Characterization of the Specificities of Human Blood Group H Gene-Specified α1,2-L-Fucosyltransferase toward Sulfated/Sialylated/Fucosylated Acceptors: Evidence for an Inverse Relationship between α1,2-L-Fucosylation of Gal and α1,6-L-Fucosylation of Asparagine-Linked GlcNAc[†]

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ABSTRACT: The assembly of complex structures bearing the H determinant was examined by characterizing the specificities of a cloned blood group H gene-specified α1,2-L-fucosyltransferase (FT) toward a variety of sulfated, sialylated, or fucosylated Galβ1,3/4GlcNAcβ- or Galβ1,3GalNAcα-based acceptor structures. (a) As compared to the basic type 2, Galβ1,4GlcNAcβ- (K_m = 1.67 mM), the basic type 1 was 137% active (K_m = 0.83 mM). (b) On C-6 sulfation of Gal, type 1 became 142.1% active and type 2 became 223.0% active (K_m = 0.45 mM). (c) On C-6 sulfation of GlcNAc, type 2 showed 33.7% activity. (d) On C-3 or C-4 fucosylation of GlcNAc, both types 1 and 2 lost activity. (e) Type 1 showed 70.8% and 5.8% activity, respectively, on C-6 and C-4 O-methylation of GlcNAc. (f) Type 1 retained 18.8% activity on α2,6-sialylation of GlcNAc. (g) Terminal type 1 or 2 of extended chain had lower activity. (h) With Gal in place of GlcNAc in type 1, the activity became 43.2%. (i) Compounds with terminal α1,3-linked Gal were inactive. (j) Galβ1,3GalNAcα- (the T-hapten) was ∼0.4-fold as active as Galβ1,4GlcNAcβ-. (k) C-6 sulfation of Gal on the T-hapten did not affect the acceptor activity. (l) C-6 sulfation of GalNAc decreased the activity to 70%, whereas on C-6 sulfation of both Gal and GalNAc the T-hapten lost the acceptor ability. (m) C-6 sialylation of GalNAc also led to inactivity. (n) β1,6 branching from GalNAc of the T-hapten by a GlcNAc residue or by units such as Galβ1,4GlcNAc-, Galβ1,4(Fucα1,3)GlcNAc-, or 3-sulfoGalβ1,4GlcNAc- resulted in 111.9%, 282.8%, 48.3%, and 75.3% activities, respectively. (o) The enhancement of enzyme affinity by a sulfo group on C-6 of Gal was demonstrated by an increase (∼5-fold) in the K_m for Galβ1,4GlcNAcβ1,6(Galβ1,3)GalNAcα-*O*-Bn in presence of 6-sulfoGalβ1,-4GlcNAcβ-*O*-Me (3.0 mM). (p) Among the two sites in Galβ1,4GlcNAcβ1,6(Galβ1,3)GalNAcα-*O*-Bn, the enzyme had a higher affinity (>3-fold) for the Gal linked to GlcNAc. (q) With respect to Galβ1,-3GlcNAcβ-*O*-Bn (3.0 mM), fetuin triantennary asialo glycopeptide (2.4 mM), bovine IgG diantennary glycopeptide (2.8 mM), asialo Cowper's gland mucin (0.06 mM), and the acrylamide copolymers (0.125 mM each) containing Galβ1,3GlcNAcβ-, Galβ1,3(6-sulfo)GlcNAcβ-, Galβ1,3GalNAcα-, Galβ1,3Galβ-, or Galα1,3Galβ- units were 153.6%, 43.0%, 6.2%, 52.5%, 94.9%, 14.7%, 23.6%, and 15.6% active, respectively. (r) Fucosylation by α1,2-L-FT of the galactosyl residue which occurs on the antennary structure of the bovine IgG glycopeptide was adversely affected by the presence of an α1,6-L-fucosyl residue located on the distant glucosaminyl residue that is directly attached to the asparagine of the protein backbone. This became evident from the 4-fold activity of α1,2-L-FT toward bovine IgG glycopeptide after ∼50% removal of α1,6-linked Fuc.

Surface-expressed H determinants exhibit precise temporal and spatial changes in their expression pattern during human and murine development (Szulman, 1964; Fenderson et al., 1986). Genetic and biochemical observations are consistent with a hypothesis that the H and secretor (Se) blood group loci correspond to distinct α1,2-L-fucosyltransferase (FT)[1] genes with tissue-specific expression patterns and close genetic linkage on chromosome 19 (Rege et al., 1964; Watkins, 1980; Oriol et al., 1981; LePendu et al., 1985; Kumazaki & Yoshida, 1984; Sarnesto et al., 1990; Larsen et al., 1990; Rouquier et al., 1995; Kelly et al., 1995; Hitoshi et al., 1995). Several reports indicate an involvement of α1,2-FTs in cancer and other malignancies. The epithelium of fetal colon and colonic adenocarcinoma, but not the normal distal colon, express blood group H type 2 (Cooper et al., 1978; 1991; Brown et al., 1984; Yuan et al., 1985). An association between tumorigenicity of rat colon cancer clones and the presence of cell surface H blood group antigen or α1,2-FT activity was observed by Zennadi et al. (1992). Subsequently, Labarriere et al. (1994) noticed a modulation

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[1] Abbreviations: FT, fucosyltransferase; Al, allyl; Bn, benzyl; Me, methyl; pNP, *para*-nitrophenyl; ONP, *ortho*-nitrophenyl; CGM, Cowper's gland mucin; AA-CP, acrylamide copolymer; TLC, thin-layer chromatography; GP, glycopeptide.

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Table 1: Specificities of a Cloned α1,2-L-Fucosyltransferase toward Galβ1,3/4GlcNAcβ-Based Structures

synthetic compounds (3.0 mM)	incorporation of [^{14}C]Fuc into the acceptor	
	CPM	%[a]
Galβ1,4GlcNAcβ-*O*-Bn	10 575	100.0
Galβ-*O*-Bn	17 202	162.7
6-sulfoGalβ-*O*-Bn	5154[b]	48.7
3-sulfoGalβ1,4GlcNAc	0	0
6-sulfoGalβ1,4GlcNAcβ-*O*-Me	23 584	223.0
6-sulfoGalβ1,4Glc	13 939	131.8
Galβ1,4(6-sulfo)GlcNAcβ-*O*-Al	3569	33.7
Galβ1,4(Fucα1,3) GlcNAcβ-*O*-Bn	0	0
6-sulfoGalβ1,4(3-*O*-Me)GlcNAcβ-*O*-Bn	8176	77.3
Galβ1,4GlcNAcβ1,6Manα1,6Manβ1,6Man	8474	80.1
Galβ1,4(Fucα1,3)GlcNAcβ1,6Manα1,6Manβ1,6Man	0	0
Galβ1,4(Fucα1,3)GlcNAcβ1,3Galβ-*O*-Me	0	0
Galβ1,3GlcNAcβ-*O*-Bn	14 430	136.5 (100.0)
Galβ1,3GlcNAcβ-*O*-Al	14 755	139.5 (102.3)
3-sulfoGalβ1,3GlcNAcβ-*O*-Bn	0	0
6-sulfoGalβ1,3GlcNAcβ-*O*-Al	20 503	193.9 (142.1)
NeuAcα2,6Galβ1,3GlcNAcβ-*O*-Bn	126	1.9 (0.9)
Galβ1,3(NeuAcα2,6)GlcNAcβ-*O*-Bn	2713	25.7 (18.8)
Galβ1,3(6-sulfo)GlcNAcβ-*O*-Al	14 910	141.0 (103.3)
Galβ1,3(6-*O*-Me)GlcNAcβ-*O*-Bn	11 509	108.9 (79.8)
Galβ1,3(4-*O*-Me)GlcNAcβ-*O*-Bn	841	7.9 (5.8)
Galβ1,3(4,6-di-*O*-Me)GlcNAcβ-*O*-Bn	2080	19.7 (14.4)
Galβ1,3(Fucα1,4)GlcNAcβ-*O*-Al	338	3.2 (2.3)
Galβ1,3GlcNAcβ1,3Galβ1,3GlcNAcβ-*O*-pNP	4144	39.2 (28.7)
Galβ1,3Galβ-*O*-Al	6237	59.0 (43.2)
Galα1,3Galβ-*O*-Al	0	0
Galα1,3Galβ1,3GlcNAcβ-*O*-Bn	0	0

[a] The values in parentheses are the relative activities expressed as percent of the activity toward the acceptor containing just the basic structure. [b] This value was obtained by TLC (silica gel GHLF; 250 μm scored 20 × 20 cm) using butanol:acetic acid:water (3/2/1) since neither the acceptor nor the product could be eluted from the Dowex-1-Cl column by NaCl.

