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Acceptor Hydroxyl Group Mapping for Human Milk α1-3 and α1-3/4 Fucosyltransferases

Sylvie Gosselin and Monica M. Palcic
Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2G2

Abstract—Two different fucosyltransferases (Fuc-Ts) have been isolated from human milk, an α1-3 Fuc-T and an α1-3/4 Fuc-T, for mapping of their acceptor binding sites. Kinetic studies employing a series of monodeoxygenated and modified Galβ1→4GlcNAcβOR and Galβ1→3GlcNAcβOR acceptor substrates showed that modifications are tolerated at every hydroxyl group in these substrates except for 6-OH of galactose and 3- or 4-OH of *N*-acetylglucosamine. Deoxygenation at these positions rendered these compounds inactive as both substrates and inhibitors. These essential hydroxyl groups, which are required for recognition of the substrates, are identical to the key polar groups that have previously been reported for cloned FucTs III, IV and V.

Introduction

The fucose-containing determinants, Lewis X (Le^x, Galβ1→4[Fucα1→3]GlcNAc) and sialyl $Lewis^x$ (Neu5Acα2→3Galβ1→4 [Fucα1→3]GlcNAc) are found on glycoproteins and glycolipids on mammalian cell surfaces. Le^x is a stage-specific embryonic antigen and a tumor-associated marker,[1–3] while sialyl $Lewis^x$ has been implicated as a ligand for selectins, a family of cell adhesion molecules.[4–6]

The last step in the biosynthesis of both determinants is catalysed by fucosyltransferases (Fuc-Ts) with the transfer of fucose from GDP-fucose to OH-3 of GlcNAc in Galβ1→4GlcNAc (LacNAc, Type II) or Neu5Acα2→3Galβ1→4GlcNAc acceptors, respectively. Fucosyltransferases are a multigene family of enzymes with differences in substrate specificity, inhibitor sensitivity, cation requirements and tissue distributions.[7–10] In human milk two different Fuc-Ts have been found that biosynthesize these structures, an α1-3 fucosyltransferase and an α1-3/4 fucosyltransferase.[8,11,12] The latter enzyme is characterized by an unusual and broad acceptor specificity since it can also transfer fucose to OH-4 of GlcNAc in Galβ1→3GlcNAc (Type I) acceptors synthesizing Le^a epitopes (Galβ1→3-[Fucα1→4]GlcNAc). Five different α1-3 and/or α1-4 Fuc-Ts have been cloned, Fuc-T III, IV, V, VI and VII (reviewed in refs 8, 13–15). Fuc-T III (EC 2.3.1.65) or Lewis enzyme is the FUT3 (X53578) encoded α1-3/α1-4 fucosyltransferase; Fuc-T IV or myeloid enzyme the FUT4 (M58596) encoded α1-3 fucosyltransferase; Fuc-T V is the FUT5 (M81485) encoded α1-3 fucosyltransferase; Fuc-T VI (EC 2.4.1.152) or plasma enzyme is the FUT6 (LO1698) encoded α1-3 fucosyltransferase; Fuc-T VII or leukocyte enzyme is the FUT7 (UO8112) encoded α1-3 fucosyltransferase; FUT3 to FUT7 are the Genome Data Base (GDB) registered names of the cloned human α1-3/4 fucosyltransferase genes. Limited kinetic and specificity characterization of the cloned and natural enzymes preclude clear correlation of the enzymes. This requires a detailed kinetic analysis of natural and cloned enzymes with defined synthetic or isolated acceptors and analogues.

Lemieux's pioneering studies have shown that a general feature in the binding of oligosaccharides by proteins is the recognition of only a few of the hydroxyl groups on the carbohydrate moiety[16–18]. These 'key polar groups'[16] can be identified by studying the effects of monodeoxygenated analogues or other modifications at each hydroxyl group in the carbohydrate ligand. In this paper a series of monodeoxygenated and substituted Type I and Type II acceptors were evaluated as substrates for isolated human milk α1-3 Fuc-T and α1-3/4 Fuc-T to identify the key hydroxyl groups required by these enzymes. Comparison with the results of similar mapping for cloned Fuc Ts III–V reveal identical key polar groups.[19]

Results and Discussion

Two different fucosyltransferases in human milk were isolated by ion-exchange chromatography, affinity chromatography using GDP-hexanolamine Sepharose as a matrix, and repetitive fractionation on Sephacryl S-200 columns[11,12]. The α1-3/4 Fuc-T has an apparent molecular weight of about 90,000–98,000 Daltons and can therefore be separated from α1-3 Fuc-T with an apparent molecular weight of 47,000–50,000 Daltons on gel filtration columns.[11,12] Figure 1 shows the separation of the two enzymes on rechromatography of an α1-3/4 Fuc-T enriched fraction. For kinetic studies, fractions 25–80 were pooled and concentrated ensuring there was no α1-3Fuc-T in the α1-3/4FucT preparation. Only fractions 140–180 were used for α1-3 Fuc-T kinetic studies; there was no detectable α1-4 activity in these fractions.

