

Ovarian Cancer α 1,3-L-Fucosyltransferase

DIFFERENTIATION OF DISTINCT CATALYTIC SPECIES WITH THE UNIQUE SUBSTRATE, 3'-SULFO-N-ACETYLLACTOSAMINE IN CONJUNCTION WITH OTHER SYNTHETIC ACCEPTORS*

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Several *N*-acetyllactosamine (LacNAc) derivatives were tested as acceptors for α 1,3-L-fucosyltransferase present in human ovarian cancer sera and ovarian tumor. The enzyme of the soluble fraction of tumor was purified to apparent homogeneity by chromatography on bovine IgG glycopeptide-Sepharose followed by Sephacryl S-200 (M_r < 67,000). As compared with 2'-methyl LacNAc, 3'-sulfo LacNAc was about 5-fold more sensitive in measuring α 1,3-fucosyltransferase in sera (K_m , 3'-sulfo LacNAc, 0.12 mM; 2'-methyl LacNAc, 6.67 mM). When ovarian cancer serum was the enzyme source, either the sulfate group or a sialyl moiety at C-3' of LacNAc enhanced the acceptor ability (341 and 242%, respectively), whereas the sulfate group at C-2' or C-6' reduced the activity (22–36%); sulfate at C-6 or fucose at C-2' increased the activity (172 and 253%). The β -benzylation of the reducing end, in general, increased the activity 2–3-fold. The enzyme of the soluble fraction of tumor exhibited more activity toward 3'-sulfo LacNAc (447%), 2'-fucosyl-LacNAc (436%), and 6-sulfo LacNAc (272%). Very low activity was observed with 3'-sialyl LacNAc (12.4%), 2'-sulfo LacNAc (33%), and 6'-sulfo LacNAc (5%); Fuc α 1,2Gal β 1,3GlcNAc β -*O*-*p*-nitrophenyl (166%), 2-methyl Gal β 1,3GlcNAc β -*O*-benzyl (204%), and 3-sulfo Gal β 1,3GlcNAc (415%) also acted as acceptors, indicating the coexistence of α 1,3- and α 1,4-fucosyltransferase. The tumor particulate enzyme behaved entirely different, exhibiting low activity with 3'-sulfo LacNAc (39%) and 2'-fucosyl-LacNAc (148%); 3'-sialyl, 6'-sulfo, 6-sulfo, or 2'-sulfo LacNAc were 3, 43, 53, and 10% active, respectively. Thus, the ovarian cancer serum α 1,3-fucosyltransferase acts equally well on H-type 2,3'-sialyl LacNAc and 3'-sulfo LacNAc, but not on H-type 1. The enzyme of soluble tumor fraction acts on H-type 2,3'-sulfo LacNAc as well as H-type 1 but poorly on 3'-sialyl LacNAc. The tumor particulate enzyme acts on H-type 2 but poorly on 3'-sulfo or 3'-sialyl LacNAc and is inactive with H-type 1.

When normal serum was examined with synthetic acceptors, >80% activity was found as α 1,2-fucosyltransferase and the rest as α 1,3-fucosyltransferase. A screening of 21 ovarian cancer and 3 normal sera (3'-sulfo LacNAc as acceptor) showed 17–572% increase (average increase, 188%) of α 1,3-fucosyltransferase activity in cancer. A parallel testing of Triton X-100 extracts of normal ovarian tissue and ovarian tumor

with synthetic acceptors revealed a high level of α 1,3-fucosyltransferase in tumor (2.17-fold increase when assayed with 3'-sulfo LacNAc and 0.42–0.56-fold increase with other specific acceptors). An examination of four ovarian tumors along with one normal ovarian tissue indicated that three tumors had 0.37–2.36-fold increase of α 1,3-fucosyltransferase activity, whereas all four tumors had 2.06–139.6-fold increase of α 1,4-fucosyltransferase activity using 2-*O*-methyl Gal β 1 \rightarrow 3GlcNAc β 1 \rightarrow *O*-benzyl as an acceptor.

An elevation of serum α 1,3-L-fucosyltransferase as well as an accumulation of Lewis X structures (Gal β 1,4(Fuc α 1,3)GlcNAc) were found to occur in a large variety of human cancers (1–12). Recently, our group (3–5) showed the existence of a correlation between this enzyme level in sera and the clinical stage and size of the tumor in gastric and lung cancers, thus indicating the usefulness of this enzyme as a diagnostic marker for cancer. The expression of mono-, di-, and trimeric X determinants in glycolipids of colon carcinoma was shown to be due to the retrogenetic expression of type 2 chain precursors (13) that were not found in normal adult colonic epithelial cells. This implies that the type of the precursor restricted the fucosyltransferase involved to transfer fucose to the C-3 position of GlcNAc. Furthermore, human colonic adenocarcinoma Colo 205 cells, in contrast to human small cell lung carcinoma NCI-H69 cells and lung carcinoma PC 9 cells (9, 10), transferred fucose in α 1,4 linkage to lacto series type 1 chain structures and in α 1,3 linkage to type 2 chain structures. A recent report (15) on the separation of α 1,3- and α 1,4-fucosyltransferase activities of human milk on Sephacryl S-200 column indicated that neither enzyme fraction was absolutely specific for type 1 or 2 chain acceptors. It, thus, becomes evident that the expression of α 1,3 and α 1,4 fucosylated lacto series carbohydrate chains involves fucosyltransferases exhibiting varying degrees of substrate specificity and differing cell and tissue distribution (14–16). Carbohydrates containing the type 2 chain Gal β 1,4GlcNAc β and the corresponding NeuAc α 2,3Gal β 1,4GlcNAc β -type structures have been used for the assay of α 1,3-L-fucosyltransferases. A recent study has shown that 2'-fucosyl LacNAc is a preferred substrate for α 1,3-L-fucosyltransferase of human neuroblastoma cells (17). Apart from the known sulfated glycoconjugates such as mucins and glycolipids, the sulfate group has been identified recently in some glycoproteins. The sulfate group in glycolipids is generally linked to a position, otherwise occupied by a sialyl residue. de Waard *et al.* (18) have found SO $_4$ \rightarrow 3Gal β 1,4GlcNAc as a terminal sequence of the asparagine-linked carbohydrate chain of porcine thyroglobulin. To date, the specificity of α 1,3-L-fucosyltransferases has not been examined with sulfated oligosaccharides, such as 3'-sulfo

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LacNAc, even though the corresponding sialylated saccharides have been studied. Thus it becomes necessary to examine the influence of the sulfate group in the oligosaccharide chain on the action of α -L-fucosyltransferases. The present study reports the discovery of sulfated saccharides as high affinity substrates for α 1,3-L-fucosyltransferase and their use in conjunction with other synthetic substrates in differentiating the distinct catalytic capabilities of α 1,3-fucosyltransferase species that exist in human ovarian cancer serum and the soluble and particulate fractions of human ovarian tumor.

EXPERIMENTAL PROCEDURES

Sera were collected from healthy females and ovarian cancer patients admitted to Roswell Park Cancer Institute. Ovarian tumor tissues were obtained during surgical procedures from patients with ovarian cancer. Both sera and tissues were stored frozen at -70°C until use.

The isolation of glycopeptides from bovine IgG (Calbiochem) by Pronase digestion, gel filtration, and concanavalin A-Sepharose chromatography and then coupling to Sepharose 4B was reported earlier (19). This affinity matrix (30 ml in bed volume) was washed and equilibrated at 4°C with 25 mM Tris-HCl, pH 7.0, containing 35 mM MgCl_2 , 10 mM $\text{Na}_2\text{S}_2\text{O}_3$, and 1 mM ATP.

Paper Chromatographic Identification of the [^{14}C]Fucose-containing Product Arising from 3'-Sulfo LacNAc

The incubation mixture (100 μl) was spotted on a Whatman 3MM paper and subjected to chromatography in ethyl acetate/pyridine/water, 12/5/4, for 48 h. The chemically synthesized authentic product, namely 3'-sulfo 3-fucosyl LacNAc, was also run in parallel on the paper. The radioactivity was located on the paper by cutting 1-cm sections, soaking them in 1 ml of water in vials, and detecting the radioactivity by scintillation counting. The nonradioactive standard was located on the paper by alkaline silver reagent (20). Protein was assayed by the Bio-Rad micromethod (21) with bovine serum albumin as the standard. Protein in the presence of Triton X-100 was determined by a modified Lowry procedure (22).