Table 2: Specificities of the Cloned α1,2-L-Fucosyltransferase toward Galβ1,3GalNAcα-Based Structures

synthetic compounds (3.0 mM)	incorporation of [^{14}C]Fuc into the acceptor	
	CPM	%[a]
Galβ1,4GlcNAcβ-*O*-Bn	10 575	100.0
Galβ1,3GalNAcα-*O*-Bn	3635	34.4 (100.0)
Galβ1,3GalNAcα-*O*-Al	4954	46.8 (136.0)
6-sulfoGalβ1,3GalNAcα-*O*-Al	4975	47.0 (136.8)
6-sulfoGalβ1,3GalNAcα-*O*-ONP	1852	17.5 (51.0)
Galβ1,3(6-sulfo)GalNAcα-*O*-Al	3525	33.3 (96.8)
6-sulfoGalβ1,3(6-sulfo)GalNAcα-*O*-ONP	37	0.4 (1.2)
Galβ1,3(NeuAcα2,6)GalNAcα-*O*-Bn	315	3.0 (8.7)
6-sulfoGalβ1,3(NeuAcα2,6)GalNAcα-*O*-ONP	81	0.8 (2.4)
GlcNAcβ1,6(Galβ1,3)GalNAcα-*O*-Bn	4070	38.5 (111.9)
Galβ1,4GlcNAcβ1,6(Galβ1,3)GalNAcα-*O*-Bn	10 288	97.3 (282.8)
Galβ1,4(Fucα1,3)GlcNAcβ1,6(Galβ1,3)GalNAcα-*O*-Me	1970	18.6 (48.3)
Galβ1,4GlcNAcβ1,6(3-sulfoGalβ1,3)GalNAcα-*O*-Bn	5623	53.2 (154.7)
3-sulfoGalβ1,4GlcNAcβ1,6(Galβ1,3)GalNAcα-*O*-Bn	2744	25.9 (75.3)

[a] The values in parentheses are the relative activities expressed as percent of the activity toward the acceptor containing just the basic structure.

of the tumorigenicity of rat colon carcinoma cells by the H blood group antigen carried by CD44V. Blaszczyk-Thurin et al. (1988) identified in gastric carcinoma cells an α1,2-FT which converts Le^a to Le^b. An α1,2-FT in colorectal cancer converted Le^a to Le^b and Le^x to Le^y (Yazawa et al. 1993). Boland and Deshmukh (1990) found about 50% reduction in mean oligosaccharide chain length in cancer-associated colonic mucins as compared to the mucin isolated from normal colons, and this observation is of interest with the finding of Henry et al. (1994) that, in the presence of reduced fucosyltransferase activity, an increased elongation of the precursor carbohydrate chains of glycolipids occurred in Lewis-negative individuals. Recently, the respiratory mucin from cystic fibrosis was shown to contain both sulfation and sialylation of mucin carbohydrate chains carrying blood group H determinant (Lo-Guidice et al., 1994). A cloned blood group H gene-specified α1,2-FT was studied for its acceptor-substrate specificity by Lowary et al. (1994) using structural analogues of octyl β-D-galactopyranoside. The present paper is a detailed investigation on the same cloned enzyme used by the above group, regarding its specificities in relation to the assembly of complex carbohydrate structures bearing the blood group H determinant.

EXPERIMENTAL PROCEDURES

Blood Group H Gene-Specified α1,2-FT. The cloned enzyme employed in the present study was the same studied by Lowary et al. (1994). This enzyme (a fusion protein with Protein A) was cloned by Glycomed in a manner similar to

Table 3: Macromolecular Carbohydrate Structures As Acceptors for the Cloned α1,2-L-Fucosyltransferase

macromolecular compounds	incorporation of [^{14}C]Fuc into the acceptor	
	CPM	%[a]
fetuin triantennary asialoglycopeptide (200 μg = 2.4 mM)	22 667	153.6
bovine IgG diantennary glycopeptide (200 μg = 2.8 mM)	6350	43.0
asialo Cowper's gland mucin (250 μg = 0.06 mM)	920	6.2
Galβ-*O*-Al/AA-CP (100 μg = 0.125 mM)	0	0
Galβ1,3GlcNAcβ-*O*-Al/AA-CP (100 μg = 0.125 mM)	7749	52.5
Galβ1,3(6-sulfo)GlcNAcβ-*O*-Al/AA-CP (100 μg = 0.125 mM)	14 004	94.9
Galβ1,3GalNAcα-*O*-Al/AA-CP (100 μg = 0.125 mM)	2173	14.7
Galβ1,3Galβ-*O*-Al/AA-CP (100 μg = 0.125 mM)	3478	23.6
Galα1,3Galβ-*O*-Al/AA-CP (100 μg = 0.125 mM)	2300	15.6

[a] The values are expressed as percent of the activity toward the simple acceptor, Galβ1,3GlcNAcβ-*O*-Bn (3.0 mM), tested at the same time.

that of Larsen et al. (1990) with the plasmid used by Wei et al. (1993). This cloned enzyme (Glycomed, Alameda, CA) was bound to IgG-Sepharose beads through Protein A which is the dimeric segment of the enzyme (1 mL of IgG-Sepharose/L of the condition media containing the Protein A-enzyme). As our present studies necessitated a soluble enzyme preparation, a 1.0 mL slurry of the Sepharose beads 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. Then 1.0 mL of 0.1 M citrate buffer, pH 4.4, was added to the beads, mixed in cold room for $^1/_2$ h using Speci-Mix (Thermolyne), and then 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. In each assay, 2 μL of this soluble enzyme preparation was used. This enzyme preparation, stored at 4 °C, showed no appreciable loss of enzyme activity for several months.

Assay of α1,2-FT. 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 2 μL of enzyme in a total volume of 20 μL; the control incubation mixtures had everything except the acceptor. At the end of incubation for 1 h at 37 °C, the mixture was diluted with 1.0 mL of water and passed through a 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 the radioactive content was determined using the scintillation mixture 3a70 (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 before. 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. Duplicate sample values obtained did not vary more than 5%.

Synthetic Compounds. We already reported the syntheses of many of the 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.

Macromolecular and Natural Acceptors. (A) Acrylamide copolymers of Galβ-*O*-Al, Galβ1,3GlcNAcβ-*O*-Al, Galβ1,3-(6-sulfo)GlcNAcβ-*O*-Al, Galβ1,3GalNAcα-*O*-Al, Galβ1,-3Galβ-*O*-Al, and Galα1,3Galβ-*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 preparations (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.

(B) Asialo CGM, bovine IgG diantennary glycopeptide, and fetuin triantennary sialoglycopeptide were available from earlier studies (Chandrasekaran et al., 1992a, 1994a,b).

(C) Modified Glycopeptides were prepared as follows.

(i) Defucosylation of bovine IgG diantennary glycopeptide. The removal of fucose from this glycopeptide was attempted by two approaches: (a) Acid treatment. The IgG glycopeptide (50 mg) was dissolved in 1 N acetic acid and heated for 2 h at 100 °C (Gibbons et al., 1955). After neutralization with NaOH, the glycopeptide from this mixture was isolated by chromatography on a Bio-Gel P2 column (1.0 cm × 105.0 cm). About 95% of the fucose was still linked to the glycopeptide [thioglycolic acid method of Gibbons (1955)]. (b) Bovine epididymis α-L-Fucosidase treatment. The bovine IgG diantennary glycopeptide (100 mg) was digested with 0.5 unit of this enzyme in 2.0 mL of the accessory buffer (Oxford Glycosystems) containing 0.03 M NaN_3 for 72 h at 37 °C. The resulting glycopeptide, isolated by chromatography on Bio-Gel P2 column, was devoid of ∼50% fucose, as compared to the original glycopeptide.

(ii) Removal of Gal from glycopeptides. Bovine IgG diantennary glycopeptide (44 mg), the IgG glycopeptide devoid of 50% fucose (48 mg), and fetuin triantennary sialoglycopeptide (200 mg) were dissolved separately in 1.0 mL each of 0.3 M sodium acetate buffer, pH 5.0, containing 30 units of β-galactosidase (ex *Aspergillus*, Calbiochem) and incubated for 48 h at 37 °C. After this treatment the resulting glycopeptide from each digest was isolated by chromatography on a Bio-Gel P6 column (1.0 cm × 105.0 cm).

