Expression of Blood Group Lewis b Determinant from Lewis a: Association of This Novel α(1,2)-L-Fucosylating Activity with the Lewis Type α(1,3/4)-L-Fucosyltransferase†

E. V. Chandrasekaran,‡ Rakesh K. Jain,‡ John M. Rhodes,‡ Cheryl A. Srnka,§ Robert D. Larsen,§ and Khushi L. Matta*,‡

Department of Gynecologic Oncology, Roswell Park Cancer Institute, Elm & Carlton Streets, Buffalo, New York 14263, and Glycomed, 860 Atlantic Avenue, Alameda, California 94501

Received September 22, 1994; Revised Manuscript Received January 17, 1995⊗

ABSTRACT: Blood group H type 1 [Fucα(1,2)Galβ(1,3)GlcNAcβ→] is known as the precursor structure of the blood group determinant, Lewis b [Fucα(1,2)Galβ(1,3)(Fucα(1,4))GlcNAcβ→]. Recently, a new biosynthetic route for Lewis b from Lewis a [Galβ(1,3)(Fucα(1,4))GlcNAc→] was identified in human gastric carcinoma cells, colon carcinoma Colo 205, and ovarian tumor. The present study demonstrates the association of this new type of α(1,2)-L-fucosyltransferase (FT) activity with the Lewis-type α(1,3/4)-L-FT as follows: (i) the α(1,4)- and novel α(1,2)-FT activities of Colo 205 were much less inhibited than the α(1,3)-FT activity by *N*-ethylmaleimide [K_i (μM) = 714.0, 119.0, and 6.5 respectively]. (ii) The α(1,4)- and novel α(1,2)-FT activities emerged from a Sephacryl S-200 column in identical positions. (iii) A specific inhibitor (copolymer from 3-sulfo-Galβ(1,3)GlcNAcβ-*O*-allyl and acrylamide) of α(1,4)-FT activity inhibited both α(1,4)- and α(1,2)-FT activities in Sephacryl S-200 column effluent to almost the same extent (~80%); (iv) separation of the Lewis-type α(1,3/4)-FT from the plasma-type α(1,3)-FT by specific elution of the affinity column (bovine IgG glycopep-Sepharose) with lactose and further purification on a Sephacryl S-100 HR column showed that (a) the α(1,3)-FT activity was the inherent capacity of the Lewis-type FT (Colo 205 fraction L) since ~90% of both the α(1,4)- and α(1,3)-FT activities is inhibited by the copolymer, (b) the unique ability of catalyzing the α(1,2)-L-fucosylation of Gal in Lewis a structure and also the α(1,3)-L-fucosylation of Glc in lactose-based structure belonged to the Lewis-type enzyme (Colo 205 fraction L), (c) a measurement of the [^{14}C]fucosyl products arising from the two acceptors Galβ(1,3)(4,6-di-*O*-Me)GlcNAcβ-*O*-Bn and 3-sulfo-Galβ(1,3)GlcNAcβ-*O*-Al (specific for α(1,2) and α(1,4), respectively) taken in the same incubation mixture showed mutual inhibition by the acceptors [K_m for the α(1,4)-specific acceptor, 3-sulfo-Galβ(1,3)GlcNAcβ-*O*-Al, increased from 32 to 50 μM in the presence of 7.5 mM Galβ(1,3)(4,6-di-*O*-Me)GlcNAcβ-*O*-Bn, whereas K_i for the mutual inhibition of α(1,2)-FT activity by the former was 102 μM], and (d) the Lewis-type FT, in contrast to the plasma-type FT, was highly effective in fucosylating complex glycopeptides. (iv) A cloned FT (FT III: Lewis type) and the Colo 205 Lewis-type FT (fraction L) showed similar activities toward various acceptors; the enzymatic product resulting from the action of cloned FT on Galβ(1,3)(Fucα(1,4))GlcNAc-β-*O*-Bn was identified by FAB mass spectrometry as the difucosyl compound. (v) An examination of six human cell lines indicated that the novel α(1,2)-FT activity associates with the α(1,4)-FT activity.

Blood group Lewis b and Lewis y antigens were not found in the distal part of the colon in human Lewis b- and Se-positive individuals, but they were expressed in the tumors arising in the lower third of the colons of these patients (Sakamoto et al., 1986; Brown et al., 1984; Abe et al.; 1986; Blazczyk et al., 1985; Ernst et al., 1984). These findings implied tumor-associated expression of an α(1,2)-L-fucosyltransferase (FT)[1] of either blood group H, secretor, or a novel type. The studies of LePendu et al. (1982) suggested the utilization of both type 1 and type 2 precursors by the secretor α(1,2)-FT, while the blood group H gene-encoded transferase might be restricted to type 2 substrates. Blaszczyk-Thurin et al. (1986) proposed that the transformation of colon epithelial cells induced the Se gene-encoded transferase, resulting in the expression of both Lewis b and Lewis y antigens. Subsequently, this group identified α(1,2)-L-FT activity in gastric carcinoma cells (Blaszczyk-Thurin et al., 1988) that can convert Galβ(1,3)(Fucα(1,4))GlcNAcβ→ (Lewis a) to Fucα(1,2)Galβ(1,3)(Fuc(1,4))GlcNAcβ→ (Lewis b), and their data suggested the existence of structural or regulatory genes other than H and Se for α(1,2)-FT. Recently, we demonstrated the existence of such a new type α(1,2)-L-FT activity in Colo 205 cells and ovarian tumor (Jain et al., 1993). Very recently, Wang et al. (1994) found, by immunoassay, appreciable amounts of Lewis b in the saliva from ABO nonsecretors, suggesting that some of the Lewis b may be formed from Lewis a in the salivary glands. The present paper reports our discovery that the Lewis-type α(1,3/4)-L-FT of colon carcinoma cells Colo 205, as well as of a cloned FT III (Lewis type), can in fact accomplish the

† This study was supported by Grants AI29326 (National Institute of Allergy and Infectious Diseases) and CA35329 (National Cancer Institute).

‡ Roswell Park Cancer Institute.

§ Glycomed.

⊗ Abstract published in *Advance ACS Abstracts,* March 15, 1995.

[1] Abbreviations: FT, fucosyltransferase; NEM, *N*-ethylmaleimide; SGGA, 3-sulfo-Galβ(1,3)GlcNAcβ-*O*-allyl; Bn, benzyl; GMGB, Galβ-(1,3)(4,6-di-*O*-Me)GlcNAcβ-*O*-benzyl; Al, allyl.

0006-2960/95/0434-4748$09.00/0

synthesis of Lewis b from Lewis a determinant.

EXPERIMENTAL PROCEDURES

Cell Culture. The Colo 205 cells were grown in 250 mL plastic T-flasks in RPMI 1640 supplemented with 5% fetal bovine calf serum (Gibco), 5% Nu serum (Collaborative Research), and 2 mM glutamine in a humidified atmosphere of 95% air and 5% CO_2 (pH 7.0) at 37 °C. Cells were subcultured with 0.05% trypsin/0.53 mM EDTA (Gibco). For experimental use, cells were pelleted at 1500 rpm for 5 min, washed twice with PBS, and stored frozen.

Enzyme Extract. A pool of Colo 205 cell pellets (1.0×10^9 cells) was suspended in 20 mL of ice cold 50 mM Tris-buffered saline (pH 7.0) containing 2% Triton X-100 and homogenized by a Dounce all-glass hand-operated grinder. The homogenate was centrifuged at 20000*g* at 4 °C for 1 h. The supernatant was dialyzed against three changes of 1 L each of 25 mM Tris-HCl (pH 7.0) containing 35 mM $MgCl_2$, 10 mM NaN_3, and 1 mM ATP at 4 °C.

Affinity Chromatography. The preceding enzyme solution (20 mL) from Colo 205 was applied to a bovine IgG glycopep-Sepharose column (30 mL bed volume), which had been washed and equilibrated with the Tris buffer containing $MgCl_2$, NaN_3, and ATP. After the column was washed with 100 mL of the equilibration buffer, sequential elution of the column was done with 100 mL each of 0.1 M lactose and 1.0 M NaCl in the preceding buffer. The lactose and NaCl eluates were concentrated to ~2.0 mL by ultrafiltration and then dialyzed against three changes of 250 mL of the preceding buffer at 4 °C and stored at 4 °C.

