The Acceptor and Site Specificity of α3-Fucosyltransferase V

HIGH REACTIVITY OF THE PROXIMAL AND LOW OF THE DISTAL Galβ1–4GlcNAc UNIT IN i-TYPE POLYLACTOSAMINES

We report here on in vitro acceptor and site specificity of recombinant α3-fucosyltransferase V (Fuc-TV) with 40 oligosaccharide acceptors. Galβ1–4GlcNAc (LN) and GalNAcβ1–4GlcNAc (LDN) reacted rapidly; Galβ1–3GlcNAc (LNB) reacted moderately, and GlcNAcβ1–4GlcNAc (Nα′,N′-diacetyl-chitobiose) reacted slowly yet distinctly. In neutral and terminally α3-sialylated polylactosamines of i-type, the reducing end LN unit reacted rapidly and the distal (sialyl)LN group very slowly; the midchain LNs revealed intermediate reactivities. The data suggest that a distal LN neighbor enhances but a proximal LN neighbor reduces the reactivity of the midchain LNs. This implies that Fuc-TV may bind preferably the tetrasaccharide sequence Galβ1–4GlcNAcβ1–3Galβ1–4GlcNAc for transfer at the underlined monosaccharide. Terminal α3-sialylation of i-type polylactosamines almost doubled the reactivities of the LN units at all positions of the chains. We conclude that, in comparison with human Fuc-TIV and Fuc-TIX, Fuc-TV reacted with a highly distinct site specificity with i-type polylactosamines. The Fuc-TV reactivity of free LNB resembled that of LNβ1–3′R of a polylactosamine, contrasting strongly with the dissimilarity of the reactivities of the analogous pair of LN and LNβ1–3′R. This observation supports the notion that LN and LNB may be functionally bound at distinct sites on Fuc-TIV surface. Our data show that Fuc-TV worked well with a very wide range of LN-glycans, showing weak reactivity only with distal (sialyl)LN units of i-type polylactosamines, biantenary N-glycans, and 1 branches of polylactosamines.

Six human α3-fucosyltransferases (Fuc-Ts)1 have been cloned (1–10), and all of them convert N-acetyllactosamine units (LN) of glycoconjugates into Lewis x (Lex) determinants, Galβ1–4(Fucα1–3)GlcNAc. Sialylated and/or sulfated versions of this trisaccharide are believed to decorate t-selectin ligands, which initiate extravasation of lymphocytes by mediating their tethering and rolling on endothelium during their recruitment into lymph nodes and into sites of inflammation (11, 12). Other leukocytes extravasate with mechanisms similar to those of lymphocytes. In some cases, also bacterial adhesion to target cells at the onset of infections (13), as well as metastasis of malignant tumor cells via blood circulation (14, 15), are known to be mediated by interactions between this family of saccharides and appropriate lectin proteins. Homotypic Lex-Lex binding has also been observed and may play a role in cell adhesion, e.g. in embryonal compaction (16–20).

Emerging differences in substrate specificities of the human Fuc-Ts (10, 21–23) may help to define the biological roles of the individual enzymes. The substrate specificities of these enzymes are of interest also for enzyme-assisted in vitro synthesis of different types of Lex-containing glycans and glycoconjugates, many of which are capable of inhibiting important selection-mediated cell adhesion processes. Among this group of enzymes, Fuc-TV has probably the broadest acceptor specificity. It transfers at significant rates to both type 1 and 2 acceptors, Galβ1–3GlcNAc (LNB) and LN, respectively, and works well also with α2,3-sialylated and α1,2-fucosylated forms of these disaccharides; in addition, it transfers to 3′-sulfo- and 6-sulfo-LN, to lactose as well as to α2′-fucosylated lactose (5, 24–26). This exceptional versatility of Fuc-TV as a synthesis catalyst prompted us to study its reactions with a large number of polylactosamines and other acceptor saccharides. Here, we report on the relative Fuc-TV reactivities of these glycans and their individual acceptor sites.