(iii) Further treatments of the fetuin triantennary sialoglycopeptide isolated after digestion with β-galactosidase: The above preparation (100 mg) was dissolved in 30 mL of 0.1 N HCl and heated for 1 h at 80 °C. After neutralization with 1 N NaOH and lyophilization to dryness, this sample was dissolved in 1.0 mL of water and the resulting glycopeptide was isolated from it by chromatography as above on a Bio-Gel P6 column. About 40 mg of this asialo glycopeptide was digested with 30 units of β-galactosidase (ex *Aspergillus*, Calbiochem) in 1.0 mL of 0.3 M sodium acetate buffer, pH 5.0, at 37 °C for 48 h. The resulting

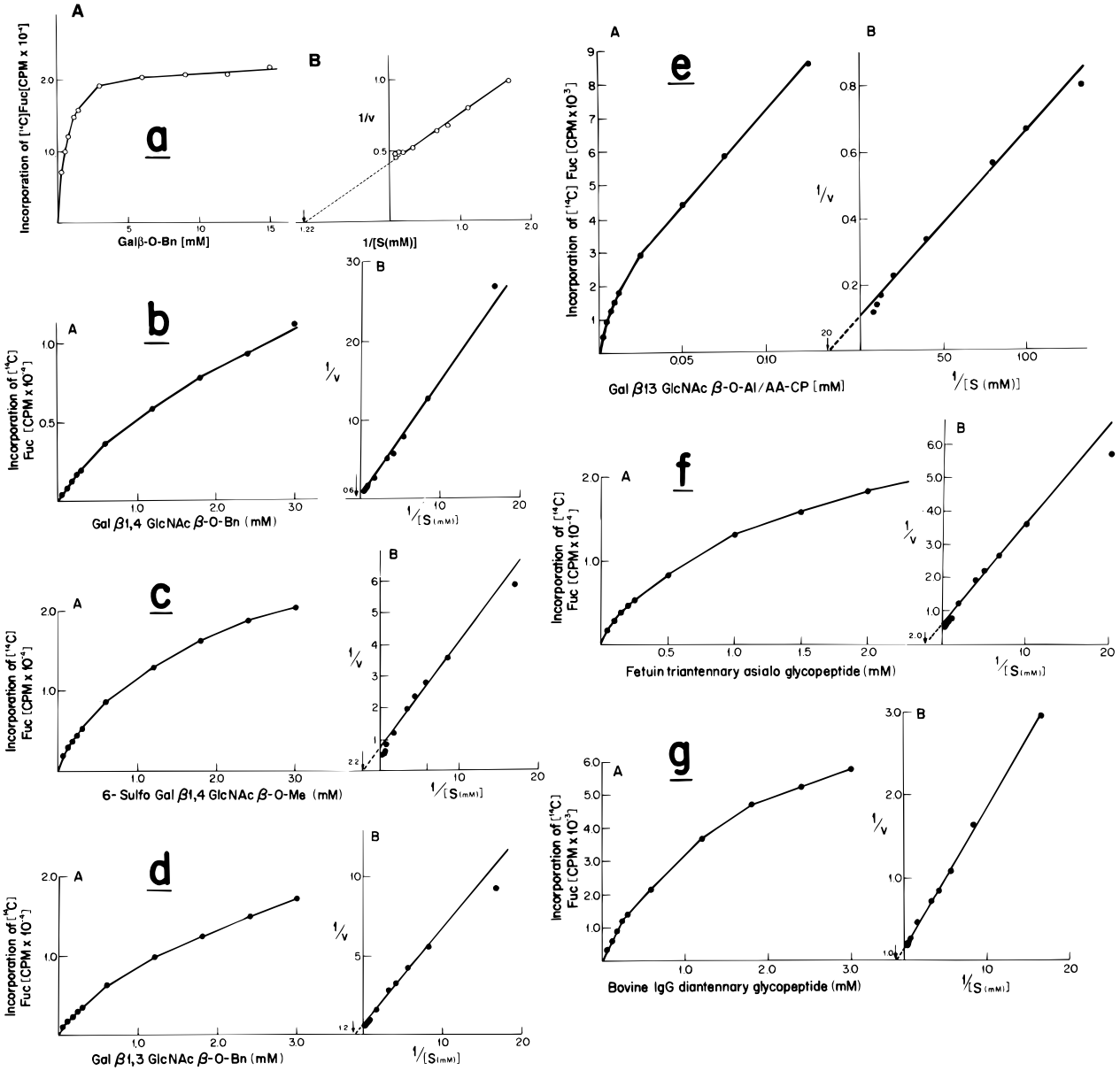

FIGURE 1: In each panel, A shows the activity of the cloned α1,2-L-fucosyltransferase at varying concentrations of acceptor and B shows a determination of K_m and V_{max} by Lineweaver−Burke plot. (a) Gal*β*-*O*-Bn. (b) Gal*β*1,4GlcNAc*β*-*O*-Bn. (c) 6-SulfoGal*β*1,4GlcNAc*β*-*O*-Me. (d) Gal*β*1,3GlcNAc*β*-*O*-Bn. (e) Gal*β*1,3GlcNAc*β*-*O*-Al/AA-CP. (f) Fetuin triantennary asialo glycopeptide. (g) Bovine IgG diantennary glycopeptide.

Table 4: K_m and V_{max} Values Obtained for the Cloned α1,2-L-Fucosyltransferase toward Various Acceptors

acceptor	K_m (mM)	V_{max} (pmol/h)
Gal*β*-*O*-Bn	0.82	38.5
Gal*β*1,4GlcNAc*β*-*O*-Bn	1.67	40.4
6-sulfoGal*β*1,4GlcNAc*β*-*O*-Me	0.45	43.1
Gal*β*1,3GlcNAc*β*-*O*-Bn	0.83	46.2
Gal*β*1,3GlcNAc*β*-*O*-Al/AA-CP	0.31	230.8
fetuin triantennary asialoglycopeptide	0.50	53.8
bovine IgG diantennary glycopeptide	1.00	23.1

glycopeptide was isolated from the above digest by chromatography on the Bio-Gel P6 column.

Large-Scale Isolation of the α1,2-FT Enzymatic Product from the Acceptor 6-SulfoGalβ1,4GlcNAcβ-O-Me. This acceptor (9.0 μmol) was incubated for 20 h at 37 °C with 3.0 μmol of GDP-Fuc and 1.25 μCi of GDP-[^{14}C]Fuc in a reaction volume of 2.0 mL under the standard incubation conditions with 600 μL of the α1,2-FT preparation. The product was isolated by fractionation on a Bio-Gel P2 column (1.0 cm × 105 cm). The position of the product in the effluent of the column was monitored by following [^{14}C]-Fuc radioactivity, galactose (anthrone reaction), and fucose (thioglycolic acid). The unreacted acceptor was followed by anthrone reaction.

Acceptor Competition Experiments. (A) Competition between the acceptor with two terminal Gal residues and the highly active acceptor with terminal 6-sulfoGal. Under the standard incubation conditions, the concentration of Gal*β*1,4GlcNAc*β*1,6(Gal*β*1,3)GalNAcα-*O*-Bn in the reaction mixture was varied between 0.6 and 12.0 mM in presence and absence of 3.0 mM 6-sulfoGal*β*1,4GlcNAc*β*-*O*-Me. Incorporation of [^{14}C]Fuc into both acceptors was measured by the Dowex-1-Cl method (Chandrasekaran et al., 1992b). The [^{14}C] product from the neutral acceptor emerges from the Dowex column in water eluate, and that from sulfated acceptor was in the 0.2 M NaCl eluate.

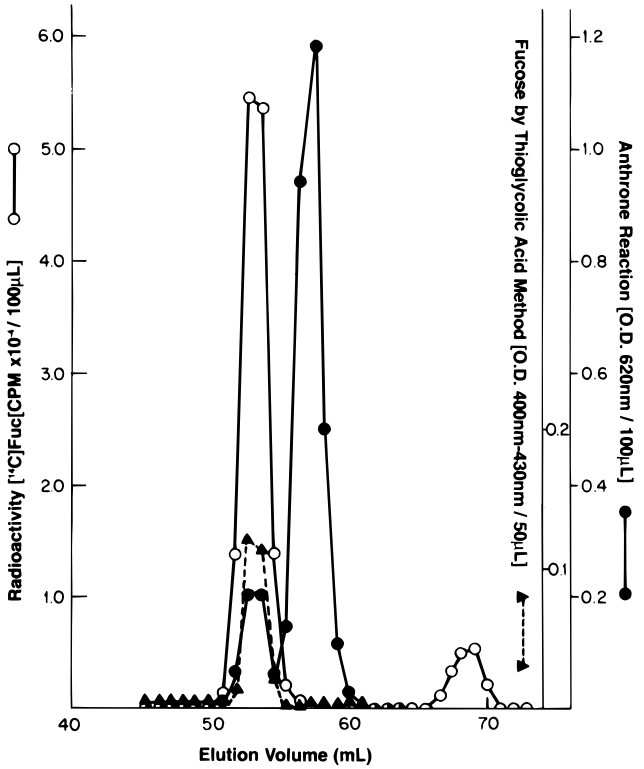

FIGURE 2: Large-scale isolation of the product resulting from the acceptor 6-sulfoGalβ1,4GlcNAcβ-*O*-Me by the action of the cloned α1,2-L-fucosyltransferase. (○) Radioactivity of [^{14}C]Fuc. (●) Galactose by anthrone reaction. (▲) Fucose by thioglycolic acid method.