A summary of the kinetic parameters for the nucleotide donor GDP-fucose and six commonly used acceptors for α1-3/4 and α1-3 Fuc-T's is given in Table 1. For α1-3/4 Fuc-T the K_m for donor is low, 6 μM, while the best acceptor is Fucα1→2Galβ1→3GlcNAcβO$(CH_2)_8COOCH_3$ (**2**) with a relative V_{max} of 100% (3.6×10^{-3} nmol/min), a K_m of 155 μM and a relative V_{max}/K_m of 0.645. The isomeric Fucα1→2Galβ1→4GlcNAcβO$(CH_2)_8COOCH_3$ (**5**) is almost as good a substrate with a V_{max} 78% of **2**, a K_m of 132 μM and a relative V_{max}/K_m of 0.59. The parent acceptors Galβ1→3GlcNAcβO$(CH_2)_8COOCH_3$ (**1**) and Galβ1→4GlcNAcβO$(CH_2)_8COOCH_3$ (**4**) have increased K_ms (780 and 340 μM, respectively) while the relative V_{max} values are decreased to 89 and 47% of acceptor **2**. Substitutions at C-3 of the galactose with sialic acid (compounds **3** and **6**) were also well tolerated; the K_ms were 580 and 100 μM with decreased V_{max} values. Johnson and Watkins[12] report a K_m of 0.5 mM for compound **1** and 3 mM for **4** for their purified α1-3/4 Fuc-T. The reasons for the discrepancies in the K_m values are unclear. We saw no evidence of biphasic kinetics expected for a preparation with two activities with different K_ms.

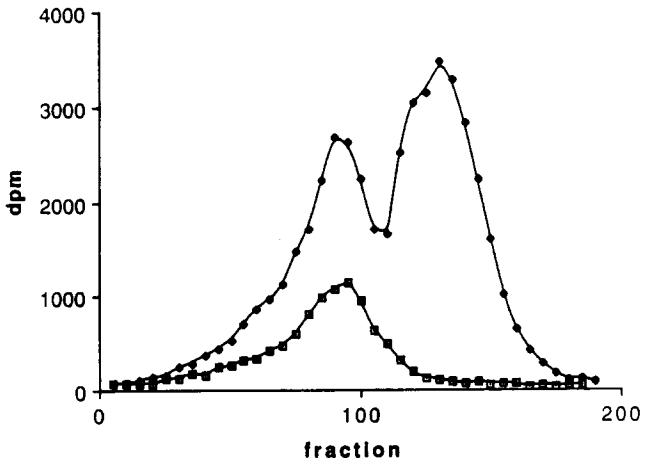

Figure 1. Chromatography of human milk fucosyltransferases on a Sephacryl S-200 column. The elution profile for the rechromatography of an α1-3/4 Fuc-T enriched fraction is shown. Standard assays for α1-3 transferase activity using Galβ1→4GlcNAcβO$(CH_2)_8COOCH_3$ as a substrate (◆); for α1-4 transferase Galβ1→3GlcNAcβO$(CH_2)_8COOCH_3$ (□) was used as a substrate. Fractions 25–80 and 140–180 were pooled and concentrated for kinetic studies.

The kinetic data for the panel of acceptors and purified α1-3 fucosyltransferase are also listed in Table 1. The K_m for the nucleotide donor GDP-fucose is 5 μM and the best substrate is compound **5** with a K_m of 130 μM and a relative V_{max} of 100% (4.8×10^{-3} nmol/min). The parent acceptor **4** has a V_{max} 71% of compound **5**, with an increase in K_m to 425 μM. Compound **6** has the lowest K_m of any substrate, 77 μM; however, the relative V_{max} is half of that of the best acceptor.