EXPERIMENTAL PROCEDURES

The Oligosaccharide Acceptors—The oligosaccharide acceptors are described here in the order of increasing molecular weight. The LN (Glycan 1) and LNB (Glycan 2) were obtained from Sigma. Manβ1–4GlcNAc (Glycan 3) was from Dextra (Reading, UK). LNβ1–Ome (Glycan

1 The abbreviations used are: Fuc, L-fucose; Fuc T, α3-fucosyltransferrase; GalNAc, N-acetyl-b-galactosamine; L, lactose; Lex, Lewis x; LN, N-acetyllactosamine (Galβ1–4GlcNAc); LNB, lacto-N-biose (Galβ1–3GlcNAc); LDN, GalNAcβ1–4GlcNAc; MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight; Man, α-mannose; Neu5Ac, N-acetylneuraminic acid; MOPS, 4-morpholinepropanesulfonic acid; CHO, Chinese hamster ovary.
can 4) was synthesized from GlcNAc-1-OMe (Sigma) by β4-galactosyltransferase (27) catalyzed by bovine milk β4-galactosyltransferase (Sigma). LDL (Glycan 5) was synthesized from GlcNAc and UDP-GalNAc as described (28); GalNAc-1-4GlcNAc-1-OMe (Glycan 6) was constructed in the same way by using GlcNAc-1-OMe as the acceptor. N,N’-Diacyl-

Enzyme-assisted β4-galactosylation of GlcNAc-β2-Man (Glyko, 

α1-sialoglycan Neu5Acα2→3Gal (not shown) confirmed the presence of the distal LNB unit (33). Subsequent gel filtration chromatography of the digest yielded 23a acetyl-chitotetraose (Glycan (32); subsequent gel filtration chromatography of the digest yielded 23a acetyl-chitotetraose (Glycan (32)); subsequent gel filtration chromatography of the digest yielded 23a acetyl-chitotetraose (Glycan (32)) was performed as described (29). Neu5Acα2→3LN (Glycan 14) was purchased from Oxford Glycosystems (Abingdon, UK). 

LNβ1-3LN (Glycan 15) was synthesized by β4-galactosylating GlcNAc-β1-3LN (Glycan 13). Synthesis of LNβ1-6Galβ1-3GlcNAc (Glycan 16) was performed essentially as described (30). The β-methyl glycoside LNβ1-3LN-1-OMe (Glycan 17) was synthesized from GlcNAc-1-OMe (Sigma) in three successive steps, involving a β4-galactosyltransferase reaction, a β3-GlcNAc transferase reaction (30), and another β4-galactosyltransferase reaction. The N,N’,N”-,N”’-tetraacetyl-chitotetraose (Glycan 18) was purchased from Seikagaku (Tokyo, Japan). Neu5Acα2→3LNβ1-2Man (Glycan 19) was obtained by incubating 600 nmol of GlcNAc-β2-Man simultaneously with 1200 nmol of Neu5Acα2→3Gal and 750 nmol of CMP-Neu5Ac as well as 12 millimolar of α3-(N)-sialyltransferase (ST3 Gal III; Calbiochem, La Jolla, CA) in 150 μl of 50 mM MOPS, pH 7.5, 10 mM MnCl2, 0.02% sodium azide at room temperature for 66 h. The acidic reaction product was isolated by gel filtration chromatography on a Mono Q column. The elution behavior and the 1H NMR spectrum of the purified Glycan 39 was synthesized as described in Ref. 40. The octasaccharide LNβ1-2Manβ1-3LNβ1-2Manβ1-6Manβ1-4GlcNAc (Glycan 40) was prepared by treating the trisaccharide Neu5Acα2→3LNβ1-2Neu5Acα2→3LNβ1-4Manα1-3( Neu5Acα2→6LNβ1-2Manα1-6Manβ1-4GlcNAc (a gift from Prof. G. Strecker) with Arthrobacter ureafaciens neuraminidase (Roche Molecular Biochemicals). The completely desialylated product was isolated by ion exchange chromatography on the Mono Q column and was subsequently desalted by chromatography on the Superdex peptide HR column. The tetra-antennary N glycans LNβ1-3LNβ1-4Manα1-3LNβ1-2LNβ1-6Manα1-6Manβ1-4GlcNAc (Glycan 41) was a gift from Prof. G. Strecker. 

