Specificity Analysis of Three Clonal and Five Non-Clonal α1,3-L-Fucosyltransferases with Sulfated, Sialylated, or Fucosylated Synthetic Carbohydrates as Acceptors in Relation to the Assembly of 3′-Sialyl-6′-sulfo Lewis x (the L-Selectin Ligand) and Related Complex Structures†

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Received September 13, 1995; Revised Manuscript Received April 11, 1996⊗

ABSTRACT: Unique specificities of the cloned α1,3-L-fucosyltransferases (FTs), FT III (Lewis type), FT IV (myeloid type), and FT V (plasma type), and the α1,3-FTs of Colo 205 (colon carcinoma), HL 60 (myeloid), B142 (lymphoid), EKVX (lung carcinoma), and calf mesenteric lymph nodes (CMLN) were discerned with sulfated, sialylated, and/or fucosylated Gal*β*1,3/4GlcNAc*β*-based acceptor moieties. (a) FT V was 1.0-, 20.8-, and 4.6-fold active in forming Lewis x, Lewis y, and 3′-α-galactosyl Lewis x, respectively. (b) FT III and FT V formed ∼4-fold 3′-sulfo Lewis x, as compared to 3′-sialyl Lewis x. (c) FT IV showed great efficiency in forming 3′-sulfo Lewis x (249%) and Lewis x (345%) in mucin-type branched chains. (d) FT III, FT IV, and FT V formed 19%, 62%, and 47% 6-sulfo Lewis x as compared to Lewis x. (e) 6′-Sulfo Lewis x and 3′-sialyl-6′-sulfo Lewis x (GLYCAM ligand) were not synthesized from their immediate precursors by FT III, FT IV, or FT V. (f) FT III, FT IV, and FT V were 311%, 9%, and 188% active, respectively, with 2′-fucosyl lactose but were not active with 2′-fucosyl-6′-sulfo lactose. (g) FT III and FT V were 7.0- and 0.5-fold active in forming Lewis a as compared to Lewis x, whereas, FT IV was inactive. (h) FT III was -2.0-fold more active in forming 3′-α-galactosyl Lewis a than Lewis b. (i) FT III synthesized 6-sialyl Lewis a (40% efficiency as compared to Lewis a) from 6-sialyl type 1. (j) FT III did not act on 6′-sulfo or 6′-sialyl type 1 but was 106% and 22% active with 3′-sulfo and 6-sulfo type 1, respectively. (k) The Colo 205 FT activities with type 1 compounds almost paralleled that of FT III except for the low activity (9%) with Gal*β*1,3(NeuAcα2, 6)GlcNAc*β*-*O*-Bn, but with type 2 considerable differences between Colo 205 FT and FT III were noticed. (l) The α1,3-FTs of CMLN, HL60, B142, and EKVX were 1.2−1.7 times active with Fucα1,2Gal*β*1,4GlcNAc*β*-*O*-pNP and Galα1,3Gal*β*1,4GlcNAc*β*-*O*-Bn with respect to Gal*β*1,4GlcNAc*β*-*O*-Al. (m) Both CMLN and HL60 FTs were 2-fold active with 3-sulfoGal*β*1,4GlcNAc in a mucin-type branch structure such as 3-sulfoGal*β*1,4GlcNAc*β*1,6(Gal*β*1,3)GalNAcα-*O*-Bn. (n) The 3′-sulfoLacNAc/acrylamide copolymer, either as an acceptor or as a competitive inhibitor, had the potential to distinguish myeloid type α1,3-FT from the plasma type.

The ligands for E- and P-selectins, two members of the selectin family of cell adhesion molecules, have been characterized as sialylated and fucosylated oligosaccharides (Lowe et al., 1990; Phillips et al., 1990; Walz et al., 1990; Polley et al., 1991; Berg et al., 1991; Takada et al., 1991). The ligand for the third member, L-selectin, has been shown to constitute a sulfate group in addition to sialic acid and fucose (Imai et al., 1991). In addition, the sulfated Lewis a tetra and pentasaccharides of ovarian cystadenocarcinoma glycoprotein were shown to be potent E-selectin ligands (Yuen et al., 1994). Presently, it is becoming evident that subtle differences in the structure of sialylated, fucosylated oligosaccharides influence their binding affinity with E- and L-selectins (Foxall et al., 1992; Needham & Schnaar, 1993). The expression of fucosylated oligosaccharides is largely controlled by regulating the expression of α1,3-fucosyltransferases (FTs)[1] (Lowe, 1991). Recent studies have led to the recognition of five different human α1,3-FTs, designated Fuc-T III to Fuc-T VII. Fuc-T III corresponds to the Lewis type α1,3/4-FT (Kukowska-Latallo et al., 1990), Fuc-T IV corresponds to the myeloid type (Goelz et al., 1990; Lowe et al., 1991; Kumar et al., 1991), Fuc-T V and Fuc-T VI correspond to the plasma type (Weston et al., 1992a,b; Koszdin & Bowen, 1992), and Fuc-T VII appears to be a unique type (Sasaki et al., 1994). Yago et al. (1993) examined the expression of Fuc-T III to Fuc-T VI at mRNA levels in various epithelial cancer and leukemia cell lines and found mixtures of multiple molecular species of these FTs. Recently, Sasaki et al. (1994) studied the expression level of the five cloned α1,3-FTs and found both Fuc-T IV

† This work was supported by Grant No. CA35329 awarded by the National Cancer Institute.

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⊗ Abstract published in *Advance ACS Abstracts,* June 15, 1996.

[1] Abbreviations: FT, fucosyltransferase; Al, allyl; Bn, benzyl; Me, methyl; pNP, *para*-nitrophenyl; AA-CP, acrylamide copolymer; CMLN, calf mesenteric lymph nodes; TLC, thin-layer chromatography.

S0006-2960(95)02194-5 CCC: $12.00

and Fuc-T VII in myeloid lineage cells. When expressed in the human Burkitt lymphoma cell line, Namalwa, both of these enzymes formed sialyl Lewis x at the cell surface, but the E-selectin binding ability was only conferred on these cells by Fuc-T VII. Sueyoshi et al. (1994) compared Chinese hamster ovary (CHO) cells, stably transfected with Fuc-T III (CHO-FT III) or Fuc-T IV (CHO-FT IV), for their carbohydrate structures and for their binding to E- or L-selectin. CHO-FT III expressed sialyl Lewis x, Lewis x, and VIM-2 structures, whereas CHO-FT IV expressed only Lewis x and a small amount of VIM-2. They also found that only CHO-FT III adhered to E-selectin and that both CHO-FT III and CHO-FT IV failed to adhere to L-selectin. The present paper reports our detailed investigation on the intricate specificities of the cloned Fuc-T III, Fuc-T IV, and Fuc-T V and also on the specificities of α1,3-FTs present in some human cell lines and calf lymph node using a variety of sulfated, sialylated, and fucosylated compounds as acceptors. The present study has led to several unique findings on the specificities of these enzymes, thus forming a sound basis to explain their distinct roles in the expression of biological carbohydrate ligands and carbohydrate antigenic determinants.