A series of monodeoxygenated analogues of Type I and Type II substrates were employed to further map the key polar groups in the acceptor that are required for recognition by both of the fucosyltransferases (Table 2). Deoxygenation at the 4-position of galactose in Type 1 and Type 2 acceptors is tolerated by both the α1-3/4 and α1-3 Fuc-T. The K_ms all increase to mM values; however, the V_{max} values are only marginally affected (60% for 4-deoxy-Galβ1→3GlcNAcβOCH_3,**7**) and even increased to 150% of the parent acceptor for **11**. Deoxygenation at the 6-position of the GlcNAc residue had similar effects for the α1-3/4 Fuc-T. With **9** the relative V_{max} was 60% while the K_m increased to 4 mM. A similar increase in K_m to 4.7 mM is observed for α1-3 Fuc-T using **13** as a substrate but the relative V_{max} is 440% of the parent compound **5**. The increases in K_m when the aglycone is OCH_3 is attributed in large part to the aglycone. The hydrophobic linker $O(CH_2)_8COOCH_3$ in an acceptor generally results in a 10-fold lower K_m value than the corresponding reducing sugar acceptor while relative V_{max} values are independent of the nature of the aglycone[20].

Deoxygenation at C-6 of galactose (**8** and **12**) and C-4 (**10**) or C-3 (**14**) of GlcNAc gives inactive compounds. Testing at 5 mM concentrations, compounds **8** and **12**

Table 1. Kinetic parameters for α1→3/4 and α1→3 Fuc-Ts and substituted Type I and Type II acceptor analogues

	α1→3/4Fuc-T			α1→3Fuc-T		
Substrate	K_m (μM)	V_{max} rel.[a] (%)	V_{max}/K_m rel.	K_m (μM)	V_{max} rel.[b] (%)	V_{max}/K_m rel.
GDP-fucose	6	—	—	5	—	—
Type I series						
Galβ1→3GlcNAcβOR[d] (**1**)	780	89	0.11	—[c]	—	—
Fucα1→2Galβ1→3GlcNAcβOR (**2**)	155	100	0.645	—	—	—
Neu5Acα2,3Galβ1→3GlcNAcβOR (**3**)	580	28	0.048	—	—	—
Type II series						
Galβ1→4GlcNAcβOR (**4**)	340	47	0.14	425	71	0.17
Fucα1→2Galβ1→4GlcNAcβOR (**5**)	132	78	0.59	130	100	0.769
Neu5Acα2,3Galβ1→4GlcNAcβOR (**6**)	100	47	0.47	77	50	0.65

[a] V_{max} rel. to 3.6×10^{-3} nmol/min for compound **2**.
[b] V_{max} rel. to 4.8×10^{-3} nmol/min for compound **5**.
[c] Not a substrate.
[d] R = $(CH_2)_8COOCH_3$.

Table 2. Kinetic parameters for α1→3/4 and α1→3 Fuc-Ts and monodeoxygenated Type I and Type II acceptor analogues

Substrate	α1→3/4Fuc-T			α1→3Fuc-T		
	K_m (μM)	V_{max} rel.[a] (%)	V_{max}/K_m rel.	K_m (μM)	V_{max} rel.[b] (%)	V_{max}/K_m rel.
Type I series						
Galβ1→3GlcNAcβOR$_1$[c] (**1**)	780	89	0.11	—[d]	—	—
4-deoxy-Galβ1→3GlcNAcβOR$_2$ (**7**)	17,000	60	3.5×10^{-3}	—	—	—
6-deoxy-Galβ1→3GlcNAcβOR$_2$ (**8**)	—	—	—	—	—	—
Galβ1→3,6-deoxy-GlcNAcβOR$_2$ (**9**)	4000	63	0.016	—	—	—
Galβ1→3,4-deoxy-GlcNAcβOR$_1$ (**10**)	—	—	—	—	—	—
Type II series						
Galβ1→4GlcNAcβOR$_1$ (**4**)	340	47	0.14	425	71	0.17
4-deoxy-Galβ1→4GlcNAcβOR$_3$ (**11**)	1400	152	0.11	1000	150	0.15
6-deoxy-Galβ1→4GlcNAcβOR$_3$ (**12**)	—	—	—	—	—	—
Galβ1→4,6-deoxy-GlcNAcβOR$_2$ (**13**)	nd[e]	nd	nd	4700	440	0.094
Galβ1→4,3-deoxy-GlcNAcβOR$_1$ (**14**)	—	—	—	—	—	—
Galβ1→4GlcNH PropionylβOR$_1$ (**15**)	270	64	0.24	280	85	0.31
Galβ1→4GlcNH$_2$βOR$_1$ (**16**)	—	—	—	—	—	—

[a] V_{max} rel. to 3.6×10^{-3} nmol/min for compound **2**.
[b] V_{max} rel. to 4.8×10^{-3} nmol/min for compound **5**.
[c] $R_1 = (CH_2)_8COCH_3$; $R_2 = CH_3$; $R_3 = (CH_2CH_2O)_2CH_2OOCH_3$.
[d] Not a substrate.
[e] Not determined.