(B) Competition between the acceptors containing terminal Gal linked β1,3 to either GlcNAc or GalNAc. The concentration of Galβ1,3GalNAcα-*O*-Al was varied in the reaction mixture between 0.6 and 12.0 mM in presence and absence of 0.6 mM 6-sulfoGalβ1,3GlcNAcβ-*O*-Al. Since this sulfated acceptor is almost five times as active as the neutral acceptor, 0.6 mM instead of 3.0 mM was chosen as the concentration of this sulfated acceptor in this competition experiment.

(C) Competition between the acceptor having two terminal Gal residues and its two derivatives, having a 3-sulfo group which acts as a block on either β1,3- or β1,4-linked Gal: The concentration of Galβ1,4GlcNAcβ1,6(Galβ1,3)GalNAcα-*O*-Bn was varied between 0.6 and 12.0 mM both in absence and in presence of either 3-sulfoGalβ1,4GlcNAcβ1,6-(Galβ1,3)GalNAc-α-*O*-Bn (3.0 mM) or Galβ1,4GlcNAcβ1,6-(3-sulfoGalβ1,3)GalNAcα-*O*-Bn (3.0 mM). The [^{14}C] products were quantitated by Dowex-1-Cl method as described above in A.

Influence of Three Different Acceptor Substrates on GDP-Fuc, the Donor Substrate. The concentration of GDP-[^{14}C]-Fuc (specific activity 290 mCi/mmol) in the reaction mixture was varied from 2 to 10 μM, and the incorporation of [^{14}C]-Fuc into each of the acceptors (3.0 mM), namely, Galβ1,-3GlcNAcβ-*O*-Al, 6-sulfoGalβ1,3GlcNAcβ-*O*-Al, and Galβ1,-3GalNAcα-*O*-Al, was measured by the Dowex-1-Cl method.

Influence of pH on the α1,2-FT Activity. The activity of the cloned α1,2-FT was compared with the α1,2-FT activity present in a human ovarian tumor extract using Galβ-*O*-Bn as the acceptor over the pH range 5.2−8.4 in Tris-maleate buffer. Galβ-*O*-Bn is a specific acceptor for α1,2-FT, and it is not reactive with α1,3- and α1,4-FTs, which are also present in the ovarian tumor extract (Chandrasekaran et al., 1992b).

RESULTS AND DISCUSSION

Activity of Cloned α1,2-FT toward Galβ1,3/4GlcNAcβ-Based Structures (Table 1). In comparison to Galβ1,-4GlcNAcβ-*O*-Bn, Galβ-*O*-Bn and its 6-sulfo derivative were 162.7% and 48.7% active, respectively. Interestingly, a sulfate group on C-6 of Gal in the type 2 structure increased activity (6-sulfoGalβ1,4GlcNAcβ-*O*-Me, 223.0%; 6-sulfoGalβ1,4Glc, 131.8%). On the other hand, a substituent on GlcNAc either decreased or abolished the acceptor ability [Galβ1,4(6-sulfo)GlcNAcβ-*O*-Al, 33.7%; Galβ1,4-(Fucα1,3)GlcNAcβ-*O*-Bn, 0%; Galβ1,4(Fucα1,3)GlcNAcβ1,-6Manα1,6Manβ1,6Man, 0%; Galβ1,4(Fucα1,3)Galβ1,-3GlcNAcβ-*O*-Me, 0%; 6-sulfoGalβ1,4(3-*O*-Me)GlcNAcβ-*O*-Bn, 77.3%]. A decrease in acceptor activity was noticed when the β1,4-linked terminal Gal is present in a long carbohydrate chain (Galβ1,4GlcNAcβ1,6Manα1,6Manβ1,-6Man, 80%).

The basic type 1 structures were better acceptors (Galβ1,-3GlcNAcβ-*O*-Bn, 136.5%; Galβ1,3GlcNAcβ-*O*-Al, 139.5%; Galβ1,4GlcNAcβ-*O*-Bn, 100.0%). C-6 sulfation of Gal as seen with the type 2 increased the acceptor activity (193.9%); to the contrary, C-6 sialylation of Gal almost abolished the activity (only 0.9% active). In contrast to the effect on the type 2 structure, C-6 sulfation on GlcNAc did not affect the activity of type 1 structure at all (activity, 103.3%). C-6 O-methylation of GlcNAc also did not have much effect on the activity (79.8% active), but C-4 O-methylation of GlcNAc decreased activity substantially (5.8% active). Even after a bulky substitution on C-6 of GlcNAc with the sialyl group, the type 1 structure still retained 18.8% of the acceptor activity.

As noticed with the type 2 structure, an extension of the chain length containing terminal Gal or fucosylation of the GlcNAc moiety decreased or almost abolished the acceptor activity [Galβ1,3GlcNAcβ1,3Galβ1,3GlcNAcβ-*O*-pNp, 28.7%; Galβ1,3(Fucα1,4)GlcNAcβ-*O*-Al, 2.3%]. When Gal is linked β1,3 to another Gal residue instead of GlcNAc, the acceptor activity decreased to 43.2%. Compounds with α1,3-linked terminal Gal did not display any acceptor activity.

α1,2-FT Activity toward Galβ1,3GalNAcα-Based Structures (Table 2). In comparison to Galβ1,4GlcNAcβ-*O*-Bn, Galβ1,3GalNAcα-*O*-Bn and Galβ1,3GalNAcα-*O*-Al were 34.4% and 46.8% active, respectively. C-6 sulfation of Gal did not affect the acceptor activity since 6-sulfo-Galβ1,3GalNAcα-*O*-Al showed the same activity as Galβ1,-3GalNAcα-*O*-Al. However, 6-sulfoGalβ1,3GalNAcα-*O*-ONP exhibited far less activity (51.0%) than did 6-sulfoGalβ1,-3GalNAcα-*O*-Al (136.8%), indicating that the aglycon *O*-nitrophenyl group has an adverse effect on this enzyme. On C-6 sulfation of the GalNAc moiety, the T-hapten still retained about 70% of its acceptor activity [Galβ1,3GalNAcα-*O*-Al, 136.0%; Galβ1,3(6-sulfo)GalNAcα-*O*-Al, 96.8%]. On the other hand, C-6 sulfation of both T-hapten sugar moieties almost abolished acceptor activity [6-sulfo-Galβ1,3(6-sulfo)GalNAcα-*O*-ONP, 1.2% activity]. Further, the T-hapten nearly lost all the acceptor activity when C-6 of GalNAc is sialylated [Galβ1,3(NeuAcα2,6)Gal-NAcα-*O*-Bn, 8.7%; 6-sulfoGalβ1,3(NeuAcα2,6)GalNAcα-*O*-ONP, 2.4%].