Transfected Cells and Lysates—The transfection and stable expression of human Fuc-TV in CHO cells has been described previously (5). 

For the enzyme assays, the transfected cells were lysed in 1% Triton X-100 in the presence of protease inhibitors. 

Fuc-TV Reactions—The assays were performed using lysates from Fuc-TV transfected CHO cells as described previously for Fuc-TIV and Fuc-TVII (22). Briefly, GDP-[14C]fucose (1 nmol) (100,000 cpm/nmol, prepared mixing GDP-fucose (Sigma) and GDP-[14C]fucose (American Radiolabeled Chemicals). In the reaction mixture containing the disialylated product and the two isomeric monosialylated products, the reaction mixture was incubated at 37 °C in the presence of GDP-Fuc as the substrate for the reaction (22). The assays were performed using lysates from Fuc-TV transfected CHO cells as described previously for Fuc-TIV and Fuc-TVII (22). Briefly, GDP-[14C]fucose (1 nmol) (10,000 cpm/nmol, prepared mixing GDP-fucose (Sigma) and GDP-[14C]fucose (American Radiolabeled Chemicals). In the reaction mixture containing the disialylated product and the two isomeric monosialylated products, the reaction mixture was incubated at 37 °C in the presence of GDP-Fuc as the substrate for the reaction (22). 

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The substrate specificity of each batch of transfected CHO cell lysates was tested with LN, LNB, and Neu5AcO3-LN before the actual experiments. The data resembled closely those described (5). All Fuc-TV reactions with CHO cell lysates were performed and analyzed at least twice. During the work up, the acceptors were directly monitored by UV absorption in all reactions, and no significant acceptor degradation was observed. No glycosidase inhibitors were used to protect the acceptors.

**Quantitation of Oligosaccharides—**Oligosaccharides were assayed by integration of the peaks obtained in Superdex Peptide HR 10/30 chromatography with UV detection. The data were related to the peaks of external GlcNAc and Neu5Ac standards. GlcNAc and GalNAc revealed equal molar absorbances.

**Chromatographic Methods—** Gel filtration was performed in a column of Superdex peptide HR 10/30 (Amersham Pharmacia Biotech) with 50 mM NH4HCO3 as the eluant; a flow rate of 1 ml/min was used. The effluent was monitored by UV absorption at 205 or 214 nm. Anion exchange chromatography on a Mono Q (5/5 column) (Amersham Pharmacia Biotech) was performed as described (30). Paper chromatography of radiolabeled oligosaccharides was performed as described (32). High pH anion exchange chromatography with pulsed amperometric detection was carried out with a Dionex series 4500i high pressure liquid chromatography system (Dionex, Sunnyvale, CA) equipped with a CarboPac PA-1 column (4 × 259 mm). The column was equilibrated and run with 40 mM NaOH at a flow rate of 1 ml/min as described (41).

**Degradations—** Oligosaccharides were treated with *A. ureafaciens* sialidase (EC 3.2.1.18; Roche Molecular Biochemicals) (42), with almond meal α-fucosidase (Oxford GlycoSystems) (43), with jack bean β-galactosidase (EC 3.2.1.23; Sigma), and with jack bean β-N-acetylhexosaminidase (EC 3.2.1.30; Sigma) as described (22). Oligosaccharide hydrolysis with *B. fragilis* endo-β-galactosidase (EC 3.2.103) was performed as described (44).