were neither inhibitors nor acceptors for the enzymes. In testing **10** as an inhibitor for α1-3/4 Fuc-T, 11% inhibition was observed for 5 mM of the inhibitor and 100 μM of **1** as an acceptor substrate. This very weak inhibition precluded complete mechanistic evaluation, however, assuming the inhibition is competitive a K_i of 38 mM is obtained using the relationship $i = 100\ [I]/([I] + K_i\{1 + [S]/K_m\})$, where i is the % inhibition, $[I]$ is the inhibitor concentration and $[S]$ is the substrate concentration.[21] When Galβ1→4, 3-deoxy-GlcNAcβO$(CH_2)_8COOCH_3$ (**14**) was evaluated as an inhibitor of α1-3 FucT, no inhibition was observed at 4 mM of the inhibitor tested with acceptor **4** at 40 μM. Since 5–10% inhibition can easily be detected, using the same equation, the K_i would be larger than 100 mM (if any inhibition exists).

The 2-amido group of the *N*-acetylglucosamine residue is required for binding to both α1-3 and α1-3/4 Fuc-T when Galβ1→4GlcNAcOR$_1$(**4**)is a substrate. Replacement with a 2-propionamido group (**15**) is favorable, giving a better acceptor, while no activity was detected for the 2-NH_2 analogue at 4 mM (**16**).

The hydroxyl group of C-6 of galactose and the hydroxyl of C-3 of GlcNAc in Type II acceptors or the C-4 of GlcNAc in Type I acceptors are essential for binding to α1-3 Fuc-T and α1-3/4 Fuc-T and are thus designated as key polar groups (Fig. 2). Removal or replacement at any one of these positions gives inactive compounds; however, substitutions or modifications are tolerated at all other positions. Identical key polar groups were mapped for the cloned FucTs III, IV and V with this panel of substrates[19] indicating that the acceptor binding site for all fucosyltranferases studied to date must be very similar.

Further comparison of the kinetic parameters for the isolated α1-3/4 and α1-3 Fuc-Ts and the cloned Fuc-Ts do not allow a clear correlation to be made. The cloned Fuc-T IV is a myeloid-type enzyme which only transfers to Type II acceptors[22–24], however, sialylated substrates are generally poor acceptors for this α1-3 Fuc-T,[7] unlike the pattern seen for the milk Fuc-Ts. The human plasma and liver enzymes[7,9,25] have a preference for fucosylated and sialylated Type II acceptors, much like our isolated α1-3 Fuc-T. These correspond to cloned Fuc-T VI,[25,26] but there has been no detailed kinetic analysis of cloned FucT-VI to allow for a comparison with the milk enzyme. The isolated milk α1-3/4 Fuc-T generally resembles the milk α1-3/4 Fuc-T characterized by Johnson et al.,[20] but no cloned enzyme appears to correlate with this activity. Fuc-T V exhibits the dual activity but acceptor K_ms are high mM rather than the μM values measured for the milk enzyme. In addition, the limited availability of the isolated milk enzymes has precluded their sequencing to allow comparisons on the basis of amino acid residues. Work is in progress to obtain partial sequence information to correlate the enzymes better.

Critical hydroxyl residues have been identified in all glycosyltransferases that have been mapped to date. These include *N*-acetylglucosaminyltransferase I,[27] II[28] and V,[29] α2-3[30,31] and α2-6 sialyltransferases[30], and core II *N*-acetylglucosaminyltransferase.[31] There have been limited mapping studies of similar enzymes from different sources, therefore it is premature to suggest that identical key polar groups might be expected for all members of an enzyme class. The cloned fucosyltransferases have a high degree of amino acid sequence identity, about 85–90% for Fuc-Ts III, V and VI and 60% for Fuc-T IV so the observation of a common acceptor binding domain is not unexpected. There is, however, a report of a source dependent variation of key polar groups for α2-3 sialyltransferases from acute myeloid leukemia cells and human placenta.[31] Of

greater importance is the limitation that key polar groups place in the utilization of enzymes for preparative synthesis, or in the design of specific inhibitors where they must be maintained to ensure binding to the enzymes.