The specific acceptor abilities of the various oligosaccharide derivatives utilized in the present study are as follows. α(1,3)-L-FT activity: Fucα(1,2)Galβ(1,4)GlcNAcβ-*O*-Bn, 2-*O*-methyl-Galβ(1,4)GlcNAc, and 3-sulfo-Galβ(1,4)GlcNAc. α(1,4)-L-FT activity: Fucα(1,2)Galβ(1,3)GlcNAcβ-*O*-pNp, 2-*O*-methyl-Galβ(1,3)GlcNAcβ-*O*-Bn, 3-sulfo-Galβ(1,3)-GlcNAcβ-*O*-Al, Galβ(1,3)GlcNAcβ-*O*-Bn, and Galβ(1,3)-(6-*O*-methyl)GlcNAcβ-*O*-Bn. Novel α(1,2)-L-FT activity: Galβ(1,3)(Fucα(1,4))GlcNAcβ-*O*-Bn, Galβ(1,3)(4-*O*-methyl)GlcNAcβ-*O*-Bn, and Galβ(1,3)(4,6-di-*O*-methyl)GlcNAcβ-*O*-Bn. Typical α(1,2)-L-FT activity: Galβ-*O*-Bn.

Glycopeptides. The diantennary glycopeptide was prepared from bovine IgG (Calbiochem) by pronase digestion, gel filtration, and Con A-Sepharose chromatography as described earlier (Yazawa et al., 1986). A similar procedure was followed to obtain, from fetuin (Sigma), the triantennary sialoglycopeptide, which did not bind to Con A-Sepharose. The asialoglycopeptide was made by heating the triantennary sialoglycopeptide at 80 °C in 0.1 N HCl for 1 h and chromatographing the neutralized solution after concentration to 1.0 mL on a Biogel P2 column (1.0 × 116.0 cm) to remove sialic acid.

Copolymer from 3-Sulfo-Galβ(1,3)GlcNAcβ-O-allyl (SGGA) and Acrylamide. This copolymer was synthesized by following the procedure of Horejsi et al. (1978). The preparation contained ~1.0 μmol of the sugar unit per milligram weight and was similar in molecular weight to dextran (average molecular weight 39 200), as was evident from column chromatography on Biogel P60.

Fractionation on the Sephacryl S-100 HR Column. Prior to using the Sephacryl S-100 HR column, initial studies were made by using a Sephacryl S-200 (superfine) column (1.0 × 116.0 cm) under the same conditions described in the following. The preceding enzyme preparations (1 mL from each sample) were fractionated separately on the Sephacryl S-100 HR column (1.0 × 116.0 cm), which had been equilibrated with 50 mM Tris-HCl (pH 7.0) containing 0.15 M NaCl, 0.1% Triton X-100, and 0.02% NaN_3. Fractions of 1.0 mL were collected, and 40 μL aliquots of alternate fractions were assayed for fucosyltransferase activity under standard incubation conditions (Chandrasekaran et al., 1992a); quantitation of the [^{14}C]Fuc-containing products resulting from the various acceptors was done by the Dowex-1-Cl method, as described previously (Chandrasekaran et al., 1992a). Protein was measured by the BCA method (Pierce Chemical Co.) with BSA as the standard.

Thin-Layer Chromatographic (TLC) Separation of [^{14}C] Compounds (Chandrasekaran et al., 1992b). TLC was run on silica gel GHLF (scored 20 × 20 cm, 250 μm, Analtech Inc., Newark, DE) using the solvent system *n*-butanol/acetic acid/H_2O (3/2/1). The authentic standards were located by spraying with sulfuric acid in ethanol and heating at 100 °C. The radioactive compounds were located by scraping the silica gel from 0.5 cm width segments into scintillation vials, soaking in 2 mL of water, and then liquid scintillation counting.

Treatment with α-L-Fucosidase. The [^{14}C]fucosyl compounds from the acceptors 2-methyl-Galβ(1,3)GlcNAcβ-*O*-Bn, 2′-fucosyl-LacNAcβ-*O*-Bn, and Galβ(1,3)(Fucα(1,4))-GlcNAcβ-*O*-Bn by the action of Colo 205 FT were isolated by the Dowex-1-Cl method followed by chromatography on a Biogel P2 column (1.0 × 116.0 cm). Treatment of the ^{14}C compound with almond meal α-L-fucosidase [specific for α(1,3/4), Oxford Glycosystems] was done with 20 μunits of the enzyme in 25 μL of incubation mixture according to the format of the supplier. The control incubation mixture contained everything except the enzyme; 100 μunits (enzyme units derived from activity toward 2′-fucosylactose) of earthworm α-L-fucosidase (Chandrasekaran et al., 1991) in 25 μL of reaction mixture using the same buffer from Oxford Glycosystems was also used. After incubation at 37 °C for 20 h, the reaction mixtures were applied as 1 cm streaks to the TLC plates and subjected to chromatography. Free fucose and monofucosylated and difucosylated benzyl *N*-acetyllactosaminides moved on the TLC plate approximately 7.5, 5.5, and 3.5 cm, respectively, from the origin under the chromatographic conditions described earlier.

Inhibition by N-Ethylmaleimide (NEM). For this purpose, the enzyme preparation (Chandrasekaran et al., 1994) resulting from elution of the affinity column with 1 M NaCl without prior elution with 0.1 M lactose was used (specific activity: 113.1 nmol of Fuc transferred to 2′-fucosyl-LacNAc per hour per milligram of protein); this preparation contained both the Lewis-type and plasma-type α(1,3)-FT, as well as the new type α(1,2)-FT activity. This preparation was completely devoid of the typical α(1,2)-FT as it did not transfer fucose to Galβ-*O*-benzyl, which is known as a good acceptor for both blood group H- and secretor-type α(1,2)-FTs (Sarnesto et al., 1992). The preceding preparation of FT from Colo 205 (5 μg of protein) was mixed with 40 μg of BSA in the incubation buffer and preincubated for 0.5 h at 37 °C with varying concentrations of NEM (0.02−0.20 mM) before the addition of the acceptor. Controls containing no NEM and those containing no acceptors were also included.

Examination of α-L-FT Activities in Some Human Cell Lines. A simultaneous comparison of the α-L-FT activities present in Colo 205 and HT 29 (colon tumor), EKVX (lung adenocarcinoma), A159 (endometrium), and JTL and B142 (B-lymphoid) was carried out. The cells were grown, extracted, and assayed for various α-L-FT activities as described earlier for Colo 205; ~5 × 10^7 cells were used in each case. The cell extracts were adjusted to 2 mg of protein/mL by adding the necessary volume of the extraction buffer, and 20 μL aliquots of the extracts were used in the assays run in duplicate. The acceptors Fucα(1,2)Galβ(1,4)GlcNAcβ-*O*-Bn, Fucα(1,2)Galβ(1,3)GlcNAcβ-*O*-pNp, and Galβ(1,3)-(Fucα(1,4))GlcNAcβ-*O*-Bn were used for measuring α(1,3)-L-FT, α(1,4)-L-FT, and the novel α(1,2)-L-FT activities, respectively.

Enzymatic Transfer of [^{14}C]Fuc to 4-O-Methyl and 4,6-Di-O-methyl Derivatives of Galβ(1,3)GlcNAcβ-O-Bn. Since the *O*-methyl group, in contrast to fucosyl linkages, is completely resistant to hydrolysis under normal experimental conditions and, further, the methylated LacNAc-based benzyl glycosides have distinctly higher mobilities on TLC in the solvent system $CHCl_3/CH_3OH/H_2O$ (13/6/1) than the corresponding nonmethylated glycosides, we simultaneously compared Galβ(1,3)GlcNAcβ-*O*-Bn, Galβ(1,3)(6-*O*-Me)-GlcNAcβ-*O*-Bn, Galβ(1,3)(4-*O*-Me)GlcNAcβ-*O*-Bn and Galβ-(1,3)(4,6-di-*O*-Me)GlcNAcβ-*O*-Bn as acceptors for Colo 205 Lewis-type FT (fraction L) under our standard incubation conditions. The reaction mixtures were then passed through Dowex-1-Cl columns. The water eluates were lyophilized to dryness and each was dissolved in 0.10 mL of water; a 10 μL aliquot from each was then subjected to silica gel TLC as described earlier using $CHCl_3/CH_3OH/H_2O$ (13/6/1). The ^{14}C products were quantitated by scraping the silica gel as described earlier. For autoradiography, 5 μL aliquots from the preceding samples were applied separately as 0.5 cm diameter spots on one TLC silica gel glass strip (5 × 20 cm) and subjected to TLC using $CHCl_3/CH_3OH/H_2O$ (13/6/1). Components on the TLC plate were detected using X-Omat AR film (Eastman Kodak) by fluorography at −70 °C after spraying with Enhance (Dupont).