Assay of α 1,3-Fucosyltransferase

The incubation mixture contained 50 mM HEPES-NaOH, pH 7.5, 5 mM MnCl_2 , 7 mM ATP, 3 mM $\text{Na}_2\text{S}_2\text{O}_3$, the acceptor 0.3 mM (for serum enzyme) or 3.0 mM (for tumor enzyme), 0.125 μCi of GDP-[^{14}C]Fuc (specific activity, 216 mCi/mmol), and enzyme in a total volume of 0.10 ml; the control incubation mixture had everything except the acceptor. At the end of incubation at 37°C for 18 h, the mixture was diluted with 1 ml of water and passed through Dowex-1-Cl column (1 ml in a Pasteur pipette). The column was washed twice with 1 ml water; the breakthrough and wash, which contained the [^{14}C] fucosylated neutral acceptor, were collected together in a scintillation vial and counted for radioactivity using the scintillation mixture 3a70 (Research Products International, Mount Prospect, IL) and Beckman LS9000. The Dowex column was then eluted successively with 3 ml each of 0.1 and 0.2 M NaCl; these eluates which contained the [^{14}C] fucosylated sulfated acceptors, were counted for radioactivity as before. Corrections were made by subtracting the radioactivity in the water and NaCl eluates of the control incubation mixture from the corresponding test fractions. The duplicate runs of samples gave almost identical values, the difference being $<5\%$.

Chemical Synthesis

β -D-Galactopyranosyl-(1 \rightarrow 4)-sodium 2-Acetamido-2-deoxy-D-glucopyranose 6-Sulfate (Compound 6)—Glycosylation of benzyl-2-acetamido-3-O-benzyl-2-deoxy-6-O-(4-methoxybenzyl)- α -D-glucopyranoside with 2,3,4,6-tetra-O-acetyl- α -D-galactosyl bromide afforded the fully protected disaccharide. The selective removal of 4-methoxybenzyl group with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone in dichloromethane provided the alcohol (compound 4). Reaction of compound 4 with 5 molar equivalents of sulfur trioxide-pyridine complex in *N,N*-dimethylformamide produced compound 5 as its sodium salt after cation (Na^+) exchange. *O*-Deacetylation of compound 5 in methanolic sodium methoxide, followed by hydrogenolysis of the benzyl group in the presence of palladium-on-carbon furnished compound 6 as an amorphous solid after passage through a cation (Na^+)-

exchange resin column. [α] $_{\text{D}}$ +26.5 (*c* 1.3, water); for ^{13}C nmr and *m/z* data, see Table I.

Sodium β -D-Galactopyranosyl 3-Sulfate-(1 \rightarrow 4)-2-acetamido-2-deoxy-D-glucopyranose (compound 9)—The reaction of benzyl 2-acetamido-2-deoxy-3,6-di-O-benzyl-4-O-(4,6-O-benzylidene-2-O-benzyl- β -D-galactopyranosyl)- α -D-glucopyranoside in *N,N*-dimethylformamide with SO_3 -pyridine complex at room temperature afforded the corresponding 3'-O-sulfo derivative which on hydrogenolysis followed by purification over Dowex-1 (acetate form) column and treatment with Na^+ resin yielded compound 9 as an amorphous solid. [α] $_{\text{D}}$ +21.3 (*c* 0.5, H_2O). For ^{13}C nmr and *m/z* data see Table I.

***O*- α -L-Fucopyranosyl-(1 \rightarrow 3)-[sodium *O*- β -D-Galactopyranosyl 3-Sulfate-(1 \rightarrow 4)]-2-acetamido-2-deoxy-D-glucopyranose (compound 17)**—Glycosylation of benzyl 2-acetamido-4-O-(2,3,4,6-tetra-O-acetyl- β -D-galactopyranosyl)-6-O-benzyl-2-deoxy- α -D-glucopyranoside with methyl 2,3,4-tri-O-benzyl-1-thio- β -L-fucopyranoside, in the presence of cupric bromide-tetrabutyl ammonium bromide, followed by *O*-deacetylation with methanolic sodium methoxide afforded known benzyl 2-acetamido-6-O-benzyl-3-O-(2,3,4-tri-O-benzyl- α -L-fucopyranosyl-4-O-(β -D-galactopyranosyl)-2-deoxy- α -D-glucopyranoside (compound 11). Isopropylidenation of compound 11 by the procedure of Catelani *et al.* (30) afforded 3',4'-O-acetyl derivative (compound 12); [α] $_{\text{D}}$ +21 (*c* 0.9, CHCl_3); ^1H nmr (CDCl_3): δ 7.42–7.16 (m, 25 H, arom.), 1.50 (s, 3 H, NAc), 1.45 and 1.25 (each s, 3 H, CMe_2), 1.13 (d, *J* \sim 6 Hz, 3 H, CMe). Acetylation of 12 with pyridine-acetic anhydride and followed by cleavage of the isopropylidene group with 80% aqueous acetic acid gave the diol: [α] $_{\text{D}}$ +5.75 (*c* 0.8, CHCl_3); ^1H nmr (CDCl_3): δ 7.45–7.10 (m, 25 H, aromatic), 2.03 (s, 3 H, OAc), 1.98 (s, 3 H, OAc), 1.54 (s, 3 H, NAc), 1.17 (d, *J* 6 Hz, 3 H, CMe). This was converted into its 3',4'-(ethyl *ortho*-acetate) which was hydrolyzed with 80% aqueous acetic acid to give 3'-hydroxy key intermediate 15; [α] $_{\text{D}}$ +10.4 (*c* 0.5, CHCl_3); ^1H nmr (CDCl_3): δ 7.38–7.08 (m, 25 H, aromatic), 2.01 (s, 6 H, 2 \times OAc), 1.91 (s, 3 H, OAc), 1.51 (s, 3 H, NAc), 1.17 (d, *J* \sim 6 Hz, 3 H, CMe). Sulfation of 15 in a manner analogous to that described for compound 4 (to give compound 5), gave 16 as its sodium salt. *O*-Deacetylation of compound 16 in methanolic sodium methoxide, followed by removal of the benzyl ether protecting groups afforded the sodium salt of *O*- α -L-fucopyranosyl-(1 \rightarrow 3)-[(*O*- β -D-galactopyranosyl 3-sulfate-(1 \rightarrow 4))-2-acetamido-2-deoxy-D-glucopyranose (17) after purification over Dowex-1 (acetate form) column followed by treatment with cation (Na^+)-exchange resin; [α] $_{\text{D}}$ -10.7 (initial) \rightarrow -8.9 (after 72 h). (*c* 0.3, H_2O); for ^{13}C nmr and *m/z* data, see Table I. Hydrogenolysis of the benzyl groups of compound 11, followed by column chromatographic purification on silica gel, gave known free trisaccharide 18; For ^{13}C nmr and *m/z* data see Table I. The chemical synthesis of other compounds used in the present study is communicated elsewhere.

3'-Sialyl LacNAc was purchased from Oxford Glycosystems, Inc., Rosedale, NY.

α -L-Fucosyltransferases in Normal Ovarian Tissue and Ovarian Tumors, as Determined with Specific Acceptors

Four tumor specimens and one normal specimen were examined simultaneously for a strict comparison. No. 2162, normal ovarian tissue from 35-year-old patient; No. 2509, ovarian tumor from 59-year-old patient. Both were snap-frozen within 1 h after surgical removal (National Disease Research Interchange, Philadelphia), transported on dry ice, and then stored at -70°C .

The three ovarian tumors JM, LM, and KK were, respectively, from 59-, 64-, and 48-year-old patients of Roswell Park Cancer Institute. After surgical removal, these tissues were stored frozen within 1 h at -70°C .