Experimental procedures

Materials

GDP[^{3}H]Fucose (8.1 Ci/mmol) was from Dupont New England Nuclear, Ecolite (+) cocktail was from ICN

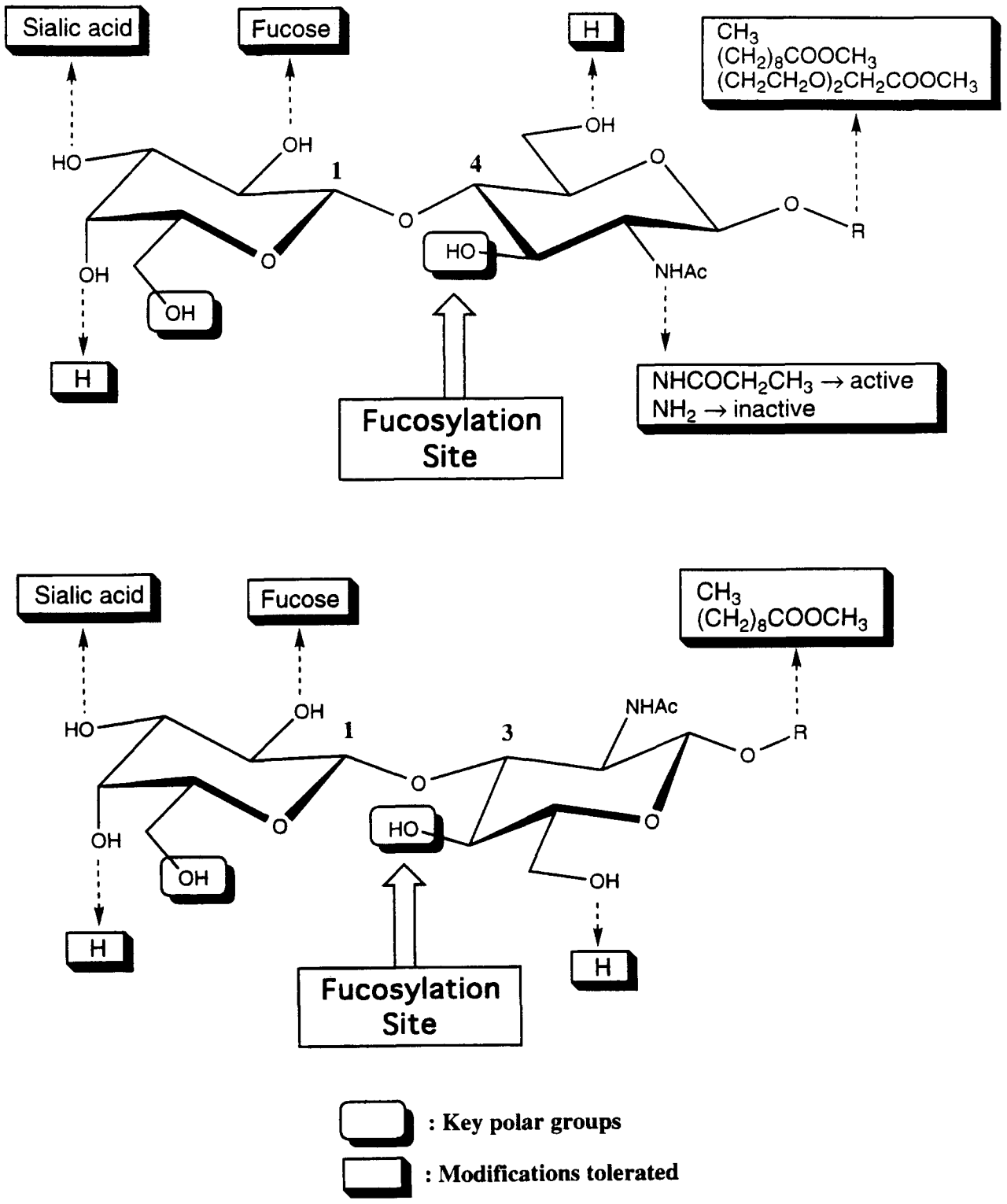

Figure 2. Structural requirements for human milk α1-3 and α1-3/4fucosyltransferases. Deoxygenation at C-6 of galactose and C-3 or C-4 of the *N*-acetylglucosamine is not tolerated; however, a variety of modifications are allowed at other positions.