Mutual Inhibition of the Enzymatic Transfer of [^{14}C]Fuc into Galβ(1,3)(4,6-di-O-Me)GlcNAcβ-O-Bn (Specific for α-(1,2)-Transfer) and 3-Sulfo-Galβ(1,3)GlcNAcβ-O-Al (Specific for α(1,4)-Transfer). Varying concentrations of 3-sulfo-Galβ(1,3)GlcNAcβ-*O*-Al were incubated with Colo 205 α(1,3/4)-L-FT in the presence of Galβ(1,3)(4,6-di-*O*-Me)-GlcNAcβ-*O*-Bn (7.5 mM) under standard incubation conditions. The radioactive products from both acceptors were measured after separation by Dowex-1-Cl, as described earlier (Chandrasekaran et al., 1992a).

Activity of the Cloned FT III with Synthetic Compounds. The cloned FT III (the Lewis type) (Glycomed, Alameda, CA) was an enzyme bound to Sepharose beads, the binding being due to protein A (the dimeric segment of the enzyme) bound to IgG coupled to Sepharose. The isolation of this enzyme was carried out as follows: DNA for FT III was isolated by PCR from human placental DNA, and its sequence was identical to that reported for the original cloned FT III by Kukowska-Latallo et al. (1990). The chimeric truncated construct of FT III showed the same kind of preference and relative activity toward the key acceptors, namely, Galβ(1,3)GlcNAc, Galβ(1,4)GlcNAc, lactose, and 2′-fucosyllactose, as the original cloned FT III (de Vries, Srnka, Palcic, Swiedler, van den Eijden, and Macher, manuscript submitted for publication). The chimeric, truncated construct of FT III was expressed as secreted product in the medium by stable transfected A293 cells and then affinity-isolated; media and extracts of control A293 cells did not transfer Fuc from GDP-Fuc to either LacNAc or 3′-sialyl-LacNAc as acceptor, indicating the absence of fucosyltransferase from these cells (Srnka, unpublished results). The soluble enzyme was prepared from 50 μL of the bead suspension; stripping of the enzyme from the beads was done with 0.1 M citrate buffer (pH 4.4) by following the protocol supplied by Glycomed. This enzyme preparation stored at 4 °C was used in the present study within a week. The activity of this soluble enzyme with various acceptors was measured in duplicate by using the standard incubation conditions and the Dowex-1-Cl method (Chandrasekaran et al., 1992a).

Demonstration of α(1,2)-L-Fucosylation of Galβ(1,3)-(Fucα(1,4))GlcNacβ-O-Bn by the Cloned Fucosyltransferase (FT-III:Lewis Type). The cloned FT (0.75 mL suspension of the Sepharose beads containing the immobilized enzyme) was taken in a reaction mixture of 2.00 mL under standard incubation conditions and mixed continuously at room temperature using Spec-Mix (Thermolyne) for 72 h. After incubation and removal of the beads by centrifugation, the supernatant was passed through Dowex-1-Cl; the water eluate, after lyophilization to dryness and then dissolution in 1.0 mL of water, was subjected to Biogel P2 column (1.0 × 116.0 cm) chromatography. The radioactive first peak was lyophilized to dryness and then subjected to FAB mass spectrometry.

RESULTS AND DISCUSSION

Differentiation of a 1,3-L-Fucosylating Enzyme Species as Distinct from α(1,4)- and α(1,2)-L-Fucosylating Species by N-Ethylmaleimide Inhibition. The incorporation of [^{14}C]Fuc into the α(1,4)-FT-specific acceptor, namely, 2-methyl-Galβ-(1,3)GlcNAcβ-*O*-Bn, the novel α(1,2)-FT activity-measuring acceptor, Galβ(1,3)(Fucα(1,4))GlcNAcβ-*O*-Bn, and the two α(1,3)-FT-specific acceptors, 2′-methyl-LacNAc and 3′-sulfo-LacNAc, was measured by incubation separately with the affinity-purified Colo 205 FT, which had been preincubated for 0.5 h at 37 °C with varying concentrations of NEM (Figure 1A).

The K_i values (μM), as determined by a Lineweaver−Burk plot, were 714.0, 119.0, 6.5, and 23.0, respectively (Figure 1B). The higher susceptibility of α(1,3)-FT to NEM suggested its distinct physical characteristics different from the others.

Characterization of the Product from Galβ(1,3)(Fucα-(1,4))GlcNAcβ-O-Bn as [^{14}C]Fucα(1,2)Galβ(1,3)(Fucα-(1,4))GlcNacβ-O-Bn. The α(1,3/4)-specific L-fucosidase (almond meal) released 100% and 70.7% [^{14}C]Fuc (Table 1) from 2-*O*-methyl-Galβ(1,3)([^{14}C]Fucα(1,4))GlcNAcβ-*O*-Bn and Fucα(1,2)Galβ(1,4)([^{14}C]Fucα(1,3))GlcNAcβ-*O*-Bn, respectively. The formation of the monofucosylated compound Galβ(1,4)([^{14}C]Fucα(1,3))GlcNAcβ-*O*-Bn was not seen (see Table 1), thus proving the inability of this fucosidase to hydrolyze the α(1,2)-linkage. When the [^{14}C]-Fuc-containing product isolated from Galβ(1,3)(Fucα(1,4))-GlcNAcβ-*O*-Bn was treated with this fucosidase, 74.9% of the product was converted into a radioactive, monfucosyl

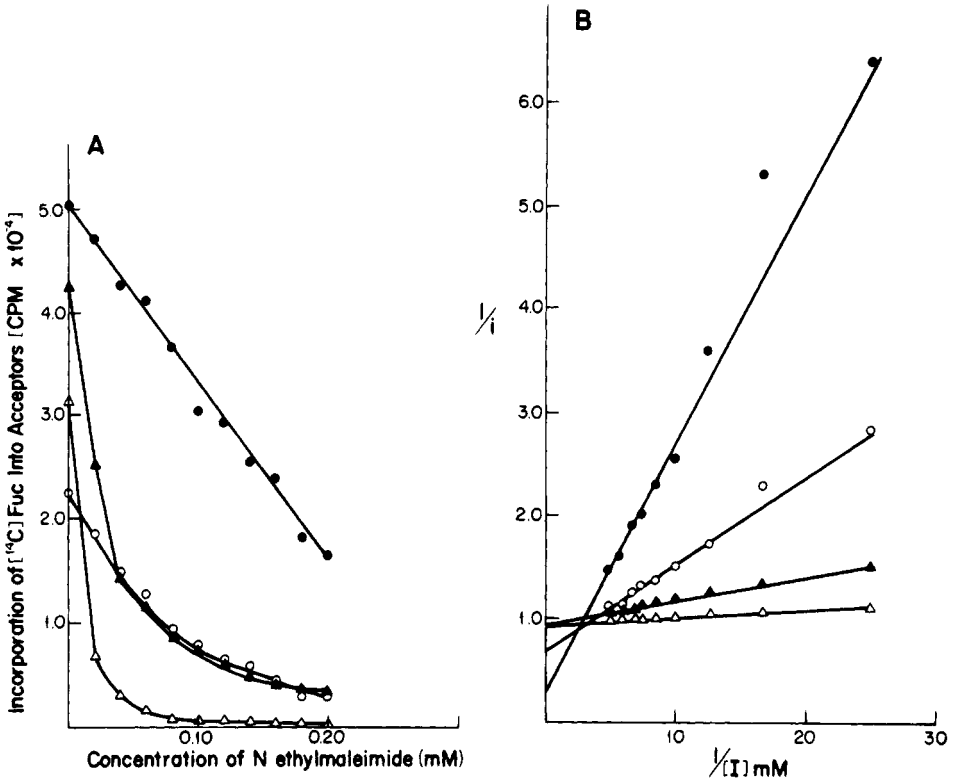


FIGURE 1: (A) Inhibition of Colo 205 FT activities by *N*-ethylmaleimide. (B) Determination of K_i by Lineweaver–Burk plots. Symbols: (●) α(1,4)-L-FT [acceptor, 2-methyl-Galβ(1,3)GlcNAcβ-*O*-Bn]; (○) α(1,2)-L-FT [acceptor, Galβ(1,3)(Fucα(1,4))GlcNAcβ-*O*-Bn]; (▲) α-(1,3)-L-FT [acceptor, 3′-sulfo-LacNAc]; (△) α(1,3)-L-FT [acceptor, 2′-methyl-LacNAc].