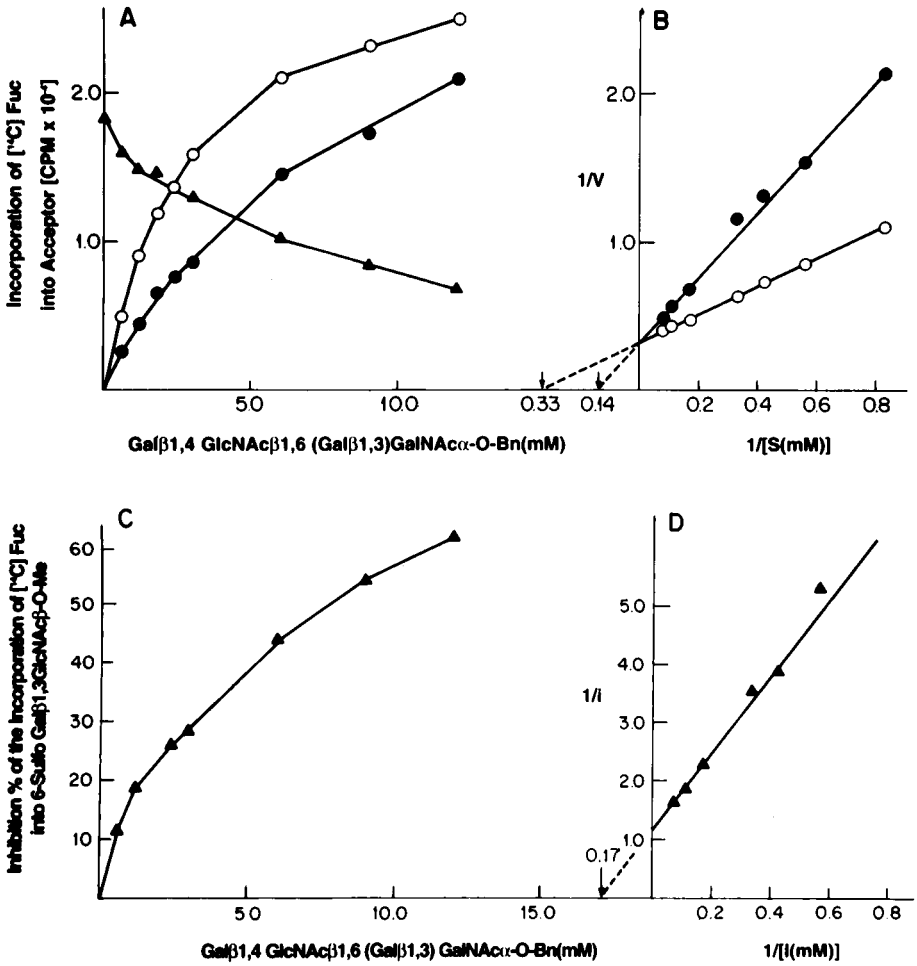

FIGURE 3: Competition between the acceptor having two terminal Gal residues, namely, Galβ1,4GlcNAcβ1,6(Galβ1,3)GalNAcα-*O*-Bn and the sulfated acceptor 6-sulfoGalβ1,4GlcNAcβ-*O*-Me for the cloned α1,2-L-fucosyltransferase. (A) Activity of the cloned α1,2-L-fucosyltransferase at varying concentrations of Galβ1,4GlcNAcβ1,6(Galβ1,3)GalNAc-α-*O*-Bn. Incorporation of [^{14}C]Fuc into Galβ1,-4GlcNAcβ1,6(Galβ1,3)GalNAcα-*O*-Bn (○) in absence of 6-sulfoGalβ1,4GlcNAcβ-*O*-Me, (●) in presence of 3.0 mM 6-sulfoGalβ1,-4GlcNAcβ-*O*-Me, and (▲) into 6-sulfoGalβ1,4GlcNAcβ-*O*-Me. (B) Determination of K_m for Galβ1,4GlcNAcβ1,6(Galβ1,3)GalNAcα-*O*-Bn by Lineweaver−Burke plot. Symbols as in A. (C) Inhibition of the α1,2-L-fucosylation of 6-sulfoGalβ1,4GlcNAcβ-*O*-Me by Galβ1,4GlcNAcβ1,6(Galβ1,3)GalNAcβ-*O*-Bn. (D) Determination of K_i for this inhibition.

When a GlcNAc residue is linked β1,6 to GalNAc in the T-hapten, the acceptor activity is not affected [Galβ1,3-(GlcNAcβ1,6)GalNAcα-*O*-Bn, 111.9% active]. When the β1,6 branch of T-hapten is Galβ1,4GlcNAc, an almost 3-fold increase in acceptor activity was noticed [Galβ1,4GlcNAcβ1,6-(Galβ1,3)GalNAcα-*O*-Bn, 282.8%; Galβ1,3GalNAcα-*O*-Bn, 100.0%]. An α1,3-Fuc on the *N*-acetyllactosamine branch reduced the acceptor activity to a great extent [Galβ1,4-(Fucα1,3)GlcNAcβ1,6(Galβ1,3)GalNAcα-*O*-Me, 48.3%]. The fact that this enzyme is more active toward Gal linked to GlcNAc is clearly evidenced from the acceptor abilities of Galβ1,4GlcNAcβ1,6(Galβ1,3)GalNAcα-*O*-Bn-based acceptors which had been blocked by a 3-sulfo group on either terminal Gal moiety. The acceptor that had a sulfate on Gal linked to GlcNAc was far less active than the other acceptor containing C-3 sulfation of Gal linked to GalNAc [Galβ1,4GlcNAcβ1,6(3-sulfoGalβ1,3)GalNAcα-*O*-Bn, 154.7%; 3-sulfoGalβ1,4GlcNAcβ1,6(Galβ1,3)GalNAcα-*O*-Bn, 75.3%].

Interaction of Macromolecular Structures with Cloned α1,2-FT (Table 3). Fetuin triantennary asialo glycopeptide, on a mole basis, was almost four times as active as bovine IgG diantennary glycopeptide (153.6% and 43.0%, respectively). Asialo CGM, containing a 3:7 ratio of T- to Tn-hapten, showed considerable acceptor activity (6.2%) even when it was used at a very low concentration (0.06 mM) when based on a molecular weight of 200 000. Most of the carbohydrate/acrylamide copolymers showed significant activities. Some noteworthy interesting observations were also made:

(A) Galβ-*O*-Al/AA-CP was completely devoid of acceptor-activity in contrast to the high acceptor activity of Galβ-*O*-Bn.

(B) Galα1,3Galβ-*O*-Al/AA-CP exhibited 15.6% activity at 0.125 mM, whereas no activity could be observed with Galα1,3Galβ-*O*-Al even at 3.0 mM.

(C) The acceptor activity of Galβ1,3(6-sulfo)GlcNAcβ-*O*-Al/AA-CP was nearly 2-fold that of Galβ1,3GlcNAcβ-*O*-Al/AA-CP (94.9% and 52.5% active, respectively), whereas Galβ1,3(6-sulfo)GlcNAcβ-*O*-Al and Galβ1,3GlcNAcβ-*O*-Al had the same activity (103.3% and 102.3% active, respectively).

Determination of K_m and V_{max} Values for Cloned α1,2-FT. Lineweaver−Burke plots of the above data are presented in Figure 1. K_m and V_{max} were calculated from the intercepts on the *X*-axis and the *Y*-axis, respectively, and the values are reported in Table 4. Among the substrates examined the most effective one was the copolymer Galβ1,3GlcNAcβ-

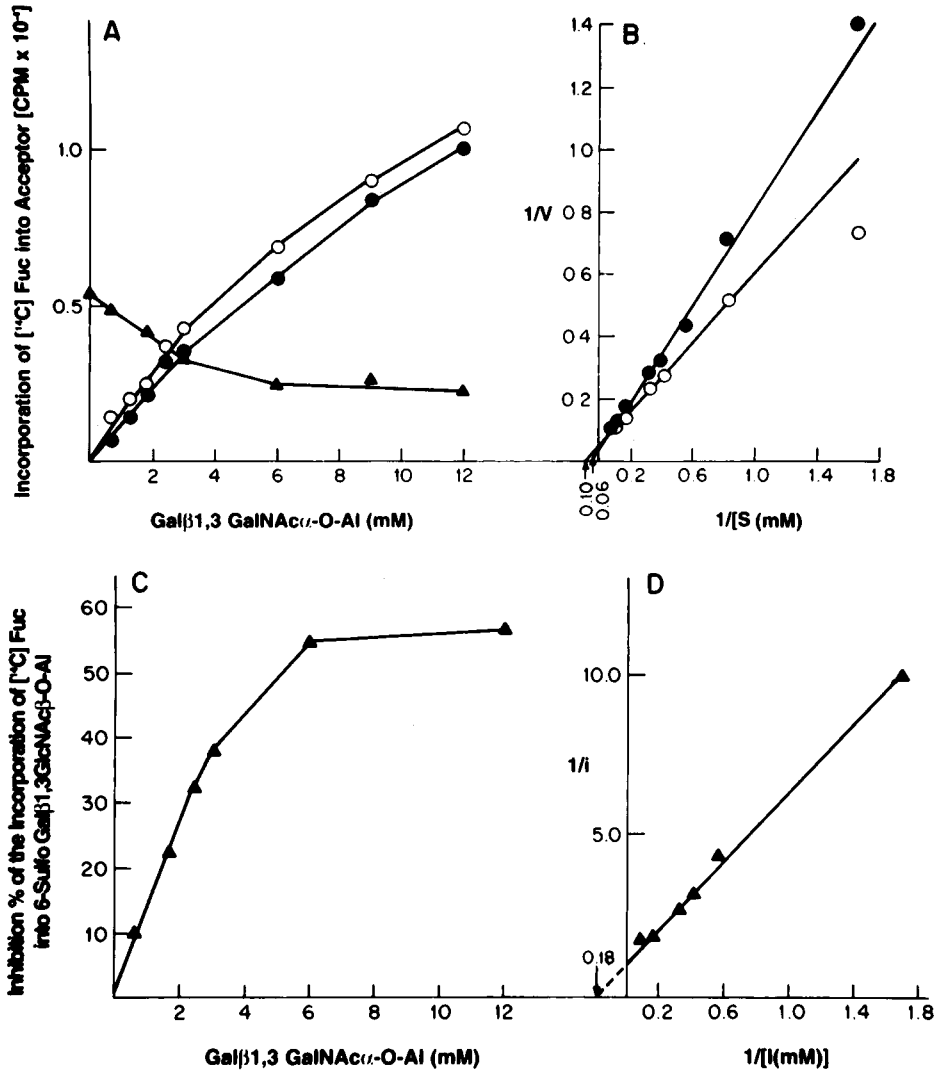

FIGURE 4: Competition between acceptors containing terminal Gal linked β1,3 to GlcNAc or GalNAc, respectively. (A) Activity of the cloned α1,2-L-fucosyltransferase at varying concentrations of Galβ1,3GalNAcα-*O*-Al. Incorporation of [^{14}C]Fuc into Galβ1,3GalNAcα-*O*-Bn (○) in absence of 6-sulfoGalβ1,3GlcNAcβ-*O*-Al, (●) in presence of 0.6 mM 6-sulfoGalβ1,3GlcNAcβ-*O*-Al, and (▲) into 6-sulfoGalβ1,-3GlcNAcβ-*O*-Al. (B) Determination of K_m for Galβ1,3GalNAcα-*O*-Al by Lineweaver−Burke plot. Symbols as in A. (C) Inhibition of α1,2-fucosylation of 6-sulfoGalβ1,3GlcNAcβ-*O*-Al by Galβ1,3GalNAcα-*O*-Al. (D) Determination of K_i for this inhibition.