and C_{18} sample preparation cartridges were from Waters Associates. GDP-fucose was synthesized as described previously[32] and was a gift from Dr O. Hindsgaul. Galβ1→3GlcNAcβO$(CH_2)_8COOCH_3$ (**1**),[33,34] Fucα1→2Galβ1→3GlcNAcβO$(CH_2)_8COOCH_3$(**2**),[35,36] Neu5Acα2→3Galβ1→3GlcNAcβO$(CH_2)_8COOCH_3$ (**3**),[37] Galβ1→4GlcNAcβO$(CH_2)_8COOCH_3$(**4**),[34] Fucα1→2Galβ1→4GlcNAcβO$(CH_2)_8COOCH_3$(**5**),[38] Neu5Acα2→3Galβ1→4GlcNAcβO$(CH_2)_8COOCH_3$ (**6**),[37] were also provided by Dr O. Hindsgaul. 4-Deoxy-Galβ1→3GlcNAcβO$(CH_2)_8COOCH_3$ (**7**), 6-deoxy-Galβ1→3GlcNAcβOCH_3(**8**),[39] Galβ1→3 6-deoxy-GlcNAcβOCH_3 (**9**),[40] Galβ1→3 4-deoxy-GlcNAcβO$(CH_2)_8COOCH_3$ (**10**),[34] 4-deoxy-Galβ1→4GlcNAcβO$(CH_2CH_2O)_2CH_2COOCH_3$ (**11**), 6-deoxy-Galβ1→4GlcNAcβO$(CH_2CH_2O)_2CH_2COOCH_3$(**12**), Galβ1→4 6-deoxy-GlcNAcβOCH_3(**13**), Galβ1→4 4-deoxy-GlcNAcβO$(CH_2)_8COOCH_3$(**14**),[34] Galβ1→4 GlcNHPropionylβO$(CH_2)_8COOCH_3$ (**15**),[41] and Galβ1→$GlcNH_2$βO$(CH_2)_8COOCH_3$ (**16**),[41] provided by Dr R. U. Lemieux (compounds **7–14**) and Dr O. P. Srivastava (compounds **15** and **16**) were available from previous studies.[19,30]

Purification of α1-3 and α1-3/4 fucosyltransferases

The purification of the human milk fucosyltransferases was carried out at 4 °C by a modification of reported procedures.[11,12] Thawed milk (4 L) was defatted by centrifugation and adsorbed onto SP-Sephadex C-50 (2 g/L) by batch adsorption overnight. The resin was then filtered over a scintered glass funnel and washed with cold water until the eluent became clear. The washed slurry was poured into a column (5 × 30 cm) and further washed with 0.1 M NaCl until no further protein eluted. The enzyme was eluted from the resin using a linear gradient of NaCl (0.1–0.6 M, 500 mL total volume). Fractions with enzyme activity, as determined by the standard radiochemical assay described below, were pooled and ultrafiltered to approximately 20 mL using an Amicon cell with a PM-10 membrane and dialysed overnight against 50 mM sodium cacodylate buffer, pH 7.0, containing 25% (v/v) glycerol. The dialysed material was then applied to a GDP-hexanolamine Sepharose column (1 × 5 cm, 4 μmol of ligand/mL of gel) equilibrated with the same buffer. The column was washed with loading buffer and then with loading buffer containing 0.8 M NaCl. The enzyme was eluted with the same buffer containing 0.8 M NaCl and 5 mM GMP directly into a tube containing BSA in order to achieve a final BSA concentration of 0.1 mg/mL. The eluent was then ultrafiltered to 10 mL.

The ultrafiltered material was then applied to a Sephacryl S-200 column (2.5 cm × 100 cm) previously equilibrated with 25 mM sodium cacodylate buffer pH 5.5 containing 0.2 M NaCl and 25% glycerol. The chromatography was performed at a flow rate of approximately 30 mL/hour, using the equilibration buffer. The first 100 mL was collected in one flask. The remaining volume was collected in 1 mL fractions. In each of the tubes, 50 μL of 'quench' buffer (0.5 M Tris–HCl buffer, pH 9.1) was placed prior to collection of the fraction. Fractions were assayed for enzyme activity using 50 μM of acceptor **1** or **4**. Activity was found in fractions 20–160. Fractions eluting early (fractions 20–70) contained both α1-3 and α1-4 activities, those eluting later (fractions 110–160) showed mostly α1-3 activity while the bulk of the activity co-eluted in fractions 71–110. Only the early and late fractions were pooled, ultrafiltered to 10 mL and re-applied to the same column. This re-chromatography ensured that the separation of the α1-3 enzyme from the α1-3/4 enzyme was complete. Final enzyme activities after concentration to about 10 mL were as follows: for the α1-3/4 Fuc-T 0.052 mU/mL α1-3 activity using 50 μM **4** and 0.048 mU/mL α1-4 activity using 50 μM **1** as an acceptor. The α1-3 Fuc-T contained 0.1 mU/mL using 50 μM **4** as an acceptor and no significant α1-4 activity. These enzymes were stable for at least 6 months at 4 °C. For poor substrates the enzymes were concd 10-fold further by ultrafiltration.