From the above tissues, 2 g (exact weight obtained) were homogenized with 10 volumes of 50 mM Tris-HCl, pH 7.0, 0.15 M NaCl, adjusted to 1% Triton X-100, and stirred for 1 h at 4°C . These extracts were centrifuged at $20,000 \times g$ for 1 h at 4°C . The supernatants were collected, stored on ice, and the protein was measured by the BCA method (Pierce Chemical Co.). The protein concentration in the extracts was adjusted to exactly 4 mg/ml with the extraction buffer. The enzyme incubation was done under the standard incubation conditions using 40 μl of each extract (160 μg of protein). Incorporation of [^{14}C]Fuc into the acceptors was measured by the Dowex-1-Cl method.

RESULTS

The purity of the synthetic sulfated compounds 6, 9, and 17 were checked by thin layer chromatography on silica gel

as well as on cellulose plates and also by paper chromatography. Their structural assignments were confirmed by ^{13}C nmr (Table I) and fast atom bombardment mass spectroscopy (Fig. 1).

In the ^{13}C spectrum of 6, the resonance for C-6 of the 2-acetamido-2-deoxy-D-glucose (GlcNAc) residue suffered a downfield shift of 6.3 ppm, by comparison with that of its counterpart in the spectrum of compound 9, evidencing that O-6 was the site of sulfation. However, in the spectra of both 9 and 17, analogous downfield shift of 7.6 and 7.7 ppm, respectively, were observed for C-3 resonances of their corresponding Gal residues, confirming that sulfation had occurred at O-3' in both the compounds.

Purification of $\alpha 1,3$ -L-Fucosyltransferase from the Soluble Fraction of Human Ovarian Tumor—As shown in the scheme (Fig. 2), the α -L-fucosidase-free extract of the tissue (40 g), after concentration by 80% $(\text{NH}_4)_2\text{SO}_4$ precipitation was fractionated on bovine IgG glycopep-Sepharose in batches of 25 ml. After washing the column with the equilibration buffer, the bound enzyme was eluted with 1 M NaCl in the same buffer. The protein positive fractions were pooled and processed as shown in the scheme; 2.0 ml of the above preparation was loaded on a Sephacryl S-200 column (Superfine, 2.6×88.0 cm) equilibrated at 4 °C with 50 mM Tris-HCl, pH 7.0, containing 0.15 M NaCl and 0.1% Triton X-100 and eluted with the same buffer. Fractions of 3.0 ml were collected. The fucosyltransferase activity was located in the effluent fractions, by assaying first every fifth fraction and then obtaining the activity profile by assaying the alternate fractions in that range, using 2'-fucosyl LacNAc as the acceptor. Fig. 3 shows the elution profile of $\alpha 1,3$ -fucosyltransferase from the column. Its elution position, which is slightly later than bovine serum albumin, run separately on the same column, indicates its molecular weight as <67,000. When subjected to SDS-polyacrylamide gel electrophoresis (Phast Gel 8-25, Pharmacia LKB Biotechnology Inc. apparatus) the preparation showed a single diffuse band (stained by Coomassie Blue) with $M_r = <67,000$ (Fig. 4) which was quite similar to the M_r values reported for $\alpha 1,3$ -fucosyltransferase of embryonal carcinoma cells (23) and human amniotic fluid (24). Table II presents the results on the purification of the enzyme. The enzyme has been purified 66-fold over the soluble extract with a recovery of 6.2%. The enzyme at this stage lost its activity either kept frozen at -20 °C or left at 4 °C for more than a week. However, the enzyme at the end of the previous step was fairly stable for at least two months at 4 °C. So this preparation of enzyme

was used in acceptor ability comparison studies. It should be pointed out that Mitsakos and Hanisch (24) reported 125-fold purification of $\alpha 1,3$ -fucosyltransferase from amniotic fluid.

3'-Sulfo LacNAc, a Unique Substrate for $\alpha 1,3$ -Fucosyltransferase—The superiority of 3'-sulfo LacNAc over 2'-methyl LacNAc as the substrate for measuring serum $\alpha 1,3$ -fucosyltransferase was tested in three different ways. First, the incubation mixtures in duplicate contained 0.3 mM 3'-sulfo LacNAc and varying amounts of 2'-methyl LacNAc (0–7.5 mM); the incorporation of [^{14}C]fucose by the serum enzyme into both substrates was measured (Table III). The incorporation of [^{14}C]fucose into 3'-sulfo LacNAc decreased and into 2'-methyl LacNAc increased with the increase in the concentration of the latter. The highest concentration of 2'-methyl LacNAc used in the experiment (7.5 mM) was able to bring about only 43% inhibition of the [^{14}C]fucose incorporation into 3'-sulfo LacNAc, whose concentration was only 0.3 mM, i.e. 25-fold less than 2'-methyl LacNAc. Second, the incubation mixtures in duplicate contained 3.0 mM 2'-methyl LacNAc and varying amounts of 3'-sulfo LacNAc (0–0.75 mM); the incorporation of [^{14}C]fucose by the same serum enzyme into both substrates was measured (Table III). The [^{14}C] fucose incorporation into 2'-methyl LacNAc decreased and into 3'-sulfo LacNAc increased by increasing the concentration of the latter. 3'-Sulfo LacNAc at 0.75 mM (the highest concentration used in the experiment; this concentration is 0.10 of the highest concentration of 2'-methyl LacNAc used in the previous experiment) brought about 68% inhibition of the [^{14}C]fucose incorporation into 2'-methyl LacNAc, whose concentration was 3.0 mM, which was four times the amount of 3'-sulfo LacNAc. Third, both 2'-methyl LacNAc and 3'-sulfo LacNAc at 0.3 mM concentration were incubated separately in duplicates with six ovarian cancer sera and one normal serum; the incorporation of [^{14}C]fucose into these acceptors were quantitated (Table IV). The [^{14}C]fucose incorporation into 3'-sulfo LacNAc was consistently higher (4–5-fold) than that into 2'-methyl LacNAc, including the assay of the normal serum. As compared with the normal serum, the cancer sera showed higher $\alpha 1,3$ -fucosyltransferase activity in the range 176–434% when measured with 2'-methyl LacNAc and 200–636% with 3'-sulfo LacNAc. These data thus establish the consistency and superiority of 3'-sulfo LacNAc as the substrate for measuring the level of serum $\alpha 1,3$ -fucosyltransferase. The K_m value calculated for 2'-methyl LacNAc in the presence of 0.3 mM 3'-sulfo LacNAc

TABLE I
Proposed ^{13}C nmr and m/z

All compounds gave satisfactory elemental analysis.

Residue	Compound	C-1	C-2	C-3	C-4	C-5	C-6	NAc	m/z
6-O-SO ₃ Na- α -D-GlcNAc	6	93.47	56.41	72.11	80.76	73.85	69.36	24.76	485.9 [M + 1] ⁺
6-O-SO ₃ Na- β -D-GlcNAc		97.78	58.98	75.18	80.45	75.48	69.30	25.04	507.9 [M + Na] ⁺
β -D-Gal(1→4)		105.41	71.47	75.36	71.14	78.17	63.86		462.2 [M - Na] ⁻
α -D-GlcNAc	9	93.48	56.66	72.06	81.48	72.20	62.97	24.87	507.9 [M + Na] ⁺
β -D-GlcNAc		97.81	59.20	75.36	81.87	77.84	63.09	25.14	462.1 [M - Na] ⁻
3-O-SO ₃ Na β -D-Gal1 → 4 β		105.49	71.31	82.98	69.81	77.74	63.82		
3-O-SO ₃ Na β -D-Gal1 → 4 α	17	105.45							
α -D-GlcNAc		93.92	56.94	74.03	77.64	72.05	62.48	24.85	654.2 [M + Na] ⁺
β -D-GlcNAc		97.53	59.77	77.41	78.16	75.51	62.57	25.10	608.3 [M - Na] ⁻
α -L-Fuc(1→3)	17	101.37	70.52	71.95	74.74	69.43	18.09		
3-O-SO ₃ Na- β -D-Gal(1→4)		104.30	72.11	83.02	69.52	76.27	64.15		
α -GlcNAc		93.88	56.89	74.01	77.78	72.05	62.53	24.81	
β -GlcNAc	18	97.52	59.77	77.72	78.26	75.64	62.62	25.06	530.1 [M + 1] ⁺
α -L-Fuc(1→3)		101.39	70.54	71.16	74.73	69.47	18.09		552.0 [M + Na] ⁺
β -D-Gal(1→4)		104.63	72.10	75.29	70.52	76.15	64.30		