EXPERIMENTAL PROCEDURES

FT III, FT IV, and FT V. These cloned and expressed enzyme−Protein A fusion products (Glycomed, Alameda, CA) were isolated by binding to IgG-Sepharose beads (1 mL of IgG-Sepharose/L of the condition media containing the protein A−enzyme), followed by dissociation of that complex for soluble enzyme. As the present studies necessitated soluble enzymes, 1.0 mL of the Sepharose bead slurry of each FT III, FT IV, and FT V was centrifuged for 1 min in a microfuge. After removal of the supernatant, 1.0 mL of 1 mM Tris-HCl−150 mM NaCl, pH 8.0, was added to the beads, mixed gently by finger tapping, and centrifuged. The supernatant was discarded, and then 1.0 mL of 0.1 M citrate buffer, pH 4.4, was added to the beads, mixed in the cold room for $^1/_2$ h using Speci-Mix (Thermolyne), and centrifuged for 1 min. The supernatant was mixed with 1.0 mL of 0.5 M Hepes, pH 7.5, containing 4% Triton X-100 and 20 mg of BSA and then dialyzed overnight at 4 °C against 1 L of 25 mM Tris-HCl, pH 7.0, containing 35 mM $MgCl_2$, 1 mM ATP, and 10 mM NaN_3. The enzyme solutions were stored at 4 °C, and there was no appreciable loss of enzyme activity for at least 2 months. In each assay, 5 μL of the soluble enzyme preparation was used.

Cell Culture. Colo 205, HL60, B142, and EKVX were grown in 250 mL plastic T-flasks in RPMI 1640 as described earlier (Chandrasekaran, 1995a,b). The cells were homogenized with Tris-buffered saline, pH 7.0, containing 2% Triton X-100 using a Dounce all-glass hand-operated grinder. The homogenate was centrifuged at 20 000*g* for 1 h at 4 °C. The supernatant was adjusted to 1 mg of protein/mL by adding the necessary volume of the extraction buffer. 5 μL aliquots of these extracts were used in assays run in duplicate. Protein was measured by the BCA method (Pierce Chemical Co.) with BSA as the standard. Calf mesenteric lymph node was made available from the animal facility in Springville, NY, through the courtesy of Dr. M. P. McGarry.

Human breast tumor and human ovarian tumor were obtained from the tissue facility of Roswell Park Cancer Institute. All tissue specimens were kept frozen at −70 °C until use.

These tissues were homogenized using Kinematica in Tris-buffered saline and then stirred for 1 h at 4 °C after adjusting the concentration of Triton X-100 to 2%. Homogenates were centrifuged at 20 000*g* for 1 h at 4 °C. The clear fat-free supernatant was adjusted to 10 mg of protein/mL by adding the necessary volume of Tris-buffered saline−2% Triton X-100. 5 μL aliquots were used in the assay.

Assay of α1,3- and α1,4-FT Activities. The incubation mixtures run in duplicate contained 50 mM Hepes-NaOH, pH 7.5, 5 mM $MnCl_2$, 7 mM ATP, 3 mM NaN_3, the acceptor (3.0 mM unless otherwise stated), 0.05% μCi of GDP-[U-^{14}C]Fuc (specific activity 290 mCi/mmol), and 5 μL of the enzyme solution in a total volume of 20 μL. Control incubation mixtures had everything except the acceptor. At the end of incubation for 4 h at 37 °C the mixture was diluted with 1.0 mL of water and passed through Dowex-1-Cl column (1 mL in a Pasteur pipet) (Chandrasekaran et al., 1992). The column was washed twice with 1 mL of water. The breakthrough and wash, which contained the [^{14}C]-fucosylated neutral acceptor, were collected together in a scintillation vial, and radioactive content was determined using the 3a70 scintillation mixture (Research Products International, Mount Prospect, IL) and a Beckman LS9000 instrument. The Dowex column was then eluted with 3.0 mL of 0.2 M NaCl to obtain the [^{14}C]fucosylated products from sialylated/sulfated acceptors and then counted for radioactivity as above. Corrections were made by subtracting the radioactivity in the water and NaCl eluates of the control incubation mixtures from the values of the corresponding eluates of the tests. Values for the duplicate runs did not vary more than 5%.

Synthetic Compounds. We already reported the synthesis of many of these compounds used in the present study (Jain et al. 1993a, 1994; Chandrasekaran et al., 1995). The synthetic details on the remaining compounds will be reported elsewhere.

Acrylamide−Sulfoglycan Copolymers. Acrylamide copolymers of 3-sulfoGal*β*1,3GlcNAc*β*-*O*-Al and 3-sulfoGal-*β*1,4GlcNAc*β*-*O*-Al were synthesized by following the procedure of Horejsi et al. (1978). About 1.0 μmol of the sugar unit was present in 1.0 mg of these copolymers (determination of Gal by anthrone reaction); these copolymers exhibited an approximate molecular weight of 40 000 as judged by chromatography on a Bio-Gel P60 column with dextran of 39 200 average molecular weight as the marker.

Acceptor Competition Experiments with Copolymers

(A) Competition between the acceptors and acrylamide−sulfoglycan copolymers for Colo 205 α1,3/4-FTs. The effect of 3-sulfoGla*β*1, 3GlcNAc*β*-*O*-Al/AA-CP on the α1,3-FT as well as α1,4-FT activities of Colo 205 was measured using the Fucα1,2Gal*β*1,4GlcNAc*β*-*O*-pNP and Fucα1,2Gal-*β*1,3GlcNAc*β*-*O*-pNP acceptors, respectively. The effect of 3-sulfoGal*β*1,4GlcNAc*β*-*O*-Al/AA-CP on the α1,3- as well as the α1,4-FT activities of Colo 205 was measured using the Gal*β*1,4GlcNAc*β*-*O*-Al and Gal*β*1,3GlcNAc*β*-*O*-Al acceptors, respectively. The concentration of copolymer in the reaction mixture varied between 2.5 and 100.0 μM (based on a molecular weight of 40 000) under the standard incubation conditions. The transfer of [^{14}C]Fuc to the neutral acceptors was measured by the Dowex method as above.

Table 1: Differentiation of the Specificities of Colo 205 and the Cloned α1,3-L-Fucosyltransferases (FT III, FT IV, and FT V) with Type 2 (Galβ1,4GlcNAcβ-)-Based Synthetic Carbohydrates as Acceptors

synthetic carbohydrate (3.0 mM)	fucosyltransferase activity, incorporation of [^{14}C]Fuc (CPM × 10^{-3})			
	Colo 205	FT III	FT IV	FT V
Galβ1,4GlcNAcβ-*O*-Al	26.95 (100)	2.88 (100)	3.75 (100)	0.62 (100)
Fucα1,2Galβ1,4GlcNAcβ-*O*-PNP	27.12 (101)	12.16 (423)	17.53 (470)	12.80 (2075)
Fucα1,2Galβ1,4Glc	16.45 (61)	8.93 (311)	0.32 (9)	1.16 (188)
Galα1,3Galβ1,4GlcNAcβ-*O*-Bn	29.11 (108)	2.66 (92)	6.45 (172)	2.83 (458)
NeuAcα2,3Galβ1,4GlcNAcβ-*O*-Bn	15.60 (58)	1.31 (46)	0.23 (6)	0.54 (88)
3-sulfoGalβ1,4GlcNAc	22.37 (83)	4.42 (154)	1.41 (37)	2.13 (345)
Galβ1,4(6-sulfo)GlcNAcβ-*O*-Al	13.02 (48)	0.55 (19)	2.32 (62)	0.29 (47)
6-sulfoGalβ1,4GlcNAc	1.84 (7)	0.06 (2)	0 (0)	0.01 (1)
6-sulfoGalβ1,4(3-*O*-Me) GlcNAcβ-*O*-Bn	0.05 (<1)	0.02 (<1)	0 (0)	0.01 (1)
6-sulfoGalβ1,4GlcNAcβ-*O*-Me	0 (0)	0.02 (<1)	0.05 (1)	0.02 (3)
NeuAcα2,3(6-sulfo)Galβ1,4GlcNAcβ-*O*-Me	0 (0)	0.04 (1)	0 (0)	0.02 (3)
Fucα1,2(6-sulfo)Galβ1,4Glc	0.03 (<1)	0.01 (<1)	0.01 (<1)	0.01 (1)
3-sulfoGalβ1,4GlcNAcβ1,6(Galβ1,3)GalNAcα-*O*-Bn	15.02 (56)	2.98 (104)	9.33 (249)	2.21 (358)
Galβ1,4GlcNAcβ1,6(3-sulfoGalβ1,3)GalNAcα-*O*-Bn	16.19 (60)	0.54 (19)	12.94 (345)	0.34 (55)