O-Al/AA-CP (K_m = 0.31 mM) followed by 6-sulfoGalβ1,-4GlcNAcβ-*O*-Me (K_m = 0.45 mM) and fetuin triantennary asialo glycopeptide (K_m = 0.50 mM).

Large-Scale Isolation and Characterization of Product Resulting from 6-SulfoGalβ1,4GlcNAcβ-O-Me by Action of α1,2-FT. Figure 2 illustrates the separation of the product from the unreacted acceptor by chromatography on a Bio-Gel P2 column. Among the two anthrone positive peaks in an approximate ratio of 1:5, the peaks containing [^{14}C]Fuc radioactivity and fucose (as determined by thioglycolic acid) coincided with peak I. The peak I material, thus recognized as the product, moved as a single spot on TLC in two solvent systems (chloroform:methanol:water, 5/4/1; butanol:acetic acid:water, 3/2/2). Mass spectrometry of this material indicated a molecular weight of 624.8 (theoretical value 624.6).

View of Enzyme (α1,2-FT) Specificity from Competition between Interesting Acceptors. Figure 3 illustrates the competition between a neutral carbohydrate substrate having two potential acceptor sites: Galβ1,4GlcNAcβ1,6(Galβ1,3)-GalNAcα-*O*-Bn and highly efficient anionic substrate 6-sulfoGalβ1,4GlcNAcβ-*O*-Me. The former is a typical mucin carbohydrate backbone, and the latter carries a sulfate group on C-6 of the Gal moiety in a lactosamine-based structure. This group is now recognized as one of the functional units of ligands for L-selectin. In the absence and presence of the sulfated acceptor, the K_m values for the other acceptor were determined as 1.33 and 6.25 mM. In spite of the presence of two sites for enzyme activity, a decrease of about 5-fold in the affinity of the neutral acceptor for the enzyme occurred in the presence of this sulfated acceptor (3.0 mM). This would imply that the affinity of this enzyme for an acceptor is greatly enhanced by a sulfo group on C-6 of Gal. This contention is further supported by the high K_i value (5.9 mM) arrived at for the mutual inhibition of fucosylation of this sulfated acceptor brought about by this neutral mucin-type acceptor.

A competition experiment between the T-hapten (Galβ1,-3GalNAcα-*O*-Al) and another highly efficient acceptor, 6-sulfoGalβ1,3GlcNAcβ-*O*-Al, was also performed (Figure 4). The sulfated acceptor, at a low concentration (0.6 mM), increased the K_m for Galβ1,3GalNAcα-*O*-Al from 10.0 to 16.7 mM. This sulfated acceptor, even at that low concentration, could compete very well with the increasing con-

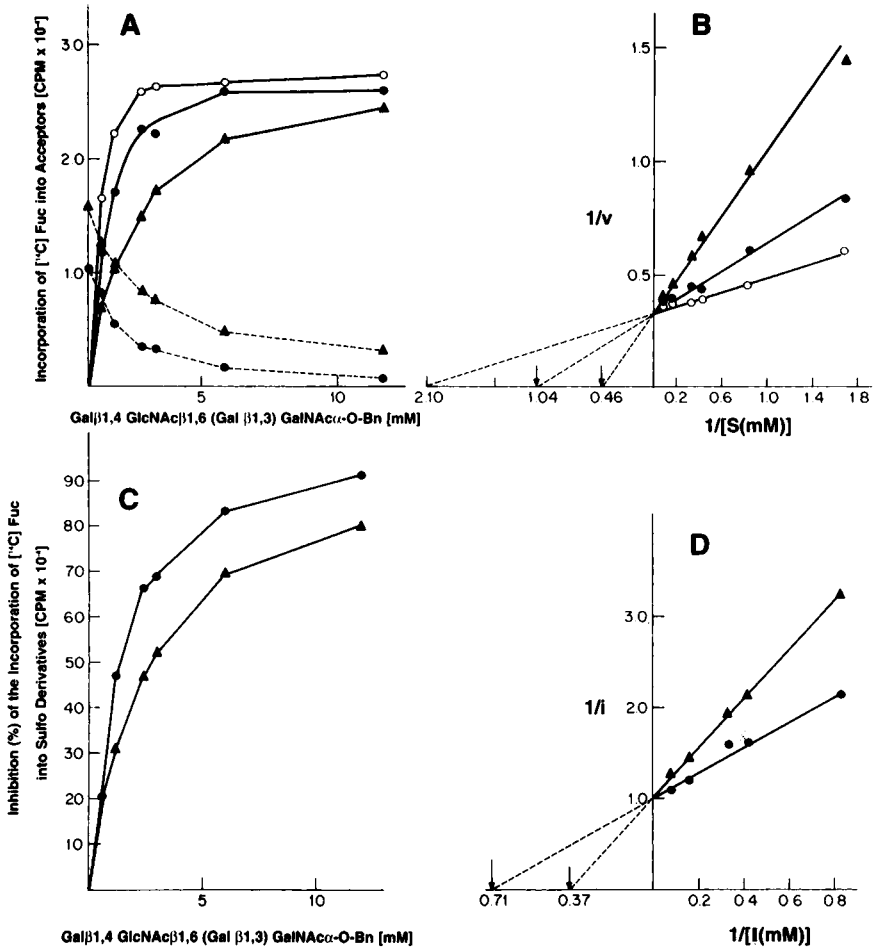

FIGURE 5: Competition between the acceptor having two terminal Gal residues, namely, Galβ1,4GlcNAcβ1,6(Galβ1,3)GalNAcα-*O*-Bn and its two derivatives in which one terminal, namely, β1,4- or β1,3-linked Gal, has been blocked by a 3-sulfo group. (A) Activity of the cloned α1,2-L-fucosyltransferase at varying concentrations of Galβ1,4GlcNAcβ1,6(Galβ1,3)GalNAcα-*O*-Bn. Incorporation of [^{14}C]Fuc into Galβ1,4GlcNAcβ1,6(Galβ1,3)GalNAcα-*O*-Bn (−○−) in absence of 3-sulfoGalβ1,4GlcNAcβ1,6(Galβ1,3)GalNAcα-*O*-Bn, (−●−) in presence of 3.0 mM 3-sulfoGalβ1,4GlcNAcβ1,6(Galβ1,3)GalNAcα-*O*-Bn, (−▲−) in presence of 3.0 mM Galβ1,4GlcNAcβ1,6(3-sulfoGalβ1,3)GaalNAcα-*O*-Bn, (- -●- -) into 3-sulfoGalβ1,4GlcNAcβ1,6(Galβ1,3)GalNAcα-*O*-Bn, and (- -▲- -) into Galβ1,4GlcNAcβ1,6-(3-sulfoGalβ1,3)GalNAcα-*O*-Bn. (B) Determination of K_m for Galβ1,4GlcNAcβ1,6(Galβ1,3)GalNAcα-*O*-Bn by Lineweaver−Burke plot. Symbols remain the same as in A. (C) Inhibitions of the α1,2-L-fucosylation of 3-sulfoGalβ1,4GlcNAcβ1,6(Galβ1,3)GalNAcα-*O*-Bn (●) and Galβ1,4GlcNAcβ1,6(3-sulfoGalβ1,3)GalNAcα-*O*-Bn (▲) by Galβ1,4GlcNAcβ1,6(Galβ1,3)GalNAcα-*O*-Bn. (D) Determination of K_i for this inhibition (symbols as in C).

centration of Galβ1,3GalNAcα-*O*-Al. This is evident from the high K_i value (5.6 mM) for its mutual inhibition by the latter. This and the previous experiment thus illustrate the enhancement of the affinity of the acceptor for the enzyme by a 6-sulfo group on Gal.