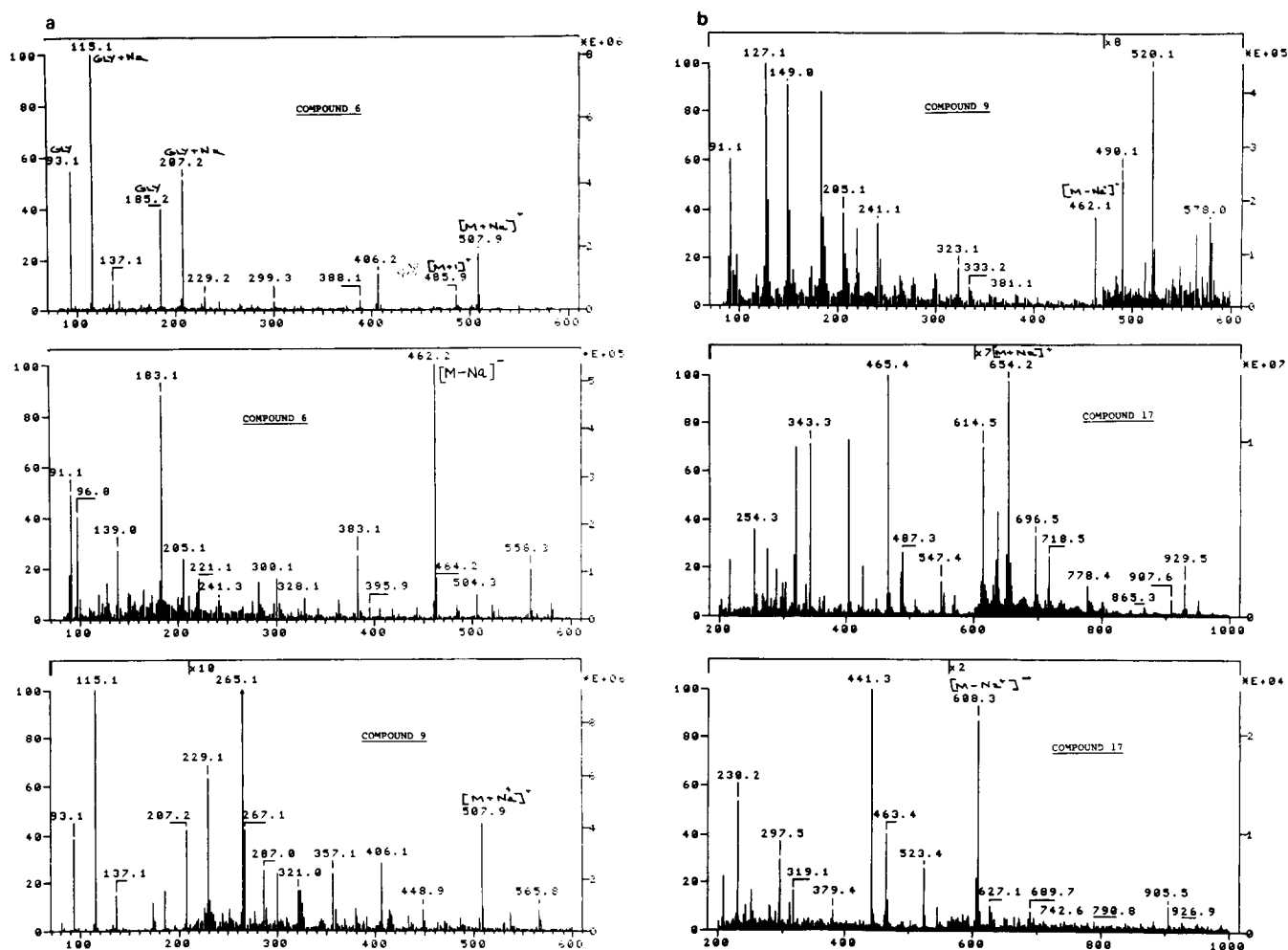


FIG. 1. *a* and *b*, fast atom bombardment ionization spectra of compounds 6, 9, and 17.

(see Table III) was 6.67 mM and for 3'-sulfo LacNAc in the presence of 3.0 mM 2'-methyl LacNAc (Table III) was 0.12 mM. These values indicate that 3'-sulfo LacNAc is a very high affinity acceptor for $\alpha 1,3$ -fucosyltransferase. The ^{14}C -fucosylated product arising from 3'-sulfo LacNAc has been tentatively identified as 3'-sulfo, 3-fucosyl LacNAc by comparing strictly its mobility on paper with the synthetic authentic compound 3'-sulfo, 3-fucosyl LacNAc (see Fig. 5).

Ovarian Cancer Serum $\alpha 1,3$ -Fucosyltransferase Activity with Various Synthetic Acceptors (See Table V)—The activity of serum $\alpha 1,3$ -fucosyltransferase was measured with the specific substrate 2'-methyl LacNAc and compared with the ability of several other synthetic substrates to act as an acceptor of fucose. LacNAc and Gal $\beta 1,3$ GlcNAc were, respectively, 93.4 and 13.1% active, suggesting that $\alpha 1,3$ -fucosyltransferase is the dominant fucosyltransferase (>90%) in the ovarian cancer serum of the present investigation. This finding was further substantiated by the data that the β -benzyl glycosides of 2'-methyl LacNAc and LacNAc were equally active (307.9 and 358.8%, respectively), whereas the same glycoside of Gal $\beta 1,3$ GlcNAc showed only 21.7% activity. The transfer of fucose to C-3 of GlcNAc was evident from the results that 2'-fucosyl LacNAc acted as a very good substrate (253% active), whereas 3-fucosyl LacNAc was almost inactive (only 4.7% active). Similar to the observation on the β -benzyl glycosides of LacNAc and 2'-methyl LacNAc, the β -benzyl glycoside of 2'-fucosyl LacNAc was a more effective substrate

(425.0%) than the parent compound (253.1%).

When the structure of the acceptor 2'-methyl LacNAc was modified by replacing the methyl group with sulfate group in C-2', -3', -6', or -6 position and tested their ability to act as the acceptor of fucose, the following novel findings emerged. 3'-Sulfo LacNAc was the most active acceptor (340.8%) as compared with other sulfated derivatives and the nonsulfated acceptors such as 2'-methyl LacNAc and 2'-fucosyl LacNAc. Among the other sulfated derivatives, 6-sulfo LacNAc was considerably active (172.4%) and 2'- and 6'-sulfo compounds were less active (22.0 and 36.2%, respectively). The β -benzyl glycoside of 3'-sulfo LacNAc was quite active (168.4%) but less than the parent compound. It is thus evident from the present study that β -benzylation causes an increase of 2–3-fold in the activity of nonsulfated LacNAc derivatives, but a decrease of 50% in the activity of sulfated LacNAc. 3'-Sialyl LacNAc was a very good acceptor (242% activity). The H-type 1 2-fucosyl Gal $\beta 1,3$ GlcNAc β -O-pNP,¹ 2-methyl Gal $\beta 1,3$ GlcNAc β -O-Bn, and 3-sulfo Gal $\beta 1,3$ GlcNAc β -O-Bn did not show any activity, thus implying the absence of $\alpha 1,4$ -fucosyltransferase activity in this serum.

Normal Serum α -L-Fucosyltransferase Activity as Measured with Synthetic Acceptors (See Table V)—As compared with 2'-methyl LacNAc, both LacNAc and Gal $\beta 1,3$ GlcNAc were, respectively, 432.9 and 410.7% active with normal sera; 3'-sialyl LacNAc was only 6.2% active, whereas 2'-fucosyl

¹ The abbreviations used are: pNP, *p*-nitrophenyl; Bn, benzyl.