K_i for the inhibition of [^{14}C]Fuc transfer to the neutral acceptor by the acrylamide sulfoglycan copolymer was determined by Lineweaver−Burke plot.

(B) Competition between the acceptors and acrylamide−sulfoglycan copolymers for HL60 α1,3-FT and the cloned FT III (α1,4-FT): The effects of both 3-sulfoGalβ1,3GlcNAcβ-*O*-Al/AA-CP and 3-sulfoGalβ1,4GlcNAcβ-*O*-Al/AA-CP on the α1,3-FT activity of HL60 and on the α1,4-FT activity of FT III were measured using Galβ1,4GlcNAcβ-*O*-Al and Galβ1,3GlcNAcβ-*O*-Al as the respective acceptors under standard incubation conditions in the presence of the copolymer as described above. K_i for the inhibition was calculated by Lineweaver−Burke plot.

RESULTS AND DISCUSSION

Type 2 (Galβ1,4GlcNAcβ-)-Based Structures as the Acceptors for Cloned Enzymes FT III, FT IV, and FT V. See Table 1.) Blood group H type 2 [Fucα1,2Galβ1,4GlcNAcβ-*O*-pNP] served as the most efficient acceptor. As compared to Galβ1,4GlcNAcβ-*O*-Al, enzyme activity with this acceptor was about 4-fold more with both FT III and FT IV. It is highly interesting to note that FT V showed more than 20-fold activity with Fucα1,2Galβ1,4GlcNAcβ-*O*-pNP as compared to the basic type 2 (Galβ1,4GlcNAcβ-*O*-Al). FT III and FT V showed 311% and 188% activity with 2′-fucosyllactose as compared to Galβ1,4GlcNAcβ-*O*-Al, whereas FT IV exhibited very low activity (9%). If the α1,2-fucosyl group in 2′-fucosylLacNAcβ- is replaced by an α1,3-linked Gal, FT III activity was reduced from 423% to 92%, FT IV activity was reduced from 470% to 172%, and FT V activity was reduced from 2075% to 458%. These results imply that even though these enzymes prefer H type 2 as the acceptor, they are also capable of synthesizing 3′-α-galactosyl Lewis x from 3′-α-galactosylLacNAc at a significant level. When a sialyl group is linked α2,3 to Gal in Galβ1,4GlcNAcβ-, FT III and FT V showed 46% and 88% activity, respectively, whereas the activity of FT IV was almost negligible (only 6%). 3′-SulfoLacNAc was highly reactive with FT III (154%) and FT V (345%) and showed less activity with FT IV (37%).

FT IV was the only enzyme that showed appreciably more activity with Galβ1,4(6-sulfo)GlcNAcβ-*O*-Al (62%) as compared to 3-sulfoGalβ1,4GlcNAc (37%), while FT III and FT V showed much lower activities (19% versus 154% and 47% versus 345%, respectively). When sulfate was present on C-6 of Gal, the acceptor activity was lost toward all of these enzymes [see the activities with the following acceptors: 6-sulfoGalβ1,4GlcNAc; 6-sulfoGalβ1,4(3-*O*-Me)GlcNAcβ-*O*-Bn; 6-sulfoGalβ1,4GlcNAcβ-*O*-Me; NeuAcα2,3(6-sulfo)-Galβ1,4GlcNAcβ-*O*-Me; and Fucα1,2(6-sulfo)Galβ1,4Glc].

A very interesting observation was made on FT IV when its activity with an acceptor containing either Galβ1,4GlcNAc or 3-sulfoGalβ1,4GlcNAc linked β1,6 to GalNAc of the T-hapten was examined. FT IV showed 249% activity with 3-sulfoGalβ1,4GlcNAcβ1,6(Galβ1,3)GalNAcα-*O*-Bn as compared to 37% activity with 3-sulfoGalβ1,4GlcNAc-, whereas FT V showed almost the same activity (358% and 345%, respectively) and FT III exhibited significantly less activity (104% and 154%, respectively). When Galβ1,4GlcNAcβ1,6-(3-sulfoGalβ1,3)GalNAcα-*O*-Bn was used as an acceptor, FT III and FT V showed much lower activity (19% and 55%, respectively), whereas FT IV was 345% active. Such a high activity exhibited by FT IV and not by FT III and FT V toward this acceptor suggests that the chain termination on the β1,3 branch has no effect on the activity of FT IV toward the β1,6 branch. It is reasonable to state that the branched structures noted above are preferred acceptors for FT IV.

Type 1 (Galβ1,3GlcNAcβ-)-Based Structures as Acceptors for Cloned Enzymes FT III, FT IV, and FT V. The acceptors containing type 1, namely, Galβ1,3GlcNAcβ-*O*-Al, Fucα1,2Galβ1,3GlcNAcβ-*O*-pNP, and Galα1,3Galβ1,3GlcNAcβ-*O*-Bn, were almost inactive with FT IV. FT V showed low activity with these acceptors (see Table 2) as compared to its activity with 2′-fucosylLacNAcβ-*O*-pNP (Table 1). On the contrary, FT III was quite active with type 1 acceptors. As compared to Galβ1,4GlcNAcβ-*O*-Al, Galβ1,3GlcNAcβ-*O*-Al was 7-fold active (CPM × 10^{-3} = 2.88 versus 20.91). H type 1 as well as the 3′-sulfo derivative of type 1 had the same degree of activity (98% and 106%, respectively), whereas Galα1,3Galβ1,3GlcNAcβ-*O*-Bn exhibited 186% activity.