Finally, a more interesting competition experiment was carried out involving the mucin carbohydrate backbone having two potential sites for enzyme activity and its two sulfo derivatives, the sulfate group acting as a block at one of the sites (see Figure 5). The K_m value for Galβ1,-4GlcNAcβ1,6(Galβ1,3)GalNAcα-*O*-Bn in the presence of 3-sulfoGalβ1,4GlcNAcβ1,6(Galβ1,3)GalNAcα-*O*-Bn (3.0 mM) increased 2-fold (from 0.48 to 0.96 mM), whereas in the presence of Galβ1,4GlcNAcβ1,6(3-sulfoGalβ1,3)-GalNAcα-*O*-Bn (3.0 mM) the K_m value increased 4-fold (from 0.48 to 2.17 mM). Thus, Galβ1,4GlcNAcβ1,6(3-sulfoGalβ1,3)GalNAcα-*O*-Bn is a more effective competitive inhibitor than 3-sulfoGalβ1,4GlcNAcβ1,6(Galβ1,3)GalNAcα-*O*-Bn. This is due to the fact that the former has, in fact, the more favorable Gal terminal for enzyme action, namely, Gal linked to GlcNAc. It is further evident from the K_i values for the mutual inhibition by Galβ1,4GlcNAcβ1,6-(Galβ1,3)GalNAcα-*O*-Bn; the fucosylation of 3-sulfoGalβ1,-4GlcNAcβ1,6(Galβ1,3)GalNAcα-*O*-Bn is inhibited more than that of Galβ1,4GlcNAcβ1,6(3-sulfoGalβ1,3)GalNAcα-*O*-Bn (K_i = 1.41 and 2.70 mM, respectively).

Influence of Three Different Acceptor Substrates on Affinity of α1,2-FT to Donor Substrate, Namely, GDP-Fuc. Figure 6A shows the incorporation of [^{14}C]Fuc into Galβ1,-3GlcNAcβ-*O*-Al, 6-sulfoGalβ1,3GlcNAcβ-*O*-Al, and Galβ1,-3GalNAcα-*O*-Al by varying amounts of GDP-[^{14}C]Fuc. A Lineweaver−Burke plot of this data (Figure 6B) gave the respective K_m (μM) and V_{max} (pmol/h) values for GDP-Fuc as 1.54 and 96.7 (acceptor, 6-sulfoGalβ1,3GlcNAcβ-*O*-Al); 1.33 and 66.7 (acceptor, Galβ1,3GlcNAcβ-*O*-Al); and 0.95 and 26.7 (acceptor, Galβ1,3GalNAcα-*O*-Al). Thus, the affinity of the enzyme for donor substrate appears to be influenced by the acceptor substrate.

Influence of pH on α1,2-FT Activity (Figure 7). Optimal activity of cloned α1,2-FT was found to occur at pH 7.2−7.6. Activity decreased below pH 7.2 (84.8% at 6.8, 59.2% at 6.4, 15.0% at 6.0, 6.3% at 5.6, and 0.3% at 5.2). When α1,2-FT activity of a human ovarian tumor extract was assayed under the same conditions with the Galβ-*O*-Bn

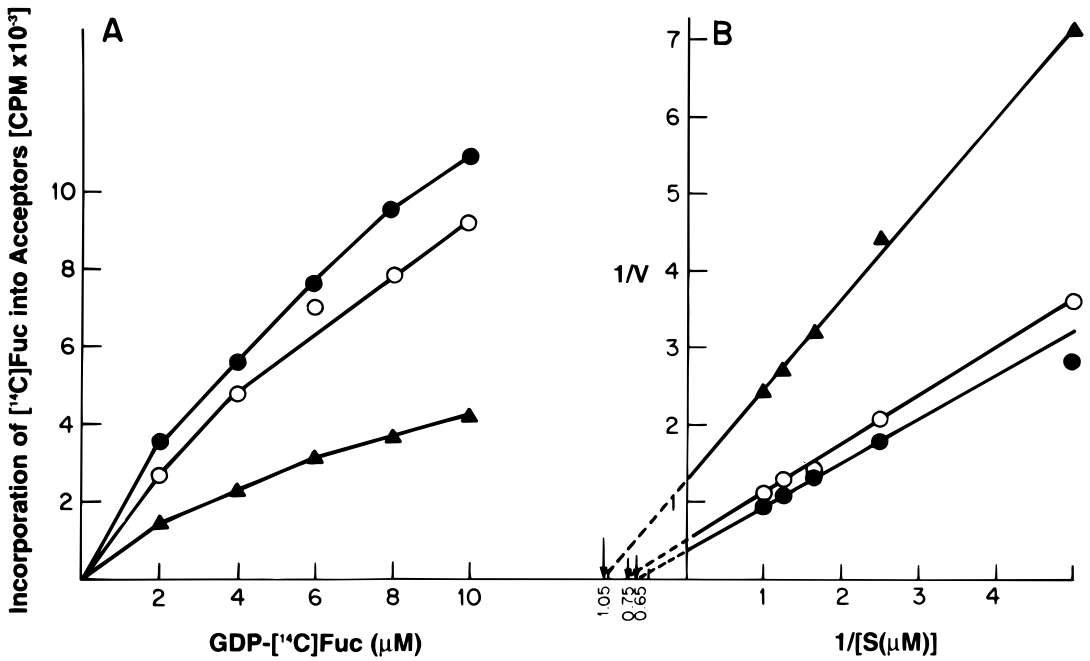

FIGURE 6: A look at the K_m and V_{max} values for GDP-Fuc by assaying the activity of the cloned α1,2-L-fucosyltransferase with three different acceptors. (A) Activity of the cloned α1,2-L-fucosyltransferase when the concentration of GDP−[^{14}C]Fuc is varied. Incorporation of [^{14}C]Fuc into (○) Galβ1,3GlcNAcβ-*O*-Al, (●) 6-sulfoGalβ1,3GlcNAcβ-*O*-Al, and (▲) Galβ1,3GalNAcα-*O*-Al. (B) Lineweaver−Burke plot for determining the K_m and V_{max} values for GDP-Fuc using the same acceptors and symbols as in A.

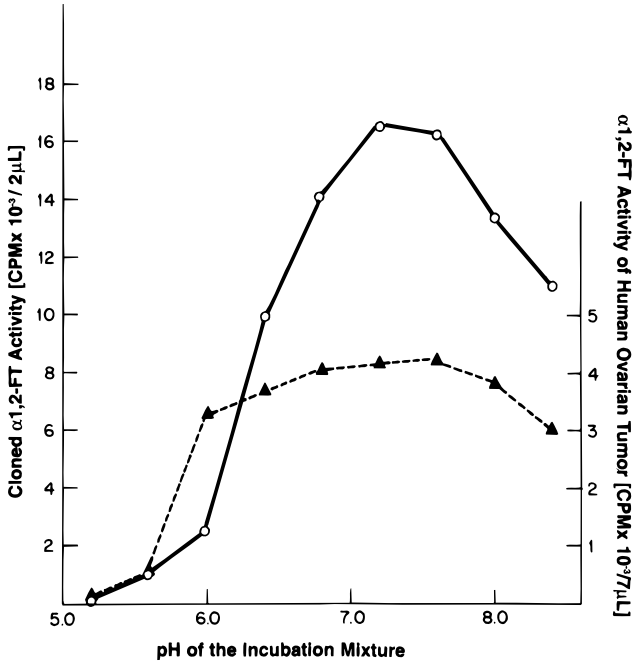

FIGURE 7: Enzyme activities of the cloned as well as human ovarian tumor α1,2-L-fucosyltransferases by varying the pH of the incubation mixture: (○) the cloned enzyme and (▲) human ovarian tumor enzyme.

acceptor, fairly good activity was seen over a wide pH range (6.0−8.0), thus indicating a significant difference between the cloned α1,2-FT and the ovarian tumor α1,2-FT.

Interaction of the Cloned α1,2-FT with Glycopeptides (Table 5). The enzyme, as anticipated, was almost devoid of activity with fetuin triantennary sialo glycopeptide (3.9%) when compared to its activity with the corresponding asialo glycopeptide (151.8%). The former after treatment with β-galactosidase (ex *Aspergillus*) in an attempt to remove any terminal Gal, which is not sialylated, resulted in 0% transfer of Fuc to the sialo glycopeptide. After complete desialylation of this glycopeptide the acceptor activity reached 146.6%. Treatment of this asialo glycopeptide with β-galactosidase to remove a considerable portion of the terminal Gal residues decreased acceptor activity to 103.6%.

Table 5: Activity of the Cloned α1,2-L-Fucosyltransferase toward Modified Glycopeptides As Acceptors

glycopeptide (GP, 200 μg)	incorporation of [^{14}C]Fuc CPM (×10^{-3}/2 h)	% [a]
fetuin triantennary asialo GP	25.1	151.8
fetuin triantennary sialo GP	0.6	3.9
after β-galactosidase (ex *Aspergillus*) treatment	0	0
after desialylation	24.2	146.6
after β-galactosidase (ex *Aspergillus*) treatment	17.1	103.6
bovine IgG diantennary GP	8.3	50.0
after acetic acid treatment	12.2	74.0
after α-L-fucosidase (bovine epididymis) treatment	30.4	184.4
after β-galactosidase (ex *Aspergillus*) treatment	3.4	20.8
after α-L-fucosidase (bovine epididymis) and then β-galactosidase (ex *Aspergillus*) treatments	0	0

[a] The values are expressed as percent of the activity toward the simple acceptor, Galβ-*O*-Bn (3.0 mM), tested at the same time.