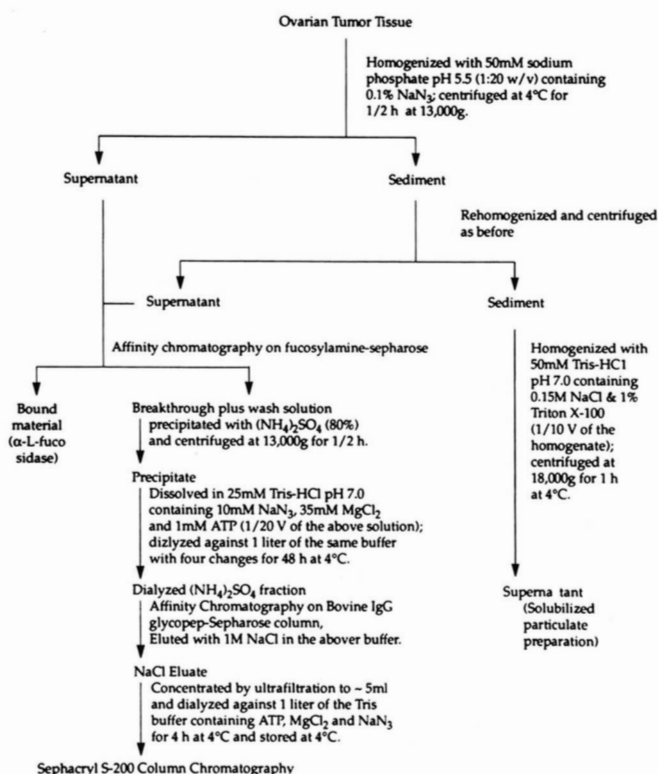


FIG. 2. The scheme for the preparation of the particulate and soluble fractions of human ovarian tumor.

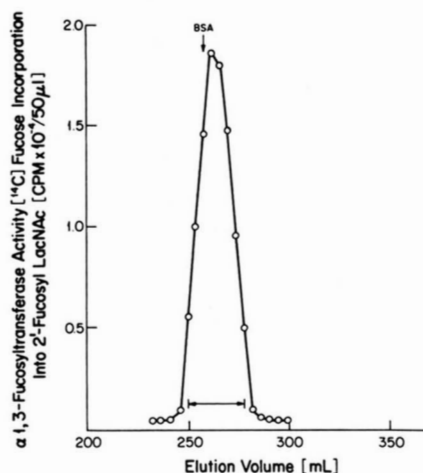


FIG. 3. Purification of α 1,3-L-fucosyltransferase on Sephacryl S-200 column. Ovarian tumor α 1,3-L-fucosyltransferase preparation, which was obtained from affinity chromatography on bovine IgG glycopep-Sephacryl (sample volume, 2.0 ml) was applied to Sephacryl S-200 column (2.6 \times 88.0 cm) equilibrated in the cold room with 50 mM Tris-HCl, pH 7.0, containing 0.15 M NaCl, 0.1% Triton X-100. Fractions of 3.0 ml were collected at a flow rate of 9.0 ml/h. The fucosyltransferase was assayed with the acceptor 2'-fucosyl LacNAc.

LacNAc and 3'-sulfo LacNAc were active 116.5 and 155.1%, respectively. 6'-Sulfo LacNAc exhibited high activity (433.7%), and 3-fucosyl LacNAc was also active (40.7%). 2-Methyl Gal β 1,3GlcNAc- β -O-Bn was inactive (1.6% activity only). The data indicate that α 1,2-fucosyltransferase is the major and α 1,3-fucosyltransferase is the minor fucosyltransferase in normal serum.

Ovarian Tumor Soluble Fraction α 1,3-Fucosyltransferase (See Table V)—When α 1,3-fucosyltransferase activity of the partially purified enzyme preparation from the soluble portion

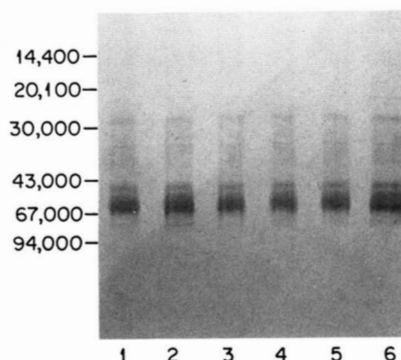


FIG. 4. SDS-polyacrylamide gel electrophoresis (Phast Gel 8-25, Pharmacia System) of α 1,3-L-fucosyltransferase purified from the soluble fraction of human ovarian tumor. Lanes 1-5, 1 μ g of the enzyme protein was applied; lane 6, 2 μ g of the enzyme protein was applied. The gel was stained with Coomassie Blue.

TABLE II
Partial purification of α 1,3-L-fucosyltransferase from the soluble fraction of human ovarian tumor

Fraction	α 1,3-L-Fucosyltransferase activity [¹⁴ C]fucose incorporated into 2'-fucosyl N-acetylactosamine			
	Activity/mg protein	Total activity	Purification	Recovery
	cpm $\times 10^{-5}$	cpm $\times 10^{-6}$	-fold	%
Ovarian tumor-soluble fraction depleted of α -L-fucosidase	0.500	40.00	1.0	100.0
Precipitation with 80% ammonium sulfate	0.719	35.95	1.4	89.9
Affinity chromatography on bovine IgG glycopep-Sephacryl column	7.782	15.56	15.6	38.9
Chromatography on Sephacryl S-200 column	33.100	2.46	66.2	6.2

of ovarian tumor was measured and compared with the activity of other acceptors, the following results were obtained. 2'-Methyl LacNAc, LacNAc, and Gal β 1,3GlcNAc were, respectively, 100.0, 60.6, and 565.5% active, indicating the presence of both α 1,3- and α 1,4-fucosyltransferase activities in this preparation. As anticipated, the β -benzyl glycosides of the above acceptors were more active than the corresponding parent compound; in particular, the β -benzyl glycoside of Gal β 1,3GlcNAc was about twice as active as Gal β 1,3GlcNAc (1004.5 and 565.5%, respectively). In contrast to the serum enzyme, which showed over 2-fold increase in activity with β -benzyl glycoside of 2'-methyl LacNAc (307.9% active), only a small increase was seen with the enzyme of the soluble tumor fraction (113.2%); 2'-fucosyl LacNAc and its β -benzyl glycoside were 436.1 and 373.0% active, respectively, whereas 3-fucosyl LacNAc was almost inactive (only 1.9% active), thus indicating the transfer of Fuc to C-3 of GlcNAc. 3'-Sialyl LacNAc was a poor acceptor (12.4% active).

Among the sulfated derivatives of LacNAc, 3'-sulfo LacNAc was the most active acceptor (447.1%) followed by 6-sulfo LacNAc (272.6%) and 2'-sulfo LacNAc (32.7%). 6'-Sulfo LacNAc was the least active acceptor (5.3%). Both 3'-sulfo and 6-sulfo LacNAc showed more activity with the tumor enzyme as compared with the serum enzyme (447.1 and 272.6% compared with 340.8 and 172.4%, respectively). As observed with the serum enzyme, the β -benzyl glycoside

TABLE III

The acceptor ability of 3'-sulfo LacNAc in the presence of the competitive substrate 2'-methyl LacNAc and vice versa with ovarian cancer sera as the source of $\alpha 1,3$ -L-fucosyltransferase

Concentration of the competitive substrate	Incorporation of [14 C]fucose	
	3'-Sulfo LacNAc (0.3 mM) ^a	2'-Methyl LacNAc
	cpm	
2'-Methyl LacNAc		
0	2520	157
1.5	2132	1143
3.0	1872	1614
4.5	1748	1973
6.0	1712	2698
7.5	1428	2788
	2'-Methyl LacNAc (0.3 mM)	3'-Sulfo LacNAc
3'-Sulfo LacNAc		
0	3664	140
0.15	2581	1294
0.30	2191	2181
0.45	1737	2417
0.65	1184	2373
0.75	1187	2560

^a There was no inhibition by 2'-methyl LacNAc when 3'-sulfo LacNAc was present at 3.0 mM concentration. The K_m value calculated for 2'-methyl LacNAc in the presence of 0.3 mM 3'-sulfo LacNAc was 6.67 mM and that for 3'-sulfo LacNAc in the presence of 3.0 mM 2'-methyl LacNAc was 0.12 mM.