Enzyme assays

Standard enzyme assays. Standard enzyme assays contained the following components, 4 μL assay buffer (200 mM Hepes pH 7.0 containing 200 mM $MnCl_2$, 2% BSA), 50 μM GDP-Fuc (including 21,000 dpm/nmol of GDP[^{3}H]Fuc), 50 μM acceptor Galβ1→3GlcNAcβO$(CH_2)_8COOCH_3$ (**1**) for α1-4 transferase assays or Galβ1→4GlcNAcβO$(CH_2)_8COOCH_3$ (**4**) for α1-3 transferase assays and enzyme and/or water to 40 μL final volume. After incubation for 30 min at 37 °C, the assays were quenched by addition of 400 μL water. Quenched samples were applied to Waters C_{18} reverse phase cartridges[42] previously washed with methanol and equilibrated with water. The cartridges were then washed with 50 mL of water and radiolabeled products eluted with 3 mL of methanol directly into scintillation vials. Samples were counted in a Beckman LS 1801 scintillation counter after the addition of 10 mL of Ecolite(+) liquid scintillation cocktail.

Determination of kinetic parameters

For donor kinetics, assays were done in duplicate as in the standard assay but the acceptor concentration was 0.765 mM Galβ1→4GlcNAcβO$(CH_2)_8COOCH_3$ (**4**) and the GDP-Fuc concentration ranged from 0.5 to 32 μM.

For acceptor kinetics, 50 μM of GDP-Fuc donor was used, with 5–6 acceptor concentrations generally ranging from 0.2 to 5 times K_m for each acceptor. For compounds **1–6**, **10–12**, and **14–16** which contain hydrophobic aglycones [R = O$(CH_2)_8COOCH_3$ or O$(CH_2CH_2O)_2CH_2COOCH_3$] at the end of incubation samples were applied to C_{18} cartridges and radiolabeled products eluted and quantitated as in the standard assays.

For compounds **7–9**, and **13** where the aglycone is not sufficiently hydrophobic for adsorption onto C_{18} cartridges ion-exchange chromatography on Dowex columns (Bio-Rad AG resin) was used to remove unreacted radiolabeled donor from reaction products.

For this assay, Dowex 1X-8 Cl^- form, 50–100 mesh was washed with water, and small 1 mL columns were prepared in Pasteur pipettes. The quenched samples were applied to the columns, and radiolabeled product eluted directly into scintillation vials with 3 mL of water. Ecolite(+) cocktail was added to eluent for counting as above.

The results obtained were analysed by fitting the initial rate data to the Michaelis–Menten equation using nonlinear regression analysis (Sigma Plot). Typical errors for V_{max} are $\pm 5\%$, and $K_m \pm 10\%$.

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References

1. Feizi, T. *Nature* **1985**, *314*, 53.

2. Muramatsu, T. *Biochimie* **1988**, *70*, 1587.

3. Hakomori, S. *Adv. Cancer Res.* **1989**, *52*, 257.

4. Phillips, M. L.; Nudelman, E.; Gaeta, F. C. A.; Perez, M.; Singhal, A. K.; Hakomori, S.-I.; Paulson, J. C. (1990) *Science* **1990**, *250*, 1130.

5. Walx, G.; Arrufo, A.; Kolanus, W.; Bevilacqua, M.; Seed, B. *Science* **1990**, *250*, 1132.

6. Brandley, B. K. *Seminars Cell Biol.* **1991**, *2*, 281.

7. Mollicone, R.; Gibaud, A.; Francois, A.; Ratcliffe, M.; Oriol, R. *Eur. J. Biochem.* **1990**, *191*, 169.

8. Macher, B. A.; Holmes, E.; Sweidler, S. J.; Stults, C. L. M.; Srnka, C. A. *Glycobiology* **1991**, *1*, 577.

9. Mollicone, R.; Canelier, J.-J.; Mennesson, B.; Couillin, P.; Venot, A. P; Oriol, R. *Carbohydr. Res.* **1992**, *228*, 265.

10. Watkins, W. M.; Skacel, P. O.; Johnson, P. H. *ACS Symp. Ser.* **1993**, *519*, 34.

11. Eppenberger-Castori, S.; Lotscher, H.; Finne, J. *Glycoconjugate J.* **1989**, *6*, 101.

12. Johnson, P. H.; Watkins, W. M. *Glycoconjugate J.* **1992**, *9*, 241.

13. Natsuka, S.; Lowe, J. B. *Curr. Opin. Struct. Biol.* **1994**, *4*, 683.

14. Kleene, R.; Berger, E. *Biochim. Biophys. Acta* **1993**, *1154*, 283.

15. Schachter, H. in *Frontiers in Molecular Glycobiology*; Fukuda, M.; Hindsgaul, O., Eds.; Oxford University: Oxford, **1994**, pp 88–162.