Substitution on C-6 of GlcNAc with a sulfo group in type 1 chain reduced the activity of FT III to 22%, whereas substitution in the same position with a sialyl group, surprisingly, retained 40% acceptor activity. Substitution with *O*-methyl retained 100% activity. Type 1 acceptors containing either the 6-sulfo or 6-sialyl group on Gal exhibited negligible amounts of activity [see the following

Table 2: Reactivity of Colo 205 and the Cloned α1,3-L-Fucosyltransferases (FT III, FT IV, and FT V) with Type 1 (Galβ1,3GlcNAcβ-)-Based Synthetic Carbohydrates

synthetic carbohydrate (3.0 mM)	fucosyltransferase activity, incorporation of [^{14}C]Fuc (CPM × 10^{-3})			
	Colo 205	FT III	FT IV	FT V
Galβ1,4GlcNAcβ-*O*-Al	26.95	2.88	3.75	0.62
Galβ1,3GlcNAcβ-*O*-Al	27.10 (100)	20.91 (100)	0.04 (100)	0.32 (100)
Fucα1,2Galβ1,3GlcNAcβ-*O*-pNP	27.49 (101)	20.47 (98)	0.30 (350)	0.31 (97)
Galα1,3Galβ1,3GlcNAcβ-*O*-Bn		38.84 (186)	0.05 (125)	0.69 (216)
Galβ1,3(Fucα1,4)GlcNAcβ-*O*-Al	3.38 (12)	1.53 (7)		
Galβ1,3(4-*O*-Me)GlcNAcβ-*O*-Bn	5.30 (20)	2.64 (13)		
Galβ1,3(6-*O*-Me)GlcNAcβ-*O*-Bn	29.98 (111)	20.85 (100)		
Galβ1,3(4,6-di-*O*-Me) GlcNAcβ-*O*-Bn	1.88 (7)	0.77 (4)		
Galβ1,3(NeuAcα2,6)GlcNAcβ-*O*-Bn	2.52 (9)	8.30 (40)		
NeuAcα2,6Galβ1,3GlcNAcβ-*O*-Bn	0.18 (<1)	0.06 (<1)		
6-sulfoGalβ1,3GlcNAcβ-*O*-Al	2.04 (8)	1.02 (5)		
Galβ1,3(6-sulfo)GlcNAcβ-*O*-Bn	8.85 (33)	4.53 (22)		
3-sulfoGalβ1,3GlcNAcβ-*O*-Al	21.98 (81)	22.23 (106)		
3-sulfoGalβ1,3(6-*O*-Me)GlcNAcβ-*O*-Al	17.65 (65)	18.13 (87)		
Fucα1,2(6-sulfo)Galβ1,3GlcNAcβ-*O*-Al	0 (0)	0.02 (<1)		

Table 3: Discerning the Specificities of α1,3-Fucosyltransferases Present in Calf Mesenteric Lymph Node (CMLN) and HL60 (Myeloid), B142 (Lymphoid), and EKVX (Lung Adenocarcinoma) Cell Lines

synthetic carbohydrate (3.0 mM)	fucosyltransferase activity, incorporation of [^{14}C]Fuc (CPM × 10^{-3})			
	CMLN	HL60	B142	EKVX
Galβ-*O*-Bn	0 (0)	0.15 (<1)	0.03 (<1)	0.08 (<1)
Galβ1,4GlcNAcβ-*O*-Al	27.15 (100)	26.81 (100)	5.54 (100)	19.37 (100)
Galβ1,3GlcNAcβ-*O*-Al	0.20 (<1)	0.20 (<1)	0 (0)	0 (0)
Fucα1,2Galβ1,4GlcNAcβ-*O*-pNP	31.22 (115)	37.80 (141)	9.20 (166)	30.41 (157)
Fucα1,2Galβ1,4Glc	1.59 (6)	2.70 (10)		0.39 (2)
Galα1,3Galβ1,4GlcNAcβ-*O*-Bn	31.20 (115)	35.70 (133)	8.81 (159)	30.40 (157)
NeuAcα2,3Galβ1,4GlcNAcβ-*O*-Bn	2.43 (9)	3.46 (13)	0.66 (12)	1.36 (7)
3-sulfoGalβ1,4GlcNAc	8.53 (31)	9.80 (37)	1.44 (26)	4.46 (23)
6-sulfoGalβ1,4GlcNAcβ-*O*-Me	0.83 (3)	2.02 (8)		0.16 (<1)
NeuAcα2,3(6-sulfo)Glaβ1,4GlcNAcβ-*O*-Me	0.01 (<1)	0.36 (1)		0 (0)
Fucα1,2(6-sulfo)Galβ1,4Glc	0 (0)	0 (0)		
3-sulfoGalβ1,4GlcNAcβ1,6(Galβ1,3)GalNAcα-*O*-Bn	18.62 (69)	20.68 (77)		
Galβ1,4GlcNAcβ1,6(3-sulfoGalβ1,3)GalNAcα-*O*-Bn	16.42 (60)	16.53 (62)		

acceptors: 6-sulfoGalβ1,3GlcNAcβ-*O*-Al; NeuAcα2,6Galβ1,3GlcNAcβ-*O*-Bn; and Fucα1,2(6-sulfo)Galβ1,3GlcNAcβ-*O*-Al].

FT V exhibited (see Table 2) the activities of 100%, 194%, and 97%, respectively, toward Galβ1,3GlcNAcβ-*O*-Al, Galβ1,4GlcNAcβ-*O*-Al, and Fucα1,2Galβ1,3GlcNAcβ-*O*-pNP. The results thus lend support to the suggestion of Henry et al. (1995) that FT V is a good candidate for the production of some Lewis antigen by Lewis negative individuals.

Activity of Colo 205 α1,3/4-FT toward Type 1 and Type 2 Structures. In Colo 205 α1,3- and α1,4-FT activities (refer Table 2) toward their respective acceptors, Galβ1,4GlcNAcβ-*O*-Al and Galβ1,3GlcNAcβ-*O*-Al, were almost equal, whereas the α1,3-FT activity was less than 15% of the α1,4-FT activity in FT III. The Colo 205 FT activities (expressed as % of the activity with Galβ1,3GlcNAcβ-*O*-Al) with various type 1 acceptors almost paralleled that of FT III except for the acceptor Galβ1,3(NeuAcα2,6)GlcNAcβ-*O*-Bn, which was less active with Colo 205 FT (9%). Considerable differences, however, were noticed in Colo 205 FT and FT III activity toward type 2 containing structures. H type 2 exhibited the same activity as the basic type 2 (Fucα1,2Galβ1,4GlcNAcβ-*O*-pNP, 101%) with Colo 205 FT, whereas FT III exhibited ∼4-fold activity with H type 2. On the contrary, while Galα1,3Galβ1,4GlcNAcβ-*O*-Bn and NeuAcα2,3Galβ1,3GlcNAcβ-*O*-Bn respectively exhibited less than $^1/_4$ and $^1/_9$ activity with FT III, their activities with Colo 205 FT were 108% and 58%, respectively, when compared to that toward Fucα1,2Galβ1,4GlcNAcβ-*O*-pNP.

Also, compared to Fucα1,2Galβ1,4GlcNAcβ-*O*-pNP, 3-sulfoGalβ1,4GlcNAc and Galβ1,4(6-sulfo)GlcNAcβ-*O*-Al respectively showed 83% and 48% activity with Colo 205 FT, whereas with FT III their respective activities were only 35% and 5%. Type 2 acceptors containing 6-sulfated Gal also showed negligible activity with Colo 205 FT.

As observed with FT III, the 3-sulfoGalβ1,4GlcNAcβ1,6(Galβ1,3)GalNAcα-*O*-Bn acceptor was less active than 3-sulfoGalβ1,4GlcNAc with Colo 205 FT (56% and 83%, respectively). In contrast to FT III which was only $^1/_5$ as active with Galβ1,4GlcNAcβ1,6(3-sulfoGalβ1,3)GalNAcα-*O*-Bn when compared to its activity with 3-sulfoGalβ1,4GlcNAcβ1,6(Galβ1,3)GalNAcα-*O*-Bn, the activity of Colo 205 FT with these acceptors was almost equal.