Table 1: Enzymatic Characterization of the Linkage of [^{14}C]Fuc Transferred to Galβ(1,3)(Fucα(1,4))GlcNAcβ-*O*-Bn with Reference to Other Acceptors

[^{14}C]fucosyl compounds: the ^{14}C products released by fucosidase	radioactivity in TLC[a] after treatment with α-L-fucosidase (CPM)	
	almond meal	earthworm[b]
(a) 2-*O*-methylGalβ(1,3)([^{14}C]Fucα(1,4))GlcNAcβ-*O*-Bn: (i) [^{14}C]Fuc;	17079 (100.0)	
(ii) 2-*O*-methylGalβ(1,3)([^{14}C]Fucα(1,4))GlcNAcβ-*O*-Bn	0	
(b) Fucα(1,2)Galβ(1,4)([^{14}C]Fucα(1,3))GlcNAcβ-*O*-Bn: (i) [^{14}C]Fuc;	12580 (70.7)	0
(ii) Galβ(1,4)([^{14}C]Fucα(1,3))GlcNAcβ-*O*-Bn;	0	2136 (24.5)
(iii) Fucα(1,2)Galβ(1,4)([^{14}C]Fucα(1,3))GlcNAcβ-*O*-Bn	5225 (29.3)	6567 (75.5)
(c) [^{14}C]fucose-containing product from Galβ(1,3)(Fucα(1,4))GlcNAcβ-*O*-Bn:		
(i) [^{14}C]Fuc;	0	1484 (81.7)
(ii) [^{14}C]Fucα(1,2)Galβ(1,3)GlcNAcβ-*O*-Bn;	1380 (74.9)	0
(iii) [^{14}C]Fucα(1,2)Galβ(1,3)(Fucα(1,4))GlcNAcβ-*O*-Bn	462 (25.1)	332 (18.3)

[a] Our TLC analysis established that there was absolutely no release of either [^{14}C]Fuc or any degraded [^{14}C]fucosyl compounds when incubation mixtures contained no fucosidase. [b] A highly purified preparation of fucosidase (Chandrasekaran et al., 1991) (specific activity = 17.8 μmol/min/mg of protein) was used.

compound without any release of [^{14}C]Fuc. On the other hand, the earthworm α-L-fucosidase, which cleaves α(1,2)-linked Fuc (Chandrasekaran et al., 1991), converted 24.5% of Fucα(1,2)Galβ(1,4)([^{14}C]Fucα(1,3))GlcNAcβ-*O*-Bn to Galβ(1,4)([^{14}C]Fucα(1,3))GlcNAcβ-*O*-Bn but did not release any [^{14}C]Fuc, indicating its inability to cleave Fuc linked α(1,3) to subterminal GlcNAc. This fucosidase hydrolyzed the [^{14}C]Fuc-containing product isolated from Galβ(1,3)-(Fucα(1,4))GlcNAcβ-*O*-Bn with a release of 81.7% [^{14}C]-Fuc; there was no formation of [^{14}C]Fucα(1,2)Galβ(1,3)β-*O*-Bn, indicating the inability of the earthworm enzyme to cleave Fuc linked α(1,4) to subterminal GlcNAc. Nonenzymatic release of Fuc from either mono- or difucosylated compounds was not observed under the incubation conditions in all cases (see Table 1).

Discerning the Association of Novel α(1,2)-FT Activity (Lewis a → Lewis b) with α(1,3/4)-FT and Not with Plasma-Type α(1,3)-FT. (a) Elution Patterns of α(1,2)-, α(1,3)- and α(1,4)-FT Activities from the Sephacryl S-200 Column. The enzyme preparation resulting from the affinity chromatography of Colo 205-solubilized extract on bovine IgG glycopep-Sepharose containing α(1,3)-, α(1,4)-, and the novel α(1,2)-FT activities was subjected to chromatography on the Sephacryl S-200 column, and the column effluent was assayed for FT activity with α(1,3)- and α(1,4)-specific acceptors and also with Galβ(1,3)(Fucα(1,4))GlcNAcβ-*O*-Bn, which measures the novel α(1,2)-FT activity. The α-(1,4)- and α(1,2)-FT activities occupied the same elution position (Figure 2A), whereas the elution patterns of α(1,3)-FT activity, as measured with two specific acceptors (Figure 2B), differed considerably from those of α(1,4) and α(1,2) activities; the α(1,3) activity showed a wider range of elution and was smaller in molecular size than the other two activities.

(b) Inhibition of the FT Activities in the Effluent from the Sephacryl S-200 Column by the Copolymer Synthesized from

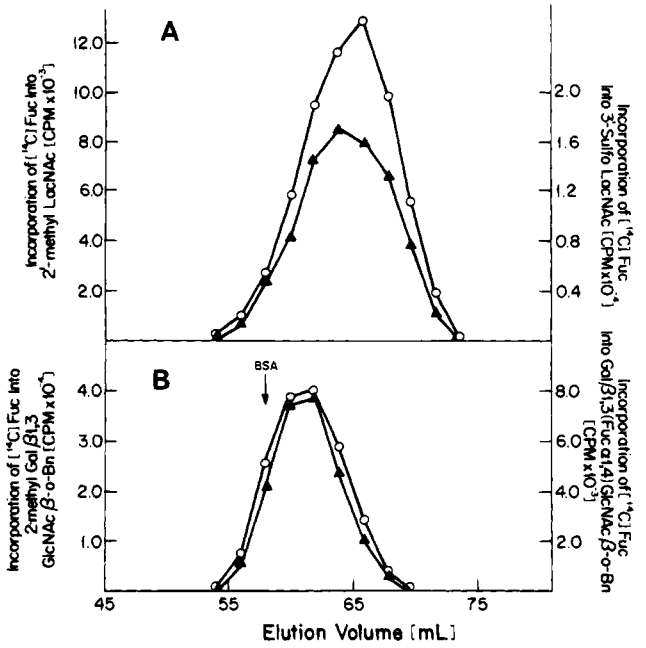


FIGURE 2: Chromatography on the Sephacryl S-200 column of the FT preparation isolated from Colo 205 extract by affinity chromatography. Incorporation of [^{14}C]Fuc into (A) (○) 2′-methyl-LacNAc and (▲) 3′-sulfo-LacNAc and (B) (○) 2-methyl-Galβ(1,3)GlcNAcβ-*O*-Bn and (▲) Galβ(1,3)(Fucα(1,4))GlcNAcβ-*O*-Bn.

SGGA and Acrylamide. When α(1,3)-, α(1,4)-, and the novel α(1,2)-FT activities were measured in both the presence and absence of the copolymer, the α(1,4) and α(1,2) activity curves followed the same patterns, and both activities were inhibited by the copolymer to a great extent and almost to the same level (Figure 3A,B). However, the α(1,3)-FT activity was inhibited only to a small extent, as observed with the two acceptors 2′-methyl-LacNAc and 3′-sulfo-LacNAc (Figure 3C,D). The results strongly suggest that the novel α(1,2) activity resides with the α(1,4)-fucosylating species.

Table 2: Affinity Chromatographic Separation of Colo 205 α(1,3/4)-L-Fucosyltransferase Activity from α(1,3) Activity

	distribution of α-L-fucosyltransferase		ratio
fraction	α(1,3)	α(1,4)	α(1,4)/α(1,3)
Colo 205 extract	50.3 (100.0)	49.7 (100.0)	0.99
IgG glycopeptide-Sepharose fractions:			
(a) lactose eluate	15.3 (30.4)	37.3 (75.0)	2.44
(b) NaCl eluate	35.0 (69.6)	12.4 (25.0)	0.35

(c) Resolution of Lewis-Type Enzyme (α(1,3/4)-FT) Containing the Novel α(1,2) Activity from the Plasma Type (α-(1,3)-FT). This goal was achieved by employing the following strategies.

(i) Selective Elution of the α(1,4)-FT-Rich Enzyme Fraction from the Affinity Column by Lactose. The elution of Colo 205 FT, which was bound to the bovine IgG glycopep-Sepharose column, with 0.1 M lactose prior to the use of 1 M NaCl for elution resulted in the recovery of 75% α(1,4)-FT activity in the lactose eluate, and this activity amounted to 2.44 times the α(1,3) activity present in this fraction (Table 2). The subsequent use of 1 M NaCl eluted 69.6% of the α(1,3)-FT activity, which was nearly 3 times the α(1,4)-FT activity contained in this fraction (Table 2).