16. Lemieux, R. U. *Chem. Soc. Rev.* **1989**, *18*, 347.

17. Lemieux, R. U. *ACS Symp. Ser.* **1993**, *519*, 5.

18. Spohr, U.; Pasziewicz-Hnatiw, E.; Morishima, N.; Lemieux, R. U. *Can. J. Chem.* **1991**, *70*, 254.

19. de Vries, T.; Srnka, C. A.; Palcic, M. M.; Swiedler, S. J.; van den Eijnden, D. H.; Macher, B. A. *J. Biol. Chem.* **1995**, *270*, 8712.

20. Johnson, P. H., Donald, A. S. R.; Feeney, J.; Watkins, W. M. *Glycoconjugate J.* **1992**, *9*, 251.

21. Segel, I. *Enzyme Kinetics*; Wiley-Interscience: New York, 1975.

22. Goelz, S. E.; Hession, C.; Goff, D.; Griffiths, B.; Tizar, R.; Newman, B.; Chi-Rosso, G.; Lobb, R. *Cell* **1990**, *63*, 1349.

23. Lowe, J. B.; Kukowska-Latallo, J. F.; Nair, R. P.; Larsen R. D.; Marks, R. M; Macher, B. A.; Kelly, R. J.; Ernst, L. K. *J. Biol. Chem.* **1991**, *266*, 17467.

24. Kumar, R.; Potvin, B.; Muller, W. A.; Stanley, P. *J. Biol. Chem.* **1991**, *266*, 21777.

25. Johnson, P. H.; Donald, A. S. R.; Clarke, J. L.; Watkins, W. M. *Glycoconjugate J.* **1995**, *12*, 879.

26. Mollicone, R.; Reguigne, I.; Fletcher, A.; Aziz, A.; Rustam, M.; Weston, B. W.; Kelly, R. J.; Lowe, J. B.; Oriol, R. *J. Biol. Chem.* **1994**, *269*, 12662.

27. Moller, G.; Reck, F.; Paulsen, H.; Kaur, K. J.; Sarkar, M.; Schachter, H.; Brockhausen, I. *Glycoconjugate J.* **1992**, *9*, 180.

28. Reck, F.; Meinjohanns, E.; Springer, M.; Wilkens, R.; van Doerst, J. A. L. M.; Paulsen, H.; Moller, G.; Brockhausen, I.; Schachter, H. *Glycoconjugate J.* **1994**, *11*, 210.

29. Kanie, O.; Palcic, M. M.; Hindsgaul, O. *RIKEN Rev.* **1995**, *8*, 53.

30. Wlasichuk, K. B.; Kashem, M. A.; Nikrad, P. V.; Bird, P.; Jiang, C.; Venot, A. P. (1993) *J. Biol. Chem.* **1993**, *268*, 13971.

31. Kuhns, W.; Rutz, W.; Paulsen, H.; Matta, K. L.; Baker, M. A.; Barner, M.; Granovsky, M.; Brockhausen, I. *Glycoconjugate J.* **1993**, *10*, 381.

32. Gokhale, U. B.; Hindsgaul, O.; Palcic, M. M. *Can. J. Chem.* **1990**, *68*, 1063.

33. Lemieux, R. U.; Bundle, D. R.; Baker, D. A. *J. Am. Chem. Soc.* **1975**, *97*, 4076.

34. Khare, D. P.; Hindsgaul, O.; Lemieux, R. U. *Carbohydr. Res.* **1985**, *136*, 285.

35. Lemieux, R. U.; Bundle, D. R.; Baker, D. A. U.S. Patent 4 137 401, 1979.

36. Lemieux, R. U. *Chem. Soc. Rev.* **1978**, 7, 423.

37. Venot, A; Ratcliffe, R. M. *Proc. Japanese–German Symp. Sialic Acids, Berlin*, **1988**, 54.

38. Hindsgaul, O.; Norberg, T.; LePendu, J.; Lemieux, R. U. *Carbohydr. Res.* **1982**, *109*, 109.

39. Lemieux, R. U.; Cromer, R.; Spohr, U. *Can. J. Chem.* **1988**, *66*, 3083.

40. Spohr, U.; Lemieux, R. U. *Carbohydr. Res.* **1988**, *174*, 211.

41. Nikrad, P. V.; Kashem, M. A.; Wlasichuk, K. B.; Alton, G.; Venot, A. P. *Carbohydr. Res.* **1993**, *250*, 145.

42. Palcic, M. M.; Heerze, L. D.; Pierce, M.; Hindsgaul, O. *Glycoconjugate J.* **1988**, *5*, 49.

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