Activities of α1,3-FT Present in Calf Mesenteric Lymph Nodes (CMLN), HL60 (Myeloid), B142 (Lymphoid), and EKVX (Lung Adenocarcinoma). The above sources (see Table 3) showed either 0% or <1% activity with the acceptors Galβ-*O*-Bn and Galβ1,3GlcNAcβ-*O*-Al, indicating that α1,2- and α1,4-FT activities are almost non-existent in these sources. Fucα1,2Galβ1,4GlcNAcβ-*O*-pNP and Galα1,3Galβ1,4GlcNAcβ-*O*-Bn were better acceptors for α1,3-FT in these sources as compared to Galβ1,4GlcNAcβ-*O*-Al. 2′-Fucosyllactose and 3′-sialylLacNAcβ-*O*-Bn exhibited low acceptor activity with these sources. Considerable

Table 4: Differentiation of α1,3-L-Fucosyltransferases on the Basis of Differences in Their Affinities toward 2′-FucosylLacNAc*β*-*O*-pNP and 3′-SulfoLacNAc*β*-*O*-Al/AA-CP

enzyme source	α1,3-L-fucosyltransferase activity, incorporation of [^{14}C]Fuc into the acceptor (CPM × 10^{-3}/mg of protein)		
	A, 2′-fucosylLacNAc*β*-*O*-pNP[a] (3.0 mM)	*B*, 3′-sulfoLacNAc*β*-*O*-Al/AA-CP[b] (62.5 μM)	ratio, *B*/*A* (%)
Colo 205 (colon carcinoma)	663.2	392.9	59.2
HL60 (myeloid)	487.5	43.5	8.9
B142 (lymphoid)	85.3	3.4	4.0
calf mesenteric lymph node	631.0	124.9	19.8
human breast tumor	122.2	9.0	7.4
human ovarian tumor	91.6	10.0	10.9

[a] The radioactive product from this acceptor was measured by Dowex-1-Cl method. [b] The radioactive product from this acceptor remained at the origin of silica gel GHLF plates after chromatography using ethylacetate:pyridine:water:acetic acid (5/5/3/1). This was quantitated by scraping the silica gel into scintillation vials containing 2.0 mL of water and then liquid scintillation counting. Correction was made by subtracting the radioactivity at the origin of TLC plates from the blanks containing no acceptor.

Table 5: Effect of Copolymers on the α1,3/4-FT Activities

copolymer	enzyme source	Inhibition of FT Activity (%)	
		α1,3	α1,4
3-sulfoGal*β*1,3GlcNAc*β*-*O*-Al/AA-CP (62.5 μM)	Colo 205	56.1	90.4
	cloned FT III (Lewis type)	ND[a]	84.3
	HL60	0	
3-sulfoGal*β*1,4GlcNAc*β*-*O*-Al/AA-CP (62.5 μM)	Colo 205	54.6	0
	cloned FT III (Lewis type)	ND	0
	HL60	0	

[a] ND, not determined.

activity (23%−37%) was observed with 3′-sulfoLacNAc. CMLN and HL60 FTs were active to a very small extent with 6′-sulfoLacNAc*β*-*O*-Me (3% and 8%, respectively), whereas no activity was observable with 3′-sialyl-6′-sulfoLacNAc*β*-*O*-Me, and 2′-fucosyl-6′-sulfolactose.

Both CMLN and HL60 showed more than 2-fold activity with 3-sulfoGal*β*1,4GlcNAc*β*1,6(Gal*β*1,3)GalNAcα-*O*-Bn as compared to 3-sulfoGal*β*1,4GlcNAc. As compared to their activities with Gal*β*1,4GlcNAc*β*1,6(Gal*β*1,3)GalNAcα-*O*-Bn, CMLN and HL60 also showed an almost equal amount of activity with Gal*β*1,4GlcNAc*β*1,6(3-sulfoGal*β*1,3)GalNAcα-*O*-Bn, indicating that the termination of the *β*1,3 branch does not inhibit the activity of α1,3-FTs of CMLN and HL 60 on the other branch.

Acceptor 3′-SulfoLacNAcβ-O-Al/AA-CP in Conjunction with 2′-FucosylLacNAcβ-O-pNP Serves as a Tool in Differentiation of α1,3-L-Fucosyltransferases. (See Table 4.) A substantial difference was noticed in each case between the α1,3-L-FT activities measured by 2′-fucosylLacNAc*β*-*O*-pNP and the copolymer. When the ratio of the activities measured by the two acceptors was calculated for each case and the resulting values were compared, it was found that the α1,3-FT of Colo 205 differs markedly from the other α1,3-FTs. Further, the α1,3-FT of calf mesenteric lymph node also differed substantially from the α1,3-FTs of HL 60, B142, and breast and ovarian tumors.

Effect of Acrylamide Copolymers Containing either 3-SulfoGalβ1,3GlcNAcβ- or 3-SulfoGalβ1,4GlcNAcβ- Units on α1,3-Fucosyltransferase Activities. (See Table 5.) When α1,3- and α1,4-FT activities of Colo 205 were measured in the presence of increasing concentrations of 3-sulfoGal*β*1,4GlcNAc*β*-*O*-Al/AA-CP, using the Gal*β*1,4GlcNAc*β*-*O*-Al and Gal*β*1,3GlcNAc*β*-*O*-Al acceptors (see Figure 1), inhibition of the α1,3-FT activity and not the α1,4-FT activity was noticed; a maximum inhibition of about 50% was reached at the minimum concentration of 25 μM of the copolymer. When α1,3- and α1,4-FT activities of Colo 205 were measured in the presence of increasing concentrations of 3-sulfoGal*β*1,3GlcNAc*β*-*O*-Al/AA-CP, using the Fucα1,2Gal*β*1,4GlcNAc*β*-*O*-pNP and Fucα1,2Gal*β*1,3GlcNAc*β*-*O*-pNP acceptors, inhibition of both α1,3- and α1,4-FT activities were seen. At 25 μM of this copolymer, ∼50% inhibition (the maximum attainable) of this α1,3-FT activity and 75% inhibition of the α1,4-FT activity were reached; a maximum inhibition (90%) of the α1,4-FT activity was possible at 75 μM of this copolymer. K_i values obtained from Lineweaver−Burke plots were (a) 1.8 μM for the inhibition of Colo 205 α1,3-FT activity by 3-sulfoGal*β*1,4GlcNAc*β*-*O*-Al/AA-CP, (b) 4.2 μM for the inhibition of Colo 205 α1,3-FT activity by 3-sulfoGal*β*1,3GlcNAc*β*-*O*-Al/AA-CP, and (c) 7.4 μM for the inhibition of Colo 205 α1,4-FT activity by 3-sulfoGal*β*1,3GlcNAc*β*-*O*-Al/AA-CP.

When α1,3-FT activity of HL60 was measured in the presence of increasing concentrations of the copolymers, namely, 3-sulfoGal*β*1,3GlcNAc*β*-*O*-Al/AA-CP or 3-sulfoGal*β*1,4GlcNAc*β*-*O*-Al/AA-CP (see Figure 2), no inhibition of this activity was seen with both copolymers using Gal*β*1,4GlcNAc*β*-*O*-Al as the acceptor.