**Analysis of [14C]Fucosaccharide Isomers Generated in Fuc-TV Reactions of Polylactosamines That Contained Multiple Potential Acceptor Sites—** Analysis of neutral [14C]fucosylated isomers generated by Fuc-TV from type 2 polylactosamines was performed as described in Fig. 1. Analogous analysis of [14C]fucosylated type 1 glycans was performed by a treatment with β3-galactosidase of *Xanthomonas manihotis* (New England Biolabs, Beverly, MA), followed by paper chromatography of the resulting [14C]oligosaccharides. Control experiments involving 2-nmol samples of type 1 reference oligosaccharides were monitored by MALDI-TOF mass spectrometry and revealed that the β3-galactosidase treatment cleaved completely the tetrasaccharide LNβ1–3LN, whereas the pentasaccharide Galβ1–3Fucα1–4GlcNAcβ1–3LN was not degraded at all (data not shown). Analysis of neutral [14C]fucosylated type 1 N-acetylated polylactosamines was performed as described (22). Corresponding analyses of type 1 and type 2 sialopolylactosamines were performed after enzymatic desialylation. Analysis of the Fuc-TV reaction products of Lexβ1–3LNβ1–3LN was performed by *B. fragilis* endo-β-galactosidase digestion (45).

**1H NMR Spectroscopy—** Prior to NMR experiments the saccharides were twice dissolved in D2O and evaporated to dryness. The samples were then transferred into 0.5 H2O (99.986 atom % D, Cambridge Isotope Laboratories, Woburn, MA), and the NMR experiments were carried out in a Varian Unity 500 spectrometer at 296 K. A modified WETT sequence was used for water suppression (46). The chemical shift data are based on internal acetone signals at 2.25 ppm.

MALDI-TOF Mass Spectrometry—MALDI-TOF mass spectrometry was performed as described previously (11, 56).

**RESULTS**

**Fuc-TV Reactivities of LN and Related Small Saccharides—** The full-length Fuc-TV, present in lysates of appropriately transfected CHO cells, fucosylated efficiently unconjugated LN (Table I). At 5 mM acceptor concentrations, [14C]fucose was incorporated at a rate of 1000–1500 pmol × h−1 × (mg lysate protein)−1 in typical experiments; better reactivity was observed with β3-sialylated LN, whereas LN reacted more slowly, confirming previous data (5). GalNAcβ1–4GlcNAc (LDN) and β3-methyl glycosides of LDN and LN reacted well, whereas N,N′,diacyctethylchitohiose reacted slowly yet significantly.

**Fuc-TV Reactivities of Distal, Monocvalent LN, and Sialyl-LN Determinants—** Two trisaccharides, mimicking branches of N-glycans showed widely different Fuc-TV reactivities. LNβ1–6Manα1-OMe, representing a substituted β-methyl glycoside of LN, was a good acceptor (Table II). Concomitantly, LNβ1–2Man, where the LN and the mannose are joined through an axial bond that generates a more crowded unit, revealed an 8-fold smaller reactivity. The corresponding α3-sialylglycans reacted better than the asialoglycans.

Two galactose-containing trisaccharides, mimicking elements of polylactosamine backbones, were also studied. Even here, the isomer representing the substituted β-methyl glycoside of LN, i.e. LNβ1–6Gal, showed good reactivity, whereas the more crowded sequence LNβ1–3Galβ1–OMe was a relatively poor acceptor. The corresponding α3-sialylglycans reacted 2–2.5 times better than the asialoglycans. The LN unit of LNβ1–4GlcNAc appeared to be a good acceptor, and so were the two O-glycans of Core 2 type, both representing molecules of the type of substituted β-methyl glycosides of LN (Table II).