TABLE IV

The utility of 3'-sulfo LacNAc as a highly sensitive acceptor for the detection of serum $\alpha 1,3$ -L-fucosyltransferase

The values in parentheses are the percentage of the ratio between the counts/min obtained for cancer and normal serum.

	Incorporation of [14 C]fucose into the acceptor (0.30 mM)		
	2'-Methyl LacNAc, A	3'-Sulfo LacNAc, B	Ratio B/A
	cpm		
Ovarian cancer sera			
1	1325 (334)	7310 (474)	5.52
2	1724 (434)	9816 (636)	6.69
3	1359 (342)	5261 (341)	3.87
4	1347 (339)	5480 (355)	4.06
5	699 (176)	3088 (200)	4.42
6	975 (246)	5483 (355)	5.62
Normal serum	397	1543	3.89

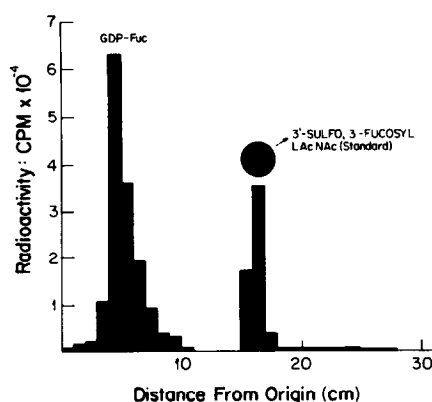


FIG. 5. Paper chromatography of the 14 C-fucosylated product arising from the incubation of 3'-sulfo LacNAc with serum $\alpha 1,3$ -L-fucosyltransferase. For details, see text.

of 3'-sulfo LacNAc was less active than the parent compound toward the soluble tumor enzyme. The H-type 1 2-fucosyl Gal $\beta 1,3$ GlcNAc β -O-pNP, 2-methyl Gal $\beta 1,3$ GlcNAc β -O-Bn, and 3-sulfo Gal $\beta 1,3$ GlcNAc β -O-Bn were highly active (166.3, 203.8, and 415.4%, respectively) as expected, since this preparation contained $\alpha 1,4$ -fucosyltransferase activity, as noted earlier when the acceptors Gal $\beta 1,3$ GlcNAc and its β -benzyl glycoside were used.

$\alpha 1,3$ -L-Fucosyltransferase Associated with the Particulate Fraction of the Ovarian Tumor (See Table V)—The particulate enzyme was less active with LacNAc (61.8%) and Gal $\beta 1,3$ GlcNAc (34.2%), indicating the major fucosyltransferase as $\alpha 1,3$ (>60%) in this fraction. This finding is quite different from what we found with the soluble fraction, where $\alpha 1,4$ -fucosyltransferase activity seems to be greater than $\alpha 1,3$ -fucosyltransferase activity. In contrast to the enzyme of the soluble fraction, which showed 436.1% activity with 2'-fucosyl LacNAc, the particulate enzyme was only 144.7% active, thus illustrating the difference in the catalytic ability of the above enzymes. The different nature of the above enzymes was made further clear, when sulfated derivatives of LacNAc were tested as acceptors. When 3'-sulfo LacNAc was found to be a highly active acceptor with the enzymes of the soluble fraction (447.1%), as well as the serum (340.8%), the particulate enzyme showed only 39% activity. As with other enzyme sources, the β -benzyl glycoside of 3'-sulfo LacNAc was less active (33.3%) than the parent compound with the particulate enzyme. Even the other sulfated derivatives exhibited differences in their affinity toward these enzymes from the tumor. When 3'-sulfo LacNAc was the most active sulfated acceptor with the enzyme of soluble fraction, 6'-sulfo and 6-sulfo LacNAc were more active (42.6 and 53.3%) than 3'-sulfo LacNAc (39.0%), and 2'-sulfo LacNAc was the least active (9.8%) with the particulate enzyme. The particulate enzyme showed very low activity (2.9%) with 3-sulfo Gal $\beta 1,3$ GlcNAc β -O-Bn and inactivity with 2-fucosyl Gal $\beta 1,3$ GlcNAc β -O-pNP and 2-methyl Gal $\beta 1,3$ GlcNAc β -O-Bn, in contrast to the soluble fraction, indicating that $\alpha 1,4$ -fucosyltransferase activity was almost absent in the particulate extract.

3'-Sulfo LacNAc, an Indicator of the Elevation in the Level of Serum $\alpha 1,3$ -Fucosyltransferase in Ovarian Cancer—Several ovarian cancer sera along with normal sera were measured for $\alpha 1,3$ -fucosyltransferase activity with 3'-sulfo LacNAc as the acceptor. In the first batch of screening (Table VI), all the seven patients showed increased activity in the range 154–360%, as compared with the normal. In batch 2, all four patients had activity in the range 204–408% with respect to the normal. Batch 3, consisting of 10 patients showed 117–672% activity.

Comparison of $\alpha 1,3$ -L-Fucosyltransferase Activities Present in Human Normal Ovarian Tissue and Ovarian Tumor Using Synthetic Acceptors—The same fixed amount of protein (160 μ g) was used from both extracts in the assays in order to make a strict comparison, and the values were then converted to activity per mg of protein. The tumor exhibited more $\alpha 1,3$ -L-fucosyltransferase activity than the normal tissue (activity per mg) as measured with various acceptors. Normal versus tumor: 2'-methyl LacNAc, 3.63 versus 5.16; 2'-methyl LacNAc β -O-Bn, 7.26 versus 10.75; 2'-fucosyl LacNAc, 9.33 versus 14.89; 2'-fucosyl LacNAc β -O-Bn, 12.86 versus 20.09; 3'-sulfo LacNAc, 1.85 versus 5.86; 3'-sulfo LacNAc β -O-Bn, 3.27 versus 5.04. The $\alpha 1,4$ -fucosyltransferase-specific acceptors 2-methylGal $\beta 1,3$ GlcNAc β -O-Bn, 3-sulfoGal $\beta 1,3$ GlcNAc β -O-Bn, Gal $\beta 1,3$ GlcNAc, and Gal $\beta 1,3$ GlcNAc β -O-Bn showed severalfold increase in activity with tumor tissue as

TABLE V

Differentiation of the distinct specificities of ovarian cancer $\alpha 1,3$ -L-fucosyltransferases present in the particulate and soluble fractions by using synthetic substrates

The values in parentheses are the actual amount (counts/min) of [14 C]Fuc incorporated into 2'-methyl LacNAc. ND, not determined.

Carbohydrate acceptor	Relative activity		Source of $\alpha 1,3$ -L-fucosyltransferase, ovarian tumor	
	Normal sera	Ovarian cancer sera	Soluble fraction, relative activity	Particulate fraction, relative activity
		%	%	%
2'-Methyl LacNAc	100 (385)	100 (1730)	100 (7392)	100 (48,252)
2'-Methyl LacNAc β -O-Bn	61.7	307.9	113.2	149.2
LacNAc	432.9	93.4	60.6	61.8
LacNAc β -O-Bn	428.4	358.8	111.6	132.9
LacNAc $\beta 1,3$ Gal β -O-PNP	309.9	166.8	87.0	70.0
3'-Sialyl LacNAc	6.2	241.6	12.4	3.4
2'-Fucosyl LacNAc	116.5	253.1	436.1	144.7
2'-Fucosyl LacNAc β -O-Bn	274.1	425.0	373.0	174.0
3'-Sulfo LacNAc	155.1	340.8	447.1	39.0
3'-Sulfo LacNAc β -O-Bn	89.3	168.4	183.8	33.3
3-SulfoGal $\beta 1,3$ GlcNAc β -O-Bn	0	0	415.4	2.9
2'-Sulfo LacNAc	95.5	22.0	32.7	9.8
6'-Sulfo LacNAc	433.7	36.2	5.3	42.6
6-Sulfo LacNAc	164.6	172.4	272.6	53.3
3-Fucosyl LacNAc	40.7	4.7	1.9	3.7
Gal $\beta 1,3$ GlcNAc	410.7	13.1	565.5	34.2
Gal $\beta 1,3$ GlcNAc β -O-Bn	503.7	21.7	1004.5	37.8
Gal $\beta 1,3$ GlcNAc $\beta 1,3$ Gal β -O-Me	758.4	42.7	ND	42.6
2-Methyl Gal $\beta 1,3$ GlcNAc β -O-Bn	1.6	0	203.8	0
2-Fucosyl Gal $\beta 1,3$ GlcNAc β -O-PNP	ND	0	166.3	0
GlcNAc $\beta 1,6$ GalNAc α -O-pNP	ND	9.5	0	ND