When α1,4-FT activity of the cloned enzyme FT III was measured in the presence of the above copolymers using Gal*β*1,3GlcNAc*β*-*O*-Al as the acceptor, the copolymer 3-sulfoGal*β*1,4GlcNAc*β*-*O*-Al/AA-CP did not inhibit the α1,4-FT activity. In fact, there was a small amount of stimulation of this activity with an increase in the copolymer concentration. On the other hand, a gradual decrease in α1,4-FT activity was noticed with the other copolymer, 3-sulfoGal*β*1,3GlcNAc*β*-*O*-Al/AA-CP. Inhibition reached 56.9% at 12.5 μM and 84.3% at 62.5 μM concentrations of this copolymer. K_i for this inhibition was found to be 13.9 μM.

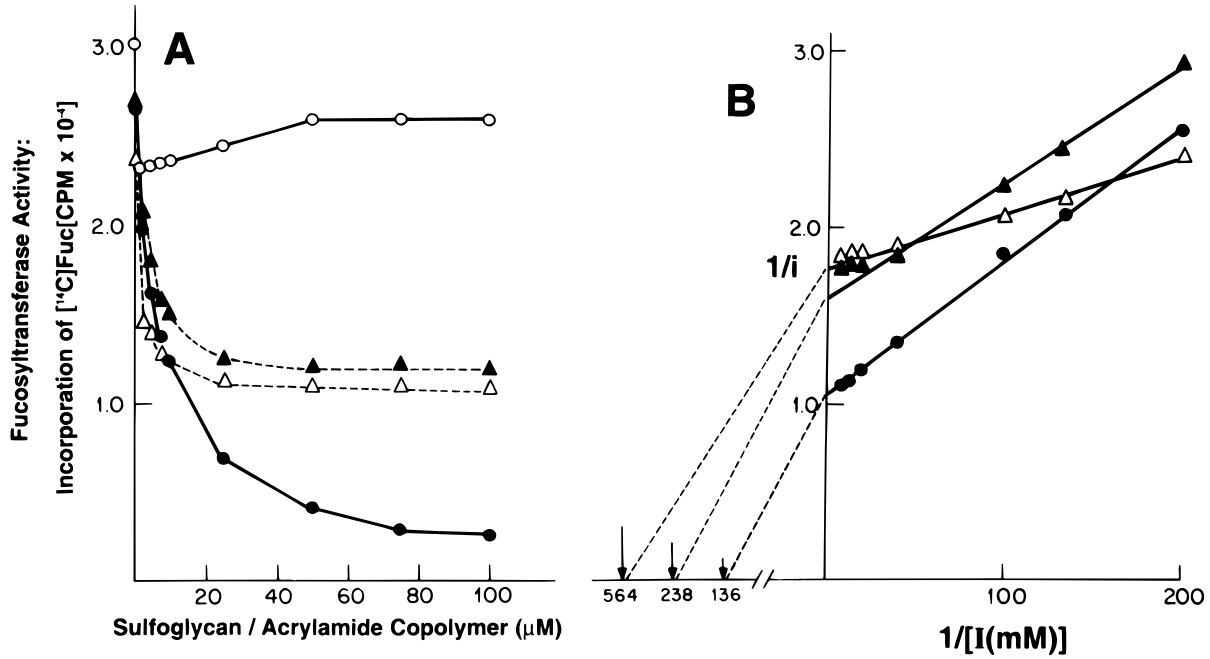

FIGURE 1: Competition between the acceptors and acrylamide−sulfoglycan copolymers for Colo 205 α1,3/4-FTs. (A) Effect of acrylamide−sulfoglycan copolymers on the α1,3-L- and α1,4-L-fucosyltransferase activities of Colo 205: (−●−) Fucα1,2Galβ1,3GlcNAcβ-*O*-pNP [(--▲--) in presence of 3-sulfoFucα1,2Galβ1,4GlcNAcβ-*O*-pNPGalβ1,3GlcNAcβ-*O*-Al/AA-CP]; (−○−) Galβ1,3GlcNAcβ-*O*-Al [(--△--) in presence of 3-sulfoGalβ1,4GlcNAcβ-*O*-AlGalβ1,4GlcNAcβ-*O*-Al/AA-CP]. (B) Determination of K_i for the inhibition of Colo 205 α1,3-FT activity by (△) 3-sulfoGalβ1,3GlcNAcβ-*O*-Al/AA-CP, Colo 205 α1,3-FT activity by (▲) 3-sulfoGalβ1,4GlcNAcβ-*O*-Al/AA-CP, and Colo 205 α1,4-FT activity by (●) 3-sulfoGalβ1,3GlcNAcβ-*O*-Al/AA-CP.

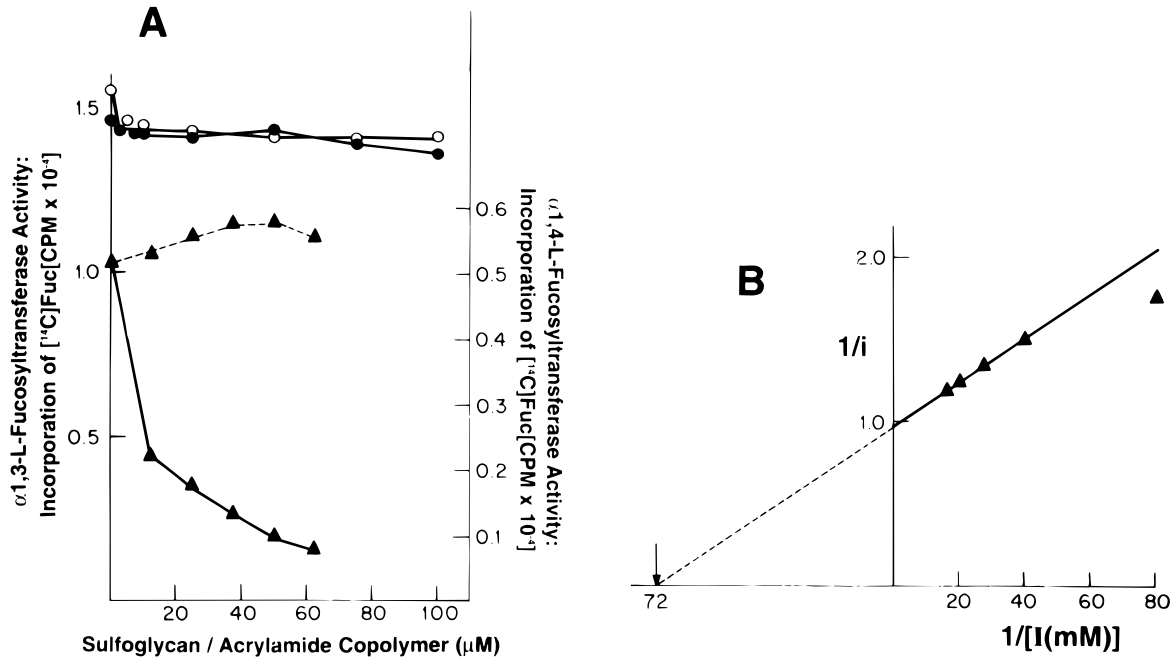

FIGURE 2: Competition between the acceptors and acrylamide−sulfoglycan copolymers for HL 60 α1,3-FT and the cloned enzyme FT III (α1,4-FT). (A) The effect of acrylamide-sulfoglycan copolymers on HL60 α1,3-FT and FT III α1,4-FT activity. HL60 α1,3-FT activity with Galβ1,4GlcNAcβ-*O*-Al as the acceptor in presence of (●) 3-sulfoGalβ1,4GlcNAcβ-*O*-Al/AA-CP and in presence of (○) 3-sulfoGalβ1,3GlcNAcβ-*O*-Al/AA-CP. FT III α1,4-FT activity with Galβ1,3GlcNAcβ-*O*-Al as the acceptor in presence of (−▲−) 3-sulfoGalβ1,3GlcNAcβ-*O*-Al/AA-CP and in presence of (--▲--) 3-sulfoGalβ1,4GlcNAcβ-*O*-Al/AA-CP. (B) Determination of K_i for the inhibition of FT III α1,4-FT activity by 3-sulfoGalβ1,4GlcNAcβ-*O*-Al/AA-CP.