Fucose is present in bovine IgG diantennary glycopeptide in an α1,6-linkage with GlcNAc, which is further attached to asparagine (Tai et al., 1975). The removal of even 5% fucose from this glycopeptide by treatment with acetic acid increased its efficiency as an acceptor for α1,2-FT (50.0% increased to 74.0%). Removal of about 50% fucose from the glycopeptide by L-fucosidase (bovine epididymis) increased its acceptor ability almost four times (from 50.0% to 184.4%). Partial removal of Gal from the original glycopeptide decreased its acceptor ability (from 50.0% to

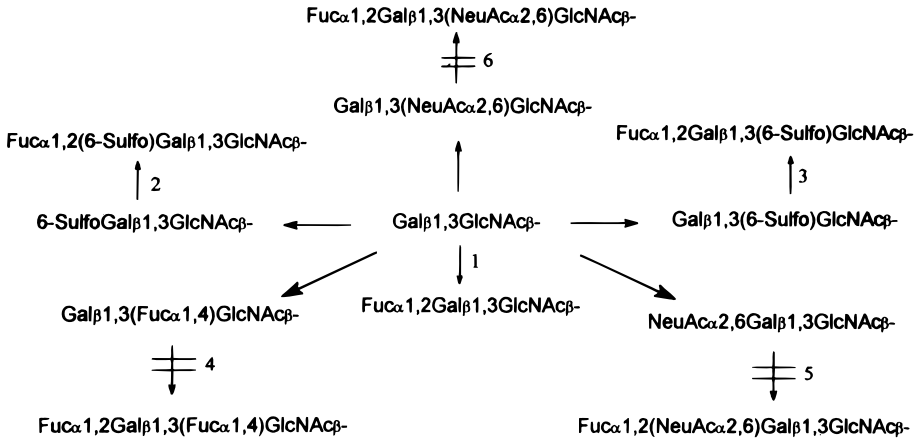

FIGURE 8: Outline of the specificities of the cloned blood group H gene-specified α1,2-FT toward Gal*β*1,3GlcNAc*β*-based structures. (Almost similar specificities were observed with type 2-based structures).

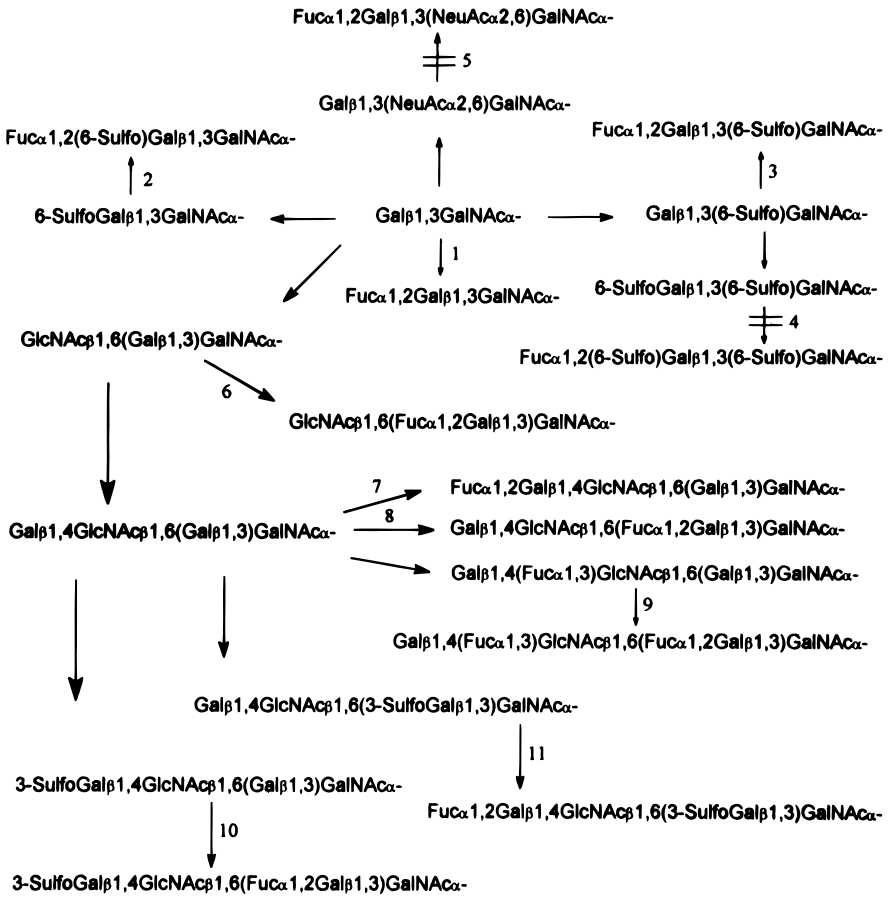

FIGURE 9: Outline of the specificities of the cloned blood group H gene-specified α1,2-FT toward Gal*β*1,3GalNAcα-based structures.

20.8%). Complete removal of Gal from the 50% defucosylated glycopeptide abolished acceptor capacity completely (from 50.0% to 0%). We were thus able to demonstrate very clearly the adverse effect of Fuc linked α1,6 to the asparagine-linked GlcNAc on α1,2-fucosylation of the nonreducing terminal Gal. In this context it is interesting to note that in recombinant human erythroprotein 95% of the N-linked chains contain α1,6-linked Fuc at the proximal GlcNAc residue. While all these chains were α2,3-sialylated on terminal Gal, not even a single chain contained either α1,2- or α1,3-linked Fuc (Watson et al., 1994).

The present investigation found the acceptor activity of 6-sulfoGal*β*-*O*-Bn (a negative charge into the C-6 position) to be 30% of that of Gal*β*-*O*-Bn. This demonstrated an adverse effect by a negative sulfate group positioned at C-6 on Gal. Lowary et al. (1994) reported that when the acceptor, Gal*β*-*O*-octyl, was modified by substituting an amino group for the hydroxyl at C-6 of Gal, which introduces a positive charge into this position, the acceptor activity dropped to 7%.

On the basis of the higher acceptor activities of 4-*O*-methyl and 6-*O*-methyl analogues of octyl *β*-D-galactopyranoside, Lowary et al. (1994) suggested that the area occupied by C-4 and C-6 hydroxyl groups in the active site of α1,2-FT must be relatively nonpolar, i.e., hydrophobic. The observations made in the present study on the α1,2-FT specificities address the real situation concerning the above issue. In contrast to the adverse effect seen on acceptor efficiency

influenced by C-6 sulfate on Gal in the monosaccharide acceptor Galβ-*O*-Bn, a sulfate group on C-6 of Gal enhanced the acceptor efficiency of both Galβ1,4GlcNAcβ- (100% increased to 223%) and Galβ1,4GlcNAcβ- (100% increased to 142%) but did not affect the efficiency of Galβ1,-3GalNAcα- (46.8% changed to 47.0%). It is apparent from our data that the area, if at all, occupied in the enzyme active site by the hydroxyl group on C-6 of Gal does not appear to be a nonpolar region. On the other hand, the free hydroxyl group at C-4 of hexosamine seems to influence the enzyme activity. The involvement of the C-4 hydroxyl group of GlcNAc in the enzyme active site binding is also evident from the finding that either methylation or fucosylation of C-4 hydroxyl of the GlcNAc moiety in Galβ1,3GlcNAcβ- almost abolished the acceptor activity (only 5.8% and 2.3% active, respectively).

The influence of the free hydroxyl group on C-4 of GlcNAc on the enzyme active site is also evident as follows: Under the influence of this hydroxyl group, a negative group like sulfate on C-6 of GlcNAc in Galβ1,-3GlcNAcβ- has no effect on the enzyme. In spite of a bulky substitution like NeuAc on C-6 of GlcNAc, the Galβ1,3-(NeuAcα2,6)GlcNAcβ-*O*-Bn acceptor retained considerable activity (18.8%). When C-6 of the GlcNAc moiety is sulfated, the acceptor activity of Galβ1,4GlcNAc became 33%.

We find that Galβ1,3GalNAcα-*O*-Al is not a better acceptor than Galβ1,3Galβ-*O*-Al (46.8% and 59.0% active, respectively), suggesting that the acetamido group may not have a significant role in binding of the enzyme to the acceptor. We find Galα1,3Galβ-*O*-Al to be inactive toward this enzyme; this would suggest that the conformational difference in a disaccharide, resulting from the α- and the β-intersaccharide linkages between the two monosaccharide residues, determines the proper fit of this enzyme with the disaccharide acceptor.

The specificities witnessed in the present investigation on the blood group H gene-specified α1,2-L-FT are outlined in Figures 8 and 9. The steps numbered were shown to occur in the present study.

The present study has shown that the acceptor Galβ1,3-(NeuAcα2$\rightarrow$6)GalNAcα-*O*-Bn exhibits an appreciable amount of activity (8.7% as compared to Galβ1,3GalNAcα-*O*-Bn) as acceptor for α1,2-L-FT. So it remains to be determined whether Fucα1,2Galβ1,3GalNAcα- can act as an acceptor for α2,6-sialyltransferase.

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