(ii) Separation of Plasma-Type FT Activity from Lewis-Type α(1,3/4) Activity by the Use of the Sephacryl S-100 HR Column. The possibility of separating these two activities on the Sephacryl S-100 HR column was discerned from our attempt to separate BSA (66 000) and ovalbumin (45 000) by FPLC on Superose 12, as well as by conventional chromatography on Sephacryl S-100 HR; the latter method gave a clean separation of these glycoproteins. Figure 4 illustrates chromatography of the affinity-purified fractions on the Sephacryl S-100 HR column. The α(1,4)- and α-(1,3)-FT activities present in the lactose eluate emerged from the column in the same position, which was similar in

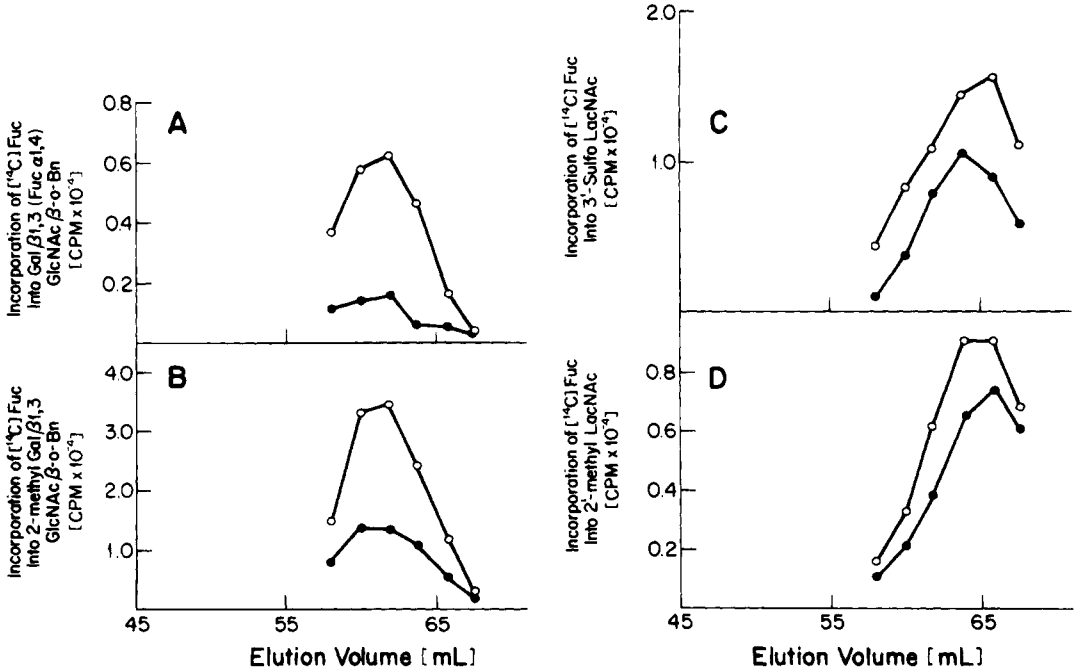


FIGURE 3: Inhibition of FT activities in Sephacryl S-200 effluent fractions by the copolymer from 3-sulfo-Galβ(1,3)GlcNAcβ-*O*-allyl and acrylamide. Incorporation of [^{14}C]Fuc into (A) Galβ(1,3)(Fucα(1,4))GlcNAcβ-*O*-Bn, (B) 2-methyl-Galβ(1,3)GlcNAcβ-*O*-Bn, (C) 3′-sulfo-LacNAc, and (D) 2′-methyl-LacNAc in presence of the copolymer (100 μg) (●) and in the absence of the copolymer (○).

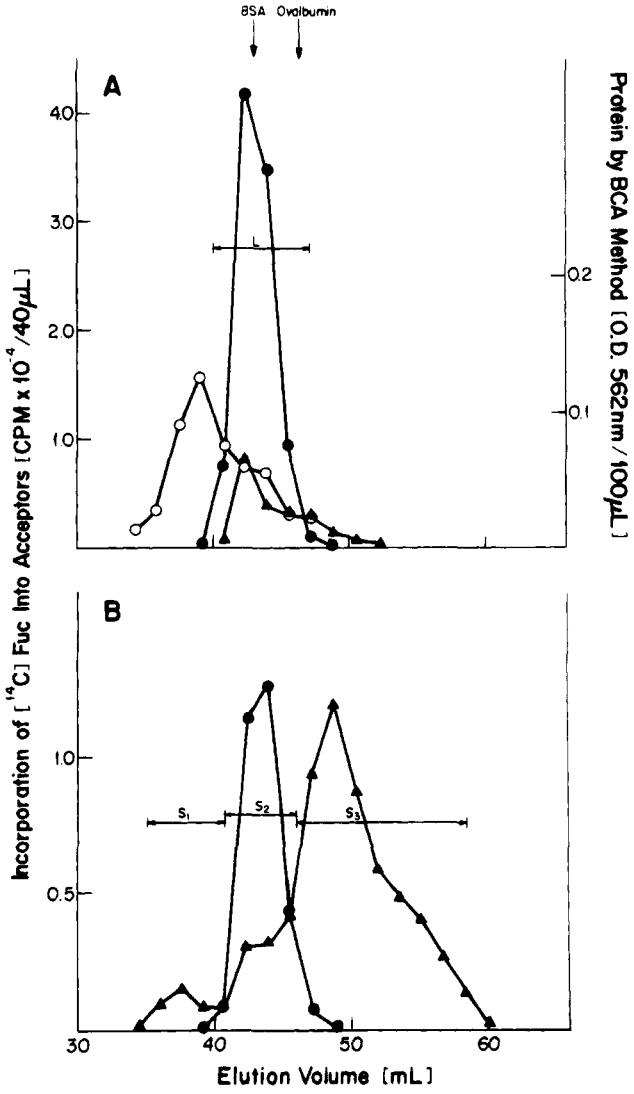


FIGURE 4: Chromatography on Sephacryl S-100 HR column: (A and B) fractionations of the lactose- and NaCl-eluted materials isolated from the Colo 205 extract, respectively. Symbols: (●) α(1,4)-L-FT [acceptor, 2-methyl-Galβ(1,3)GlcNAcβ-*O*-Bn]; (▲) α-(1,3)-L-FT [acceptor, 2′-fucosyl-LacNAcβ-*O*-Bn]; (○) protein.

molecular size to BSA (Figure 4A). A major portion of the protein contaminant in the lactose eluate was eliminated from the enzymes during this fractionation. Most of the α(1,4)-FT activity and a small amount of α(1,3)-FT activity present in the NaCl eluate emerged from the column in the same position as the FT of the lactose eluate (Figure 4B); a major portion of α(1,3) activity emerged from the column later than ovalbumin, and a minor portion came in front of the α(1,4)-FT-containing peak (Figure 4B).

(iii) Assignment of Fraction L as Lewis Type and Fraction S_3 as Plasma Type and Identification of the Novel α(1,2) Activity in the Former. Table 3 shows the distribution of α(1,3)- and α(1,4)-FT activities in the fractions resulting from fractionation on the Sephacryl S-100 HR column. Fraction L had 67.8% and S_2 had 23.3% of the α(1,4)-FT activity present in Colo 205. Fractions S_3, S_2, and L had 55.9%, 17.9%, and 19.7%, respectively, of the α(1,3)-FT activity present in Colo 205. The ratios of the activities (α(1,3)/α-(1,4)) given in the last column of Table 3 showed that fraction L had α(1,4)-FT activity that was 4 times its α-(1,3)-FT activity; S_2 consisted of 60% α(1,4)-FT activity and

Table 3: Levels of Colo 205 α(1,3)- and α(1,4)-L-Fucosyltransferase Activities in the Fractions Resulting from Chromatography on the Sephacryl S-100 HR Column

fraction	α(1,3)-L-FT activity (% total α(1,3) activity)	α(1,4)-L-FT activity (% total α(1,4) activity)	ratio of activities [α(1.3)/α(1.4)] in each fraction
L	19.7	67.8	0.25
S_1	6.5	3.5	1.63
S_2	17.9	23.3	0.66
S_3	55.9	5.4	8.89

40% α(1,3)-FT activity, and S_3 had α(1,3) activity that was about 9 times its α(1,4) activity.