Recently, Sasaki et al. (1994) reported the expression of both Fuc-T IV and Fuc-T VII in myeloid lineage cells, and they also identified the 3′-sialyl type 2 structure as an exclusive acceptor for Fuc-T VII. The α1,3-FTs present in CMLN, HL60, B142, and EKVX were shown in the present study to be exclusively active with type 2 acceptors. These enzymes exhibited some activity toward NeuAcα2,3Galβ1,4GlcNAcβ-*O*-Bn (7%−13%). It is known from several studies that 3′-sialyl type 2 does not serve as an acceptor for myeloid type Fuc-T IV. The present study also found that Fuc-T IV was least reactive with this structure as compared to its activity with 2′-fucosyl type 2. Thus, our results appear to be consistent with the findings of Sasaki et al.

Sasaki et al. (1994) have shown that Fuc-T VII does not react with neutral type 2, Galβ1,4GlcNAcβ- structure, so the

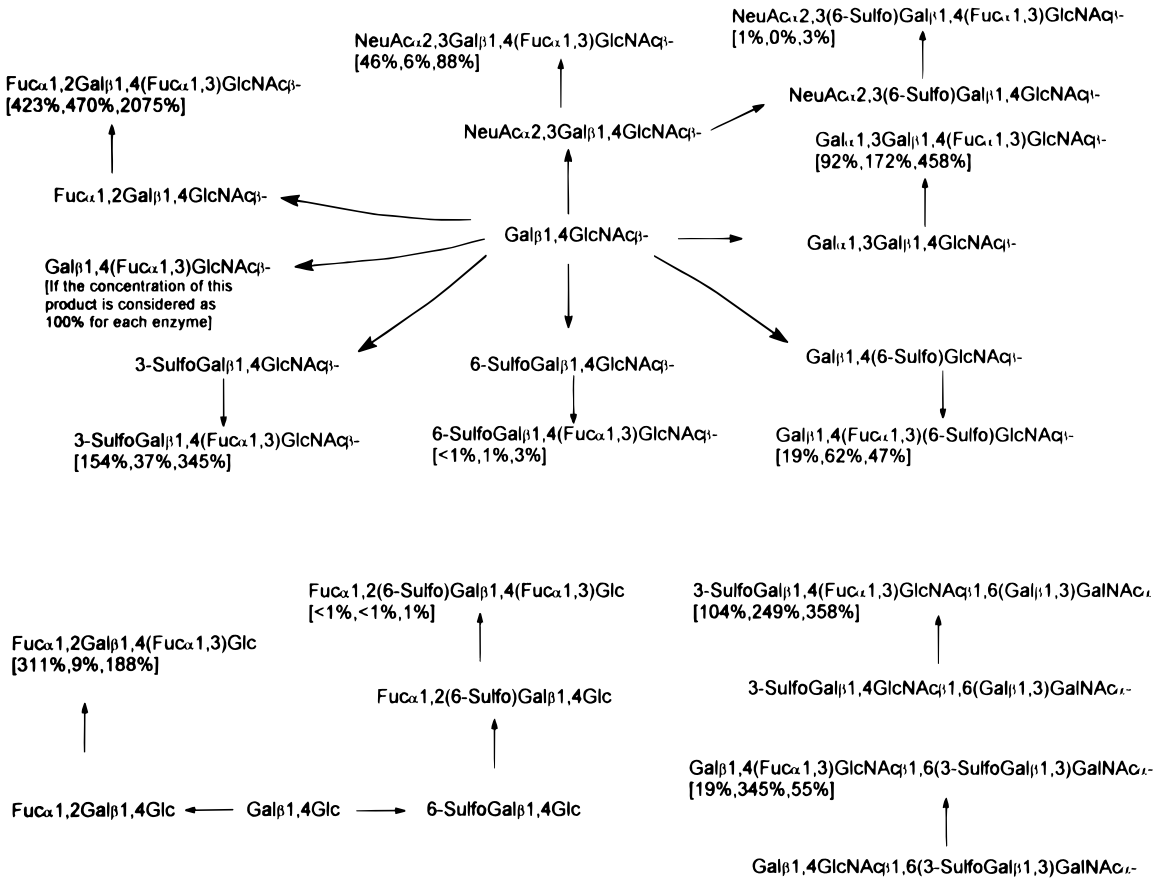

FIGURE 3: Specificities of the cloned enzymes FT III (Lewis type), FT IV (myeloid type), and FT V (plasma type) toward blood group type 2 chain-based structures (The amount of product formed from each compound tested as the acceptor is shown in parentheses as the percent of the activity with the basic structure Gal*β*1,4GlcNAc*β*-; the three values in the order shown in parentheses are obtained for FT III, FT IV, and FT V, respectively).

activity measured in CMLN, HL60, B142, and EKVX with neutral type 2 acceptors must be attributed to Fuc-T IV. We find that the activities shown by the above FTs with Fuc-α1,2Gal*β*1,4GlcNAc*β*-*O*-pNP and Galα1,3Gal*β*1,4GlcNAc*β*-*O*-Bn were almost the same in each case. These results would indicate that Fuc-T is capable of forming Lewis y as well as 3′-α-galactosyl Lewis x at the same rate.

Assuming from the available evidence that Fuc-T IV reacts with neutral type 2 and Fuc-T VII with 3′-sialyl type 2, a structure such as 3′-sulfoLacNAc, which is similar to 3′-sialylLacNAc, is expected to serve as an efficient substrate for Fuc-T VII and not for Fuc-T IV. We also found that with 3′-sulfoLacNAc Fuc-T IV is only 37% active as compared to its 470% activity shown with 2′-fucosylLacNAc*β*-*O*-pNP. On the contrary, the high activity shown by Fuc-T IV toward 3-sulfoGal*β*1,4GlcNAc*β*1,6(Gal*β*1,3)-GalNAcα-*O*-Bn would suggest that the enzyme affinity for a particular structure is greatly influenced by the neighboring sugars.