**Fuc-TV Reactivities of LN and LNB Units in Neutral Polylactosamines—** Relative Fuc-TV reactivities of individual LN units of multisite polylactosamine acceptors were determined using an approach described in Fig. 1, where an analysis of mono-[14C]fucosylated products of the octasaccharide LNβ1–3LNβ1–3LN was shown. The isomeric Fuc-TV products were digested with a mixture of β-galactosidase and β-N-acetylhexosaminidase, which cleaved off almost all distal, [14C]fucose-free LN units but did not attack the α3-fucosylated LN units. The resulting mixture of [14C]fucosylated glycans was then resolved into [14C]Fucα1–3LNβ1–3LNβ1–3LNβ1–3LN (Peak 1), [14C]Fucα1–3LNβ1–3LNβ1–3LNβ1–3LNβ1–3LN (Peak 2), [14C]Fucα1–3LNβ1–3LNβ1–3LN (Peak 3), and [14C]Fucα1–3LN (Peak 4) by paper chromatography. The amount of radiolabel in the distinct [14C]fucosylglycan fractions indicates the relative Fuc-TV reactivity of each LN unit along the octasaccharide chain.

The data in Table III show that the reactivity of GlcNAcβ1–3LN was 3-fold enhanced relative to free LN and was further up-regulated at the reducing end of the tetrasaccharide LNβ1–3LN (the reactive sites being underlined). Among the LN units of the hexasaccharide LNβ1–3LNβ1–3LN and the octasaccharide LNβ1–3LNβ1–3LNβ1–3LN, the best reactivity was also found at the reducing end LN. However, the levels of reducing end reactivity in these large glycans were not quite as...
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**TABLE II**

| Neutral acceptor | Relative Fuc-TV reactivity\(^a\) | Sialylated acceptor | Relative Fuc-TV reactivity\(^b\) |
|------------------|----------------------------------|---------------------|----------------------------------|
| LN (1)           | 1.0                              | Neu5Acα2–3LN (14)   | 1.7                              |
| LNβ1–6Manα1–OMe (11) | 1.6                         | Neu5Acα2–3LNβ1–6Manα1–OMe (22) | 3.7                              |
| LNβ1–2Man (8)    | 0.2                              | Neu5Acα2–3LNβ1–2Man (19) | 0.6                              |
| LNβ1–6Gal (9)    | 1.4                              | Neu5Acα2–3LNβ1–6Gal (20) | 3.5                              |
| LNβ1–3Galβ1–OMe (10) | 0.39                        | Neu5Acα2–3LNβ1–3Galβ1–OMe (21) | 1.1                              |
| LNβ1–6Galβ1–3GalNAc (16) | 3.0                         | Neu5Acα2–3LNβ1–6Galβ1–3GalNAc (25) | 2.5                              |
| LNβ1–4GlcNAc (12) | 0.9                            |                     |                                  |

\(^a\) Initial transfer rates were measured at 5 mM acceptor concentrations and were compared with that of 5 mM LN.

\(^b\) Initial transfer rates were measured at 3 mM acceptor concentrations and were compared with that of 3 mM LN.

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**FIG. 1. Degradative analysis of the isomeric mono-[14C]fucosylated products generated by Fuc-TV from the linear octasaccharide LNβ1–3LNβ1–3LNβ1–3LN.** The [14C]fucosylated products were treated with a mixture of jack bean β-galactosidase and jack bean β-N-acetylglucosaminidase, were desalted on a mixed bed of ion exchangers, and were chromatographed on paper for 105 h with Solvent A. The radioactivity was recovered. Peak 2 chromatographed approximately like the trisaccharide Lexβ1–3LNβ1–3LN. The data imply that most of the [14C]fucose was transferred from GDP-[14C]Fuc to the reducing end LN of the octasaccharide acceptor, and only very little [14C]fucose was introduced to the distal LN of the acceptor, whereas intermediate amounts were transferred to both midchain LN units.

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high as in the tetrasaccharide LNβ1–3LN, possibly because of the presence of higher amounts of total LN units. The high reactivity of the proximal LN unit in the unconjugated saccharides was preserved in the β-methylglycoside of the tetrasaccharide LNβ1–3LN. The second LN in the tetrasaccharide LNβ1–3LN and and its β-methyl glycoside, LNβ1–3LN–OMe reacted very slowly. This property of the second LN was related to its most