Colo 205 enzyme fractions L, S_2, and S_3 were tested with synthetic carbohydrates (Table 4). Fraction L was 506.1% active toward SGGA (another α(1,4)-specific acceptor), in comparison to its activity with the α(1,3)-specific acceptor, 2′-fucosyl-LacNAcβ-*O*-Bn; 2′-fucosyllactose was quite active (133.2%), in fact, better than 2′-fucosyl-LacNAcβ-*O*-Bn as an acceptor for fraction L. The Lewis a determinant structure Galβ(1,3)(Fucα(1,4))GlcNAcβ-*O*-Bn served as a good acceptor (87.8%) for fraction L. As anticipated, the α(1,4)-FT-specific acceptor was only 11.2% active compared to the α(1,3)-FT-specific acceptor when tested with fraction S_3. S_3, which was thus identified as predominantly α(1,3)-FT, was also active with the other α(1,3)-FT-specific acceptor, 3′-sulfo-LacNAc, but this activity was considerably lower compared to fraction L (L, 93.1%, and S_3, 40.2%); S_3 showed only negligible activity with 2′-fucosyllactose (3.0%) and the acceptor containing Lewis a structure (1.6%). Fraction S_2, as evident from Table 4, exhibited activity intermediate between that of fractions L and S_3 when tested with the acceptors reported earlier.

The effect of the copolymer (a specific inhibitor of α-(1,4)-FT activity) from SGGA and acrylamide on the FT activities present fractions in L, S_2, and S_3 was measured (Table 5) in order to ascertain that α(1,3) activity present in fraction L is inherent to the α(1,4)-fucosylating species containing the novel α(1,2) activity. The α(1,3)-FT activity present in fraction S_3 was inhibited to a small extent only by the copolymer (10 μg, 11.4%; 100 μg, 13.7%), whereas the α(1,4)-FT activity of fraction S_3 was inhibited to a great extent (69.8%) at the 100 μg level of the copolymer. On the contrary, both the α(1,3)- and α(1,4)-FT activities of fraction L were inhibited to a large extent (60.1% and 38.7%, respectively) at the 10 μg level; the inhibition of both activities reached almost 90% at the 100 μg level. The α-(1,3)-FT activity of fraction S_2 was inhibited 20.1% and 26.5% and the α(1,4) was inhibited 18.8% and 79.7% at 10 and 100 μg of the copolymer, respectively. The extensive inhibition of α(1,3)-FT activity in fraction L to the same level as the α(1,4)-FT activity present in L at two different concentrations of the copolymer and the nonobservance of such an inhibition of α(1,3)-FT activity in fraction S_3 strongly suggested that α(1,3)-FT activity in fraction L is inherent to the α(1,4)-FT species of L, which carry the novel α(1,2)-FT activity.

Further Evidence for α(1,2)-L-Fucosylation Catalyzed by Colo 205 Lewis-Type FT. (a) Autoradiography as Well as Quantitation by Gel Scraping. We used Galβ(1,3)GlcNAcβ-*O*-Bn, Galβ(1,3)(6-*O*-Me)GlcNAcβ-*O*-Bn, Galβ(1,3)(4-*O*-Me)GlcNAcβ-*O*-Bn, and Galβ(1,3)(4,6-di-*O*-Me)GlcNAcβ-

Table 4: Reactivity of Sephacryl S-100 HR Major Fractions Isolated from Colo 205 with Synthetic Acceptors[a]

	enzyme activity of Sephacryl S-100 HR fractions		
	Colo 205 L	Colo 205 S_2	Colo 205 S_3
2′-fucosyl-LacNAcβ-*O*-Bn	100 (5175)	100 (3870)	100 (11559)
2′-fucosyl-Lac	133.2	37.8	3.0
3′-sulfo-LacNAc	93.1	62.0	40.2
3-sulfo-Galβ(1,3)GlcNAcβ-*O*-allyl	506.1	181.7	11.2
Galβ(1,3)(Fucα(1,4))GlcNAcβ-*O*-Bn	87.8	30.3	1.6

[a] The values in parentheses are the actual CPM of [^{14}C]Fuc in the product formed from 2′-fucosyl-LacNAcβ-*O*-Bn (3.0 mM) in the experiments. The activities of the other acceptors for each fraction are expressed as a percent of this value.

Table 5: Inhibition of the Fucosyltransferase Activities Present in Sephacryl S-100 HR Fractions from Colo 205 by Synthetic Copolymer

	fucosyltransferase activity of Sephacryl S-100 HR fractions					
	Colo 205 L		Colo 205 S_2		Colo 205 S_3	
reaction mixture	CPM	inhibition (%)	CPM	inhibition (%)	CPM	inhibition (%)
	α(1,3)-L-Fucosyltransferase Activity					
(a) 2′-fucosyl-LacNAcβ-*O*-Bn	8623		6429		21207	
(b) (a) plus SGGA−acrylamide copolymer						
(i) 10 μg	3442	60.1	5136	20.1	18791	11.4
(ii) 100 μg	930	89.2	4726	26.5	18301	13.7
	α(1,4)-L-Fucosyltransferase Activity					
(a) 2-methyl-Galβ(1,3)GlcNAcβ-*O*-Bn	39869		12828		2478	
(b) (a) plus SGGA−acrylamide copolymer						
(i) 10 μg	28456	38.7	10411	18.8	2313	6.7
(ii) 100 μg	4553	88.6	2605	79.7	748	69.8

O-Bn as the acceptors (for reasons, see Experimental Procedures) and compared the product by autoradiography and also by quantitation of the radioactivity by gel scraping. The preceding acceptors on a separate TLC run showed single spots (sprayed with H_2SO_4 in ethanol and then heated) of different mobilities, indicating their high purity (Figure 5A). The radioactive products from all of the methylated compounds showed spots of higher mobility compared to that from Galβ(1,3)GlcNAcβ-*O*-Bn, indicating that [^{14}C]Fuc is really incorporated into the methylated compounds (Figure 5B). There was another radioactive spot from the acceptor Galβ(1,3)(4-*O*-Me)GlcNAcβ-*O*-Bn, corresponding to the radioactive spot from Galβ(1,3)GlcNAcβ-*O*-Bn. In view of the high degree of purity of all of these compounds, the nature of this product is unclear at present. A measurement of radioactivity by scraping the gel showed that, as compared to Galβ(1,3)GlcNAcβ-*O*-Bn and Galβ(1,3)(6-*O*-Me)GlcNAcβ-*O*-Bn (the acceptors for α(1,4)-FT), 33−39% radioactivity had been incorporated into Galβ(1,3)(4-*O*-Me)GlcNAcβ-*O*-Bn and Galβ(1,3)(4,6-di-*O*-Me)GlcNAcβ-*O*-Bn, which can accept Fuc only on Gal since C-4 of GlcNAc is substituted by a methyl group. The product with the lower mobility from the acceptor Galβ(1,3)(4-*O*-Me)GlcNAcβ-*O*-Bn contained 55% radioactivity with respect to the higher mobility spot.

(b) Mutual Inhibition. We demonstrated the inhibition of α(1,4)-FT activity by the α(1,2)-specific acceptor, Galβ(1,3)-(4,6-di-*O*-Me)GlcNAcβ-*O*-Bn, as well as the inhibition of α(1,2)-FT activity by 3-sulfo-Galβ(1,3)GlcNAcβ-*O*-Al, which is a specific acceptor for α(1,4)-FT, by incubating varying concentrations of 3-sulfo-Galβ(1,3)GlcNAcβ-*O*-Al with Colo 205 fraction L in the presence of Galβ(1,3)(4,6-di-*O*-Me)-GlcNAcβ-*O*-Bn and measuring the incorporation of [^{14}C]-Fuc into both acceptors (Figure 6A). The K_m values for 3-sulfo-Galβ(1,3)GlcNAcβ-*O*-Al in the absence and presence of Galβ(1,3)(4,6-di-*O*-Me)GlcNAcβ-*O*-Bn (Figure 6B) were found to be 32 and 50 μM, respectively, thus revealing an