TABLE VI

Elevation of serum $\alpha 1,3$ -L-fucosyltransferase activity in ovarian cancer patients as measured with 3'-sulfo-LacNAc as an acceptor

Sera	$\alpha 1,3$ -Fucosyltransferase activity	
	Incorporation of [14 C]fucose	As Percent of normal
	cpm	
Batch 1		
Patients:		
L.O.	3,182	302
J.I.	1,620	154
L.K.	3,789	360
L.M.	2,102	199
L.S.	2,571	244
A.S.	3,295	313
E.D.	3,083	293
Normal		
R.M.	1,054	100
Batch 2		
Patients:		
41	1,782	250
287	1,453	204
9	2,900	408
258	1,642	231
Normal		
157	710	100
Batch 3		
Patients:		
298	4,618	224
247	2,456	117
74	2,514	120
15	10,382	496
235	9,973	477
72	5,265	252
A.R.	5,894	282
B.B.	6,018	288
S.C.	14,070	672
M.H.	3,268	156
Normal		
159	2,093	100

compared with the normal tissue. Normal *versus* tumor: 2-methylGal $\beta 1,3$ GlcNAc β -O-Bn, 0.91 *versus* 19.68; 3-sulfoGal $\beta 1,3$ GlcNAc β -O-Bn, 0 *versus* 8.09; Gal $\beta 1,3$ GlcNAc, 0.91 *versus* 8.40; Gal $\beta 1,3$ GlcNAc β -O-Bn, 1.23 *versus* 19.71. The activities of $\alpha 1,3$ - and $\alpha 1,4$ -fucosyltransferases as measured, respectively, by the specific acceptors 2'-methyl LacNAc β -O-Bn and 2-methyl Gal $\beta 1,3$ GlcNAc β -O-Bn were 7.26 and 0.91/mg in normal tissue and 10.75 and 19.68/mg in tumor; when expressed as percent of the activity for the acceptor 2'-methyl LacNAc, they were 200.0 and 2.5 in normal and 208.5 and 381.7 in tumor.

Expression of α -L-Fucosyltransferases in Human Normal Ovarian Tissue and Ovarian Tumors—We examined one normal and four tumors for the level of $\alpha 1,2$ -, $\alpha 1,3$ -, and $\alpha 1,4$ -fucosyltransferases using specific acceptors in order to ascertain the above (Table VII) finding on the elevation of $\alpha 1,4$ -fucosyltransferase activity in ovarian tumor. Table VIII presents the results of this experiment. Except for one tumor (No. 2509), the others showed 1.37–3.36-fold (3'-sulfo LacNAc as acceptor) and 1.38–1.65-fold (2'-methyl LacNAc β -O-Bn as acceptor) $\alpha 1,3$ -L-fucosyltransferase activity, as compared with the normal. On the other hand, all four tumors exhibited high elevation of $\alpha 1,4$ -fucosyltransferase activity which ranged 3.06–140.60-fold. Both normal and tumor tissues had low $\alpha 1,2$ -fucosyltransferase activity.

DISCUSSION

The present investigation has led to the discovery of 3'-sulfo LacNAc as a unique acceptor for $\alpha 1,3$ -L-fucosyltransferase for the following reasons: 3'-sulfo LacNAc behaves like H-type 2 (2'-fucosyl LacNAc) and 3'-sialyl LacNAc in its ability to serve as an acceptor for ovarian cancer serum $\alpha 1,3$ -fucosyltransferase (activity 341, 253, and 242%, respectively). While H-type 2 was more active than 2'-methyl LacNAc toward the tumor particulate enzyme (145% active), 3'-sulfo LacNAc exhibited considerably low activity (39%). Both the particulate and soluble enzymes of the tumor did not show

TABLE VII

A comparison of α 1,3-L-fucosyltransferase activities present in human normal ovarian tissue and ovarian tumor using synthetic acceptors. The values in parentheses are the ratio of the activities between tumor and normal for each acceptor.

Carbohydrate acceptor	Normal ovarian tissue extract		Ovarian tumor extract	
	Incorporation of [14 C]Fuc catalyzed by 1 mg protein	Relative activity	Incorporation of [14 C]Fuc catalyzed by 1 mg protein	Relative activity
	$\text{cpm} \times 10^{-4}$	%	$\text{cpm} \times 10^{-4}$	%
2'-Methyl LacNAc	3.63	100	5.16 (1.42)	100
2'-Methyl LacNAc β -O-Bn	7.26	200.0	10.75 (1.48)	208.5
LacNAc	3.04	83.8	3.23 (1.06)	62.7
LacNAc β -O-Bn	6.96	191.8	9.91 (1.42)	192.1
2'-Fucosyl LacNAc	9.33	256.9	14.89 (1.60)	288.7
2'-Fucosyl LacNAc β -O-Bn	12.86	354.2	20.09 (1.56)	389.7
3'-Sulfo LacNAc	1.85	51.1	5.86 (3.17)	113.6
3'-Sulfo LacNAc β -O-Bn	3.27	90.1	5.04 (1.54)	97.8
2'-Sulfo LacNAc	0.54	15.0	1.43 (2.65)	27.8
6'-Sulfo LacNAc	1.04	28.6	0.73 (0.70)	14.2
6-Sulfo LacNAc	3.26	89.7	4.64 (1.42)	90.0
Gal β 1,3GlcNAc	0.91	25.0	8.40 (9.23)	163.0
Gal β 1,3GlcNAc β -O-Bn	1.23	33.9	19.71 (16.02)	382.3
2-Methyl Gal β 1,3GlcNAc β -O-Bn	0.91	2.5	19.68 (21.63)	381.7
3-Sulfo Gal β 1,3GlcNAc β -O-Bn	0	0	8.09	156.9

TABLE VIII

Expression of α -L-fucosyltransferases in normal ovarian tissue and ovarian tumors

The values in parentheses are the -fold of each α -L-fucosyltransferase activity in tumors when compared to that present in normal tissue.

Ovarian tissue	Incorporation of [14 C]Fuc catalyzed by 1 mg protein			
	α 1,2-FT, acceptor: Gal β 1,3(Fuc α 1,4), GlcNAc β -O-Bn	α 1,3-FT		α 1,4-FT, acceptor: 2-methylGal β 1,3-GlcNAc β -O-Bn
		Acceptors: 2'-methyl LacNAc, β -O-Bn	3'-Sulfo LacNAc	
		$\text{cpm} \times 10^{-4}$		
Normal				
No. 2162	0.35	9.96	2.03	0.14
Tumors				
No. 2509	0.37 (1.06)	5.17 (0.52)	0.97 (0.48)	0.44 (3.06)
JM	0.97 (2.78)	16.48 (1.65)	4.52 (2.23)	7.88 (55.07)
LM	0.42 (1.21)	13.77 (1.38)	2.78 (1.37)	0.66 (4.59)
KK	0.53 (1.52)	14.55 (1.46)	6.81 (3.36)	20.12 (140.60)

any activity with 3'-sialyl LacNAc. On the other hand, 3'-sulfo LacNAc was highly active with the enzyme of the soluble fraction (447%) as compared with the particulate enzyme (39%). The results thus demonstrate that 3'-sulfo LacNAc as compared with H-type 2 and 3'-sialyl LacNAc has the unique ability of differentiating the various catalytic species of α 1,3-L-fucosyltransferase.