Sasaki et al. found Fuc-T III (Lewis-type), Fuc-T IV (myeloid-type) and Fuc-T VI (plasma-type) to be expressed at a significant level in Colo 205 in ratios of 2.0:0.2:1.1, respectively. They also found levels of Fuc-T IV and Fuc-T VII (3′-sialylLacNAc, an exclusive substrate) in HL60 in a ratio of 1.3:0.8. The absence of Fuc-T III and the presence of Fuc-T VII in HL 60 are also evident from the present study which demonstrated the absence of α1,4-FT activity and the presence of α1,3-FT acting on 3′-sialylLacNAc in HL60. We have shown in an earlier study the occurrence of both Lewis type and plasma type FTs in Colo 205 by separating the two enzymes through affinity and gel filtration columns (Chandrasekaran et al., 1995). The present study has shown the unique ability of Colo 205 FT to use the copolymer 3′-sulfoLacNAc*β*-*O*-Al/AA-CP as an acceptor. When it was tested as a competitive inhibitor, this copolymer inhibited the α1,3-FT activity of Colo 205 to a great extent but did not inhibit HL60 α1,3-FT. Further, this acceptor was not able to inhibit the α1,4-FT activity of Colo 205 which has inherent α1,3-FT activity. These results indicate the usefulness of this copolymer as an acceptor and as a competitive inhibitor for identifying Fuc-T VI (plasma type) in tissues and cell lines.

The activities of FT III, FT IV, and FT V toward blood group type 2-based compounds as well as the activity of FT III toward blood group type 1-based compounds are depicted in Figures 3 and 4, respectively. For each enzyme the concentration of the product formed from various acceptors is depicted as the percentage of product arising from basic type 1 (Gal*β*1,3GlcNAc*β*-) or type 2 (Gal*β*1,4GlcNAc*β*-). This is also indicated in the figures. These illustrations would enable one to understand the intricate specificities of these enzymes through a direct comparison of their activities with each compound tested as an acceptor and also by a comparison of the activities of each enzyme toward different acceptors. Several unique differences in the specificities of these enzymes were noticeable as listed below:

(A) The participation of FT V is extremely shifted toward the formation of Lewis y (2075%) and 3′-α-galactosyl Lewis x (458%) as compared to the formation of Lewis x (100%).

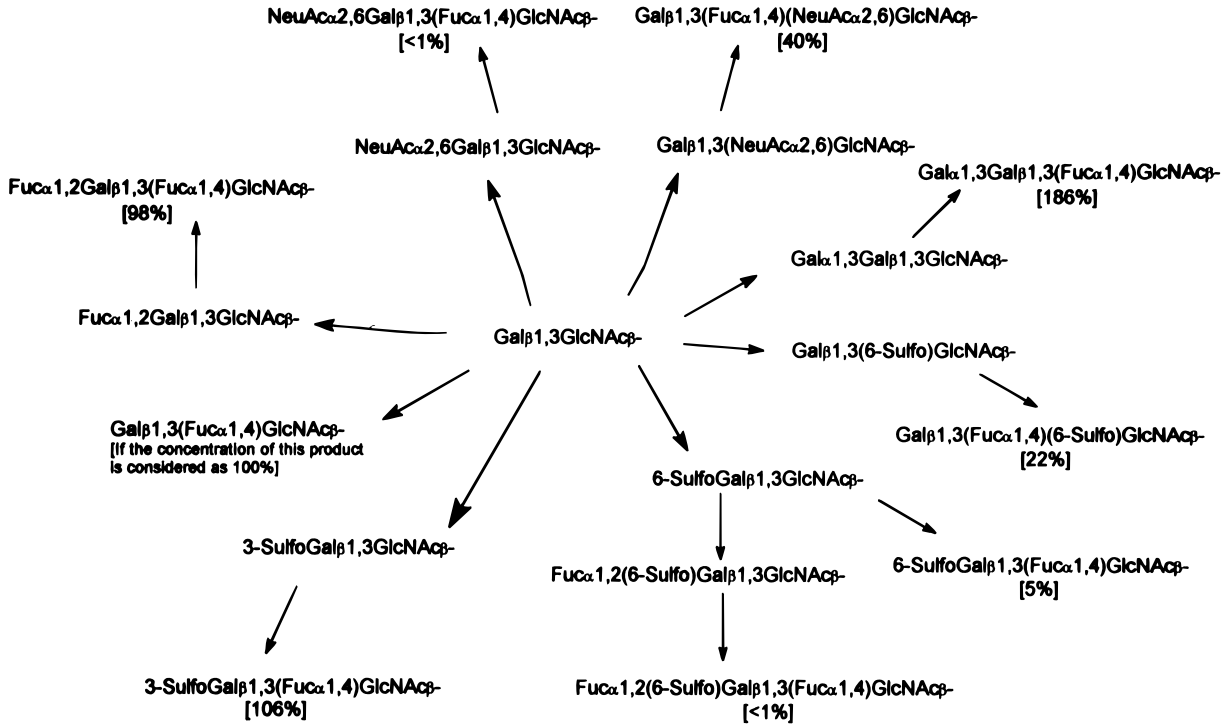

FIGURE 4: Specificities of the cloned enzyme FT III (Lewis type) toward blood group type 1 chain-based structures. (The amount of product formed from each compound tested as the acceptor is shown in parentheses as the percent of the activity with the basic structure Galβ1,3GlcNAcβ-).

(B) Both FT III and FT V are quite efficient in synthesizing 3′-sialyl Lewis x (46% and 88%, respectively), but they show higher efficiency in forming 3′-sulfo Lewis x (154% and 345%, respectively).

(C) All three enzymes catalyze the formation of 6-sulfo Lewis x, but FT IV appears to be the most efficient (62%).

(D) It is noteworthy that the biosynthesis of either 6′-*O*-sulfo Lewis x or 3′-sialyl-6′-*O*-sulfo Lewis x from their immediate precursors does not seem to be catalyzed by these FTs. The latter structure occurs as a part of the carbohydrate moiety in GLYCAM-I (Hemmerich et al., 1995), and on the basis of our observations sulfation appears to be the last step in its synthesis (Chandrasekaran et al., 1995).

(E) Both FT III and FT V are highly efficient in converting 2′-fucosyllactose to 2′-fucosyl-3-fucosyllactose (311% and 188%, respectively), but all three enzymes do not form 2′-fucosyl-6′-sulfo-3-fucosyl lactose from the immediate precursor.

(F) FT IV appears to be unique in showing vast preference for forming 3′-sulfo Lewis x (249%) as well as Lewis x (345%), where the precursor structures occur as part of a mucin type chain.

(G) The activities of FT III and FT V with the basic type 1 are ~7 times and 0.5 times those of the basic type 2, respectively, whereas FT IV is almost inactive with type 1.

(H) The acceptor activity of H-type 1 with FT III is not greater than that of the basic type 1, whereas the formation of 3′-α-galactosyl Lewis x is nearly twice.

(I) The most interesting finding is the facile synthesis of Galβ1,3(Fucα1,4)(NeuAcα2,6)GlcNAcβ- from Galβ1,3-(NeuAcα2,6)GlcNAcβ- by FT III (40%).

(J) Either C-6 sulfation or C-6 sialylation of Gal in type 1 leads to almost complete loss of acceptor activity toward FT III.

(K) On C-3 sulfation of Gal or C-6 sulfation of GlcNAc in type 1, the acceptor activities became 106% and 22%, respectively, toward FT III.

Thus, the above findings on the specificities of these enzymes would serve as a sound basis to explain the distinct role of these enzymes in the expression of biologically relevant carbohydrate ligands and antigenic determinants.

ACKNOWLEDGMENT

The authors thank J. M. Rhodes for his assistance in preparing Figures 3 and 4. The editorial assistance of C. Piskorz is deeply appreciated.

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