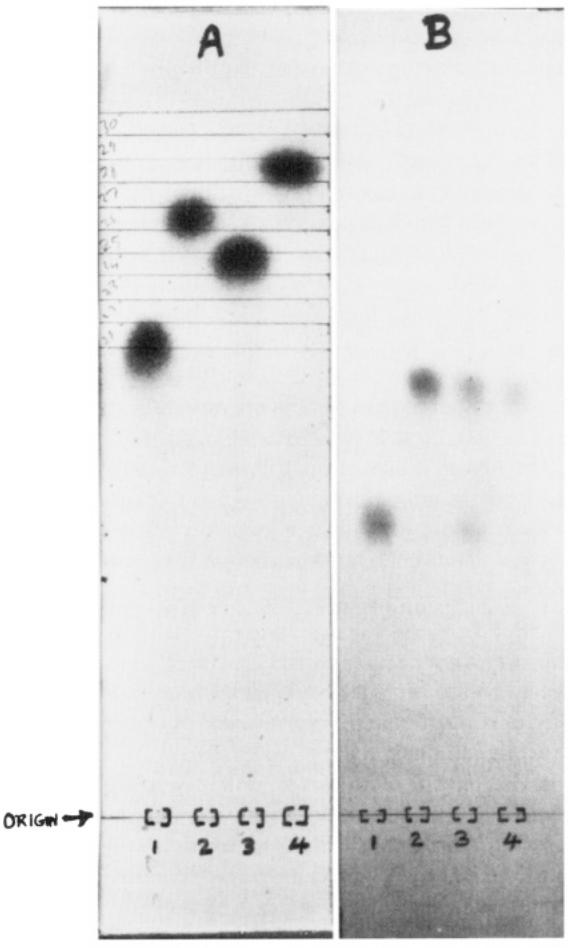


FIGURE 5: Thin-layer chromatography of the radioactive products arising from *O*-methyl derivatives of Galβ(1,3)GlcNAcβ-*O*-Bn. (A) Lanes 1−4: Galβ(1,3)GlcNAcβ-*O*-Bn, Galβ(1,3)(6-*O*-Me)GlcNAcβ-*O*-Bn, Galβ(1,3)(4-*O*-Me)GlcNAcβ-*O*-Bn, and Galβ(1,3)(4,6-di-*O*-Me)GlcNAcβ-*O*-Bn (GMGB), respectively. (B) Lanes 1−4: Autoradiography of the [^{14}C]fucosyl compounds arising from the above preceding, respectively.

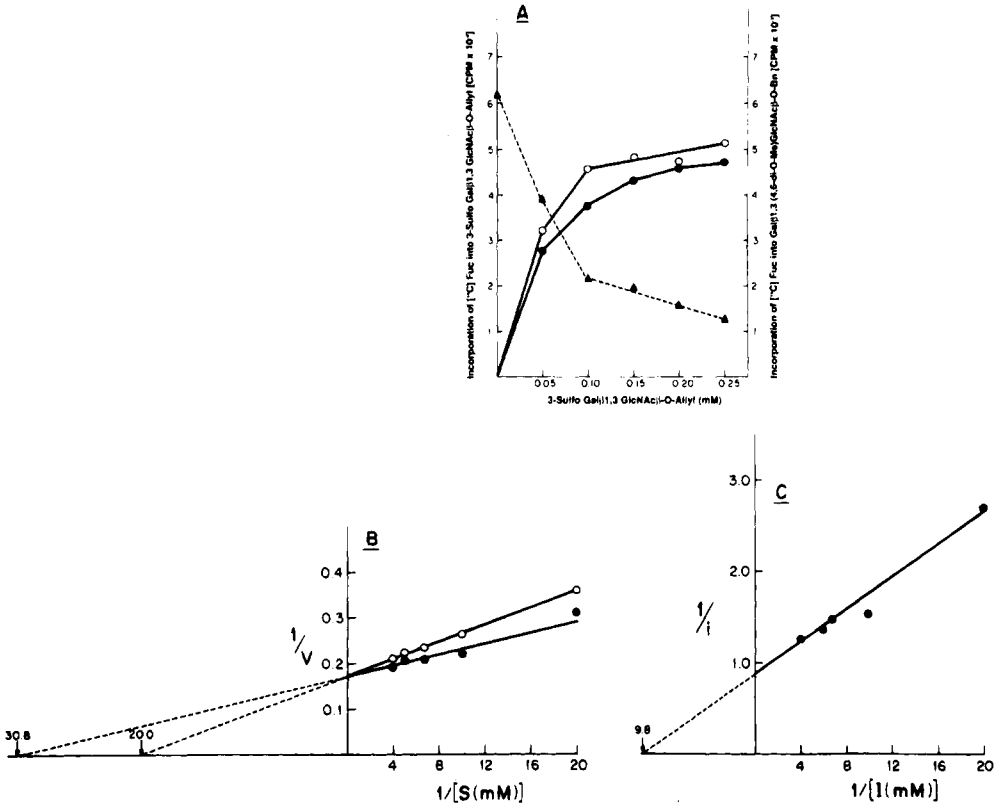


FIGURE 6: Acceptor activity of GMGB in the presence of increasing concentrations of SGGA. Determinations of K_m for SGGA in the presence and absence of GMGB and K_i for SGGA as the inhibitor were made. (A) Incorporation of [^{14}C]Fuc into SGGA (○); SGGA in presence of GMGB (7.5 mM) (●); GMGB in presence of SGGA (▲). (B) Lineweaver–Burk plot of [^{14}C]Fuc incorporation into SGGA in the presence (○) and absence (●) of GMGB. (C) Lineweaver–Burk plot of [^{14}C]Fuc incorporation into GMGB in the presence of increasing concentrations of SGGA.

Table 6: Reactivity of Sephacryl S-100 HR Major Fractions Isolated from Colo 205 with Glycopeptides

glycopeptide	α(1,3)-L-fucosyltransferase activity[a] of Sephacryl S-100 HR fraction		
	Colo 205 L	Colo 205 S_2	Colo 205 S_3
fetuin triantennary sialoglycopeptide with 3′-sialyl-LacNAc terminals			
20 μg	5.7	7.6	5.6
200 μg (0.40 mM)[b]	3.8	0.8	1.2
fetuin triantennary asialoglycopeptide			
20 μg	10.7	11.2	5.5
200 μg (0.48 mM)[c]	76.8	44.5	23.0
bovine IgG diantennary glycopeptide			
20 μg	0.3	4.4	1.9
200 μg (0.56 mM)[d]	6.0	13.8	12.3

[a] Expressed as percent of the activity toward 2′-fucosyl-LacNAcβ-*O*-Bn (3.0 mM). [b–d] Denote the concentration of glycopeptides in the reaction mixture, based on approximate molecular weights of 5000, 4100, and 3600, respectively.

increase in K_m due to acceptor competition. A gradual decrease in the incorporation of [^{14}C]Fuc into Galβ(1,3)-(4,6-di-*O*-Me)GlcNAcβ-*O*-Bn was seen with an increase in the concentration of 3-sulfo-Galβ(1,3)GlcNAcβ-*O*-Al. K_i for this inhibition (Figure 6C) was found to be 102 μM.

Next, the possibility of a difference in the reactivity of 1,3-FT species present in Colo 205 L, S_2, and S_3 fractions with three different glycopeptides was examined (Table 6); fractions L, S_2, and S_3 were active to some extent at 20 μg levels of fetuin triantennary sialoglycopeptide and its corresponding asialo derivative. Much less activity was seen in all cases at 200 μg of fetuin triantennary sialoglycopeptide. On the contrary, an increase in acceptor activity was noticed when the concentration of fetuin triantennary asialoglycopeptide was increased from 20 to 200 μg (L, 10.7% → 76.8%; S_2, 11.2% → 44.5%; S_3, 5.5% → 23.0%). As compared to fetuin triantennary asialoglycopeptide, bovine IgG glycopeptide was a poor acceptor for fractions L, S_2, and S_3, and this behavior was especially pronounced with fraction L (refer to Table 6 for a comparison). It becomes evident from the present data that the Lewis-type enzyme (α(1,3/4)-FT) containing the novel α(1,2)-FT activity differs significantly from α(1,3)-FT present in fraction S_3, the former showing a vast preference for the triantennary structure as a substrate (20 μg level, asialo triantennary → 10.7% and diantennary → 0.3%; 200 μg level, 76.8% and 6.0%, respectively).