The present study indicates that 3'-sulfo LacNAc can distinguish the α 1,3-L-fucosyltransferase of the ovarian tumor particulate fraction from that of the soluble fraction, in that these enzymes show extreme difference of activities toward this substrate in comparison with 2'-methyl LacNAc. 3'-Sulfo LacNAc was 447 and 39% active, respectively, with soluble and particulate fractions of the ovarian tumor; in this respect the serum enzyme resembled the soluble tumor fraction (340% active with 3'-sulfo-LacNAc). Recently, α 1,3-L-fucosyltransferase from human neuroblastoma cells was shown to be more active with Fuc α 1 \rightarrow 2Gal β 1 \rightarrow 4GlcNAc/Glc in comparison with Gal β 1 \rightarrow 4GlcNAc/Glc but was inactive with NeuAc2 \rightarrow 3- and NeuAc2 \rightarrow 6Gal-linked compounds. The present study shows that the synthetic H-type 2 acceptor (2'-fucosyl LacNAc), as well as 3'-sialyl LacNAc, react differently with our α -L-fucosyltransferases (particulate, 144 and 3%; soluble, 436 and 12%; serum, 253 and 242%, respectively). For human serum enzyme, our results are consistent with the report of

Johnson and Watkins (16) that α -3-L-fucosyltransferase purified from human serum was 162 and 147% active, respectively, toward 2'-fucosyl LacNAc and 3'-sialyl LacNAc as compared with the parent LacNAc. In fact, NeuAc α 2 \rightarrow 3Gal-linked oligosaccharides have been frequently used for differentiating α -L-fucosyltransferase from different sources (25). In sialylated nLc₆ NeuAc α 2 \rightarrow 3Gal β 1 \rightarrow 4GlcNAc β 1 \rightarrow 3Gal β 1 \rightarrow 4GlcNAc β 1 \rightarrow 3Gal β 1 \rightarrow 4Glc-Cer, α 1,3-fucosyltransferase of one mutant of hamster transfers fucose to the internal GlcNAc of both nLc₆ and NeuAc α 2 \rightarrow 3nLc₆ (25). But α 1,3-L-fucosyltransferase from the other mutant, which acts only on the external GlcNAc in nLc₆, could not fucosylate NeuAc nLc₆ (25). Mollicone *et al.* (26) reported the existence of at least three distinct types of α 1,3-L-fucosyltransferase in human tissues, the myeloid enzyme acting on H-type 2 acceptor and poorly on sialylated LacNAc, the plasma and hepatocyte enzyme acting, in addition, on sialylated LacNAc, and the Lewis enzyme (gallbladder, kidney, and milk) acting, in addition, on H-type 1 acceptor. The particulate enzyme of the present study acts on H-type 2 acceptor but poorly on 3'-sulfo LacNAc and 3'-sialyl LacNAc, indicating that this enzyme belongs to the category of myeloid enzyme. On the other hand, the soluble tumor enzyme acts on H-type 2, 3'-sulfo LacNAc, as well as H-type 1, resembling the Lewis enzyme. The serum enzyme of the present study behaves like the

plasma enzyme reported by Mollicone *et al.* (26); it acts equally well on H-type 2 3'-sulfo LacNAc and 3'-sialyl, but not on H-type 1.

According to Holmes and Levery (14), α 1,3-L-fucosyltransferase of Colo 205 cells behaves like human milk α 1,3/4-fucosyltransferase acting on both type 1 and type 2, including NeuAc α 2 \rightarrow 3Gal-containing structures. It has also been known that 2'-L-fucosyl type 1 and type 2 can act as acceptors for soluble human milk α 1,3/4-fucosyltransferases. Interestingly, our synthetic 3'-sulfo LacNAc type 2 and 3'-sulfo lacto-*N*-biose type I structures and H-type 1 2-fucosyl Gal β 1,3GlcNAc β -O-pNP and 2-methyl Gal β 1,3GlcNAc β -O-Bn acted as acceptors for the ovarian tumor-soluble enzyme.

Several noteworthy findings emerged when we used other sulfated disaccharide derivatives of Gal β 1 \rightarrow 4GlcNAc. Our synthetic 6'-sulfo LacNAc was a very poor acceptor for α 1,3-fucosyltransferase as compared with 2'-methyl LacNAc. As far as we know, NeuAc α 2 \rightarrow 6Gal β 1 \rightarrow 4GlcNAc does not act as acceptor for α 1,3-L-fucosyltransferase. In fact, a reciprocal relation has been strongly suggested between 2,6-sialyltransferase and α 1,3-L-fucosyltransferase (17, 27). On the other hand, sialylation followed by fucosylation is a well recognized biosynthetic pathway for the assembly of NeuAc α 2 \rightarrow 3Gal β 1 \rightarrow 4(Fuc α 1 \rightarrow 3)GlcNAc and NeuAc α 2 \rightarrow 3Gal β 1 \rightarrow 3(Fuc α 1 \rightarrow 4)GlcNAc type glycoconjugates. In this context it is interesting to note that 3'-sialyl LacNAc was highly active with the serum enzyme (242% activity) and almost inactive with the soluble (12%) and particulate (3%) fractions of tumor. It has been found that LacNAc can act as an effective acceptor for 3-O-sulfotransferase of calf thyroid microsomes to give 3'-sulfo-LacNAc (28). Based upon our present study of showing 3'-sulfo LacNAc as an efficient acceptor for α 1,3-L-fucosyltransferase, it is likely that glycoproteins in some tissues may have the carbohydrate sequence, 3-O-sulfo Gal β 1 \rightarrow 4(Fuc α 1 \rightarrow 3)GlcNAc.

Aryl- β -D-galactosides have been frequently used as an acceptor for α 1,2-L-fucosyltransferase. We found that our synthetic *p*-nitrophenyl-3-O-sulfo- β -D-galactoside did not accept any fucose when incubated with GDP-fucose and human serum containing α 1,2-L-fucosyltransferase. To the best of our knowledge, no one has reported NeuAc α 2 \rightarrow 3Gal β -linked compounds as acceptors for α 1,2-L-fucosyltransferase, and there is no carbohydrate structure containing NeuAc α 2,3(Fuc α 1,2)Gal β , where both sialyl and fucosyl residues are linked to the same terminal galactose. It is also noteworthy that our 2'-sulfo LacNAc showed a very poor activity for α 1,3-L-fucosyltransferase, and we are not aware of any carbohydrate structures containing the NeuAc α 2 \rightarrow 2Gal β 1 \rightarrow 4GlcNAc β sequence. On the other hand, we found that 6-O-sulfo LacNAc, in which sulfate is at C-6 position of GlcNAc, acts as an acceptor for soluble α 1,3-L-fucosyltransferase. As discussed earlier, these soluble α -L-fucosyltransferases can also act on type 1 chain structures; thus, we think that 6-O-sulfo-lacto-*N*-biose Gal β 1 \rightarrow 3(6-sulfo)GlcNAc will act as acceptor. It may be pointed out that the Gal β 1 \rightarrow 3(NeuAc α 2 \rightarrow 6)(Fuc α 1 \rightarrow 4)GlcNAc sequence has been reported as an inner core structure of certain glycolipids (29).

It is really striking to find that our synthetic sulfate analogs corresponding to existing sialylated type linkage type structures showed similar activity with our α -L-fucosyltransferase. Although the present study is limited, it provides adequate and valuable information on the synthetic sulfated analogs for the study of fucosyltransferases.

The present study showed that all ovarian cancer patients had an elevated level of serum α 1,3-fucosyltransferase, when assayed with 3'-sulfo LacNAc; this finding illustrates that this acceptor has the potential for use in the clinical laboratory involved in cancer diagnosis.

The most noteworthy finding of the present investigation, which was possible by the use of various synthetic analogs of LacNAc, is that apart from the moderate increase in the expression of α 1,3-fucosyltransferase activity, the human ovarian tumors are endowed with an explosive expression of α 1,4-fucosyltransferase.

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