretor" individuals supported the hypothesis [6, 7] that "secretor" status was determined by the presence or absence of the α-2-L-fucosyltransferase responsible for the synthesis of blood group H-active structures. The early work also established that irrespective of their tissue origin the α-2-L-fucosyltransferases utilise both Type 1 and Type 2 oligosaccharide structures as acceptor substrates [27, 33]. However, a number of factors, including competition for donor and acceptor substrates arising from the presence in crude preparations of other fucosyltransferases, the unavailability of non-radioactive GDP-L-fucose, and the difficulties encountered in purification of the enzymes, have resulted in comparatively little being established about the kinetic properties and precise substrate preferences of the human α-2-L-fucosyltransferases. Improvements in the assay techniques have now enabled low levels of this enzyme activity to be detected in submaxillary glands of "non-secretors" and have thus made possible a comparison of the properties of the α-2-L-fucosyltransferases which are expressed in tissues of the same origin when the secretor Se gene is either operative or inoperative.

Homogenates of submaxillary glands from blood group A "non-secretors" had only about 5% of the amount of α-2-L-fucosyltransferase activity detected in homogenates of glands from group A "secretor" individuals (Table 1). The levels of blood group *A* gene-associated α-3-*N*-acetyl-D-galactosaminyltransferase, blood group *Le* gene-associated α-3/4-L-fucosyltransferases [25], the Lewis independent α-3-L-fucosyltransferase [25] and the β-4-D-galactosyltransferase were not appreciably different in the two types of glands. Thus, of those tested, the only glycosyltransferase in submaxillary gland tissue affected by the expression of the different alleles at the *Sese* locus is the α-2-L-fucosyltransferase.

Fractionation of the homogenates of the glands from "secretor" individuals showed that the majority of the α-2-L-fucosyltransferase was membrane bound and very little was present in the soluble fraction (Fig. 1). The α-3/4- and α-3-L-fucosyltransferases were considerably more soluble, as might be expected since these enzymes are readily detectable in saliva, whereas α-2-L-fucosyltransferase cannot be demonstrated in this secretion [34]. There is very much less total α-2-L-fucosyltransferase in the "non-secretor" glands but a larger proportion of the total activity occurred in the soluble fraction. The greater solubility of the enzyme in the "non-secretor" glands was also observed when detergent was added to the homogenates; Triton X-100 solubilised about 25% of the α-2-L-fucosyltransferase activity from the "secretor" glands and about 70% from the "non-secretor" glands (Table 2). No appreciable differences were observed in the detergent solubilities of the other glycosyltransferases tested and hence these results suggest that there may be differences in the α-2-L-fucosyltransferases in "secretor" and "non-secretor" glands which influence their attachment to the membrane. The detection of lower molecular weight forms of the enzyme in the preparations from "non-secretor" glands (Fig. 5) also points to possible differences in the hydrophobic membrane attachment areas of the α-2-L-fucosyltransferases from the two sources. A further difference in physical properties of the preparations was apparent from their behaviour on ion exchange chromatography; only a small proportion of the "secretor" enzyme was bound to DEAE-cellulose whereas nearly all the activity from the "non-secretor" tissues was retained on the column (Fig. 4). This result therefore indicates some difference in the total net surface charge of the enzymes from the two sources.

The most striking differences in the properties of the α-2-L-fucosyltransferases from the two classes of submaxillary gland were reflected in their substrate specificities. The separation of the α-2-L-fucosyltransferase from the α-3- and α-3/4-fucosyltransferases on GDP-adipate-Sepharose 4B enabled partially purified preparations to be obtained which were largely free of the contaminating enzymes that compete for the donor and acceptor substrates. Both "secretor" and "non-secretor" enzymes used Type 1 and Type 2 oligosaccharides as acceptor substrates, but whereas the rate of transfer to Type 1 acceptors was markedly higher for the "secretor" enzyme, the reverse was true for the "non-secretor" α-2-L-fucosyltransferase (Table 3). Thus the transferase in the glands where the *Se* gene was expressed had a preference for Type 1 chain acceptors whereas the enzyme in the glands from individuals homozygous for *se* had a preference for Type 2 chains. The apparent K_M value for GDP-fucose also differed for the two preparations and was two- to three-fold higher for the "secretor" enzyme than for the "non-secretor" enzyme.

These results have demonstrated that expression of the secretor gene *Se* in submaxillary glands results in an α-2-L-fucosyltransferase which differs in some of its properties from the corresponding enzyme found in glands where the *Se* gene is not expressed. The difference in acceptor substrate specificity is that expected from the modified hypothesis of Oriol *et al.* [8], if it is assumed that the enzyme in the "non-secretor" tissue represents some residual expression of the *H* gene in epithelial tissues and that the transferase in the "secretor" tissue is the enzyme coded by the alternative structural gene *Se*. Le Pendu *et al.* [35, 36] studied the α-2-L-fucosyltransferases in the serum of individuals with rare ABO phenotypes in which H antigenic activity is absent from the red cells but present in secretions and concluded that this enzyme differed in its kinetic properties from the α-2-L-fucosyltransferase in the serum of individuals with normal ABO phenotypes. The inference was that the enzyme in the serum of the H deficient individuals was shed into the plasma from secretory tissues and thus represented the *Se* gene product. More recently Kumazaki and Yoshida [37] compared the properties of partially purified α-2-L-fucosyltransferases from human plasma with that from human milk and concluded that their studies also supported the model in which *H* and *Se* are both structural genes. The milk enzyme had a twenty-fold higher affinity for a Type 1 chain acceptor than for Type 2 chain analogues whereas the plasma enzyme did not show such a distinction. Thus the results described in the present paper, and those obtained by others, support the interpretation that more than one form of the α-2-L-fucosyltransferase exists and that the species found in epithelial tissues and secretions when *Se* is expressed has a preference for Type 1 acceptor substrates which is not shown in the absence of *Se*. The occurrence of enzymes coded by multi-loci gene families is well documented [38] and the human α-2-L-fucosyltransferase may fall into this category. Nevertheless, further investigations are required before this explanation can be regarded as established. That the substrate specificity of a glycosyltransferase can be modified by the product of a different gene locus is exemplified by lactose synthetase [39]. The *Se* gene product could be a similar modifier acting on the *H* gene-specified α-2-L-fucosyltransferase in such a way that the specificity is directed towards Type 1 chain acceptors. Alternatively, post-translational modification of the enzyme protein or differential processing of the DNA or messenger RNA might give rise to the observed differences in molecular forms of the *H* gene product. However, any regulatory model for the function of the *Se* gene must now take into account not only the very limited expression of α-2-L-fucosyltransferase in the absence of *Se* but also the production of a variant form of this enzyme in the presence of *Se*.

Acknowledgements

We thank Dr. A.S.R. Donald and Miss P. Rathbone, Clinical Research Centre, U.K. for the preparation of oligosaccharide substrates and Drs. G. Slavin and A.B. Price, Northwick Park Hospital, U.K. for the supply of submaxillary glands.

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