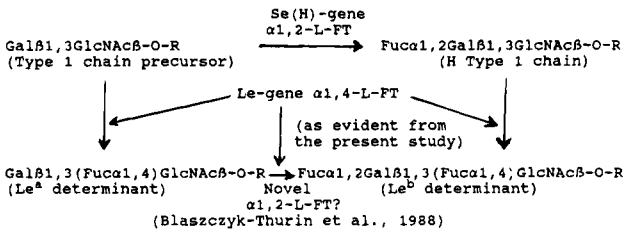
Activity of the Soluble Enzyme Preparation from the Immobilized, Cloned FT III. This enzyme showed 103.4%, 22.9%, 13.5%, 10.5%, and 7.7% activity toward 3-sulfo-Galβ(1,3)GlcNAcβ-*O*-Al, 2′-fucosyl-Lac, Galβ(1,3)(Fucα-(1,4))GlcNAcβ-*O*-Bn, 2′-fucosyl-LacNAcβ-*O*-Bn, and 3′-sulfo-LacNAc, as compared to 2-methyl-Galβ(1,3)GlcNAcβ-*O*-Bn, which measures α(1,4)-FT activity. The results

indicated that the specificity of this enzyme was similar to that of Colo 205 fraction L (Table 4).

Identification of the Product Resulting from Galβ(1,3)-(Fucα(1,4))GlcNAcβ-O-Bn by the Action of Cloned FT III (Lewis Type). The FAB mass spectrum of the enzymatic product showed *m/z* 766.2 [$(M + 1)^+$], which was consistent with the difucosyl derivative. A loss of fucose (mass of 147) from the positively charged molecular ion (M + 1) at 766.2 resulted in an ion at *m/z* 619.8 and, a loss of two fucose moieties resulted in an ion at *m/z* 472.8, which after the loss of galactose showed an ion at *m/z* 331.0.

Additional Evidence for the Coexistence of the Novel α-(1,2)-L-FT Activity with α(1,4)-L-FT Activity. We examined a few human cell lines for this purpose. The α(1,3)-, α-(1,4)-, and novel α(1,2)-L-FT activities [transfer of [^{14}C]Fuc (CPM × 10^{-5}) catalyzed by 1 mg of protein of the cell extract] were, respectively, as follows: Colo 205 (6.6, 5.6, 5.5); HT29 (6.2, 0.9, 0.2); EKVX (2.6, 0, 0); A159 (2.7, 0.01, 0.03); JTL (1.7, 0.05, 0); and B142 (2.2, 0.05, 0). The data show that a high level of α(1,4) and the novel α(1,2)-L-FT activities was found only in Colo 205. This parallel occurrence strongly suggested the coexistence of the novel α(1,2)-L-FT activity in α(1,4)-L-FT species. Further, the distinct nature of the Colo 205 enzyme from those of other cell lines of this study is also evident from its great ability to transfer Fuc (CPM × 10^{-5}) to both the sialo and asialo triantennary glycopeptides of fetuin: Colo 205 (6.0, 8.3); HT29 (0.01, 1.9); EKVX (0, 1.8); A159 (0.3, 2.7); JTL (0, 4.0); and B142 (0, 2.1).

The present study has thus provided convincing evidence for an alternate exciting pathway for the biosynthesis of Lewis b determinant, as outlined in the following scheme:



The present study has also shown that the Lewis-type enzyme of Colo 205 catalyzes α(1,3)-L-fucosylation of Glc in lactose-based structures, thus clarifying the recent report by Holmes (1993) that α(1,3)-L-fucosylation of LC_3, GlcNAcβ(1,3)Galβ-(1,4)Glc1→1Cer, by the Colo 205 enzyme occurs on the Glc moiety and not on the terminal GlcNAc.

Our successful attempt at separating the Lewis-type enzyme from the plasma type of Colo 205 cells thus enabled us to assign a unique role to the former in the expression of various fucosylated glycoconjugates in cancer and probably other malignancies for the following reasons. This enzyme can form several kinds of fucosylated structures, such as Lewis a, Lewis x, sialyl Lewis a, sialyl Lewis x, Lewis b from H type 1, Lewis y from H type 2, Lewis b from Lewis a (the novel α(1,2)-FT activity), and R-Galβ(1,4)(Fucα(1,3))-Glcβ→ (R = Fucα(1,2) or GlcNAcβ(1,3)); further, this enzyme seems to be highly effective in fucosylating triantennary (probably multiantennary) complex structures. All of the preceding broadest specificity and efficiency of this enzyme demonstrate its potential involvement in malignancy.

The genes encoding for the three α(1,3)-L-fucosyltransferases, namely, Fuc-T III (Lewis type), Fuc-T V, and Fuc-T VI, cloned by Lowe and co-workers (Weston et al., 1992), were located on chromosome 19. These enzymes were nearly identical in carboxyl-terminal regions. Most variations among these enzymes were found in a region between the transmembrane segment and the amino-terminal end of the catalytic domain. Thus, the amino acids in this hypervariable region may be crucial in the binding interaction between enzyme and acceptor and thus may determine the efficiency of activity with different acceptor substrates. The broad specificity of the Lewis-type FT, as witnessed by several investigators, and the present discovery of the additional specificities, namely, the novel α(1,2)-FT activity and the α(1,3)-FT activity on lactose-based structures for the Lewis-type enzyme of Colo 205 would indicate the importance of addressing the amino acid sequence determinants that are responsible for the broad specificity.

ACKNOWLEDGMENT

The authors thank Drs. R. J. Bernacki and R. A. DiCioccio of the Roswell Park Cancer Institute for providing the cell lines.

REFERENCES

Abe, K., Hakomori, S.-i., & Oshiba, S. (1986) *Cancer Res. 46*, 2639−2644.

Blaszczyk, M., Pak, K. Y., Herlyn, M., Sears, H. F., & Steplewski, Z. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 3552−3556.

Blaszczyk-Thurin, M., Thurin, J., Hindsgaul, O., Karlsson, K.-A., Steplewski, Z., & Koprowski, H. (1986) *J. Biol. Chem. 262*, 372−379.

Blaszczyk-Thurin, M., Sarnesto, A., Thurin, J., Hindsgaul, O., & Koprowski, H. (1988) *Biochem. Biophys. Res. Commun. 151*, 100−108.

Brown, A., Ellis, I. O., Embleton, M. J., Baldwin, R. W., Turner, D. R., & Hardcastle, J. D. (1984) *Int. J. Cancer 33*, 727−736.

Chandrasekaran, E. V., Jain, R. K., Ramakrishnan, P. R., Chatrath, K., Piver, M. S., & Matta, K. L. (1991) Eleventh International Symposium on Glycoconjugates, Toronto, Abstr. 15.45.

Chandrasekaran, E. V., Jain, R. K., & Matta, K. L. (1992a) *J. Biol. Chem. 267*, 23806−23814.

Chandrasekaran, E. V., Jain, R. K., & Matta, K. L. (1992b) *J. Biol. Chem. 267*, 19929−19937.

Chandrasekaran, E. V., Rhodes, J. M., Jain, R. K., Bernacki, R. J., & Matta, K. L. (1994) *Biochem. Biophys. Res. Commun. 201*, 78−89.

Ernst, C., Atkinson, B., Wysocka, M., Blaszczyk, M., Herlyn, M., Sears, H. F., Steplewski, Z., & Koprowski, H. (1984) *Lab. Invest. 50*, 394−400.

Holmes, E. H. (1993) *Glycobiology 3*, 77−81.

Horejsi, V., Smolek, P., & Kocourek, J. (1978) *Biochim. Biophys. Acta 538*, 293−298.

Jain, R. K., Pawar, S. M., Chandrasekaran, E. V., Piskorz, C. F., & Matta, K. L. (1993) *Bioorg. Med. Chem. Lett. 3*, 1333−1338.

Kukowska-Latallo, J. F., Larsen, R. D., Nair, R. P., & Lowe, J. B. (1990) *Genes Dev. 4*, 1288−1303.

LePendu, J., Lemieux, R. U., Lamberg, F., Dalix, A.-M., & Oriol, W. (1982) *Am. J. Hum. Genet. 34*, 402−415.

Sakamoto, J., Furukawa, K., Cordon-Cardo, C., Yin, B. W. T., Rettig, W., Oettgen, H. F., Old, L. J., & Lloyd, K. O. (1986) *Cancer Res. 46*, 1553−1561.

Sarnesto, A., Kohlin, T., Hindsgaul, O., Thurin, J., & Blaszczyk-Thurin, M. (1992) *J. Biol. Chem. 267*, 2737−2744.

Wang, B., Akiyama, K., & Kimura, H. (1994) *Vox Sang. 66*, 280−286.

Weston, B. W., Smith, P. L., Kelly, R. J., & Lowe, J. B. (1992) *J. Biol. Chem. 267*, 24575−24584.

Yazawa, S., Madiyalakan, R., Chawda, R. P., & Matta, K. L. (1986) *Biochem. Biophys. Res. Commun. 136*, 563−569.

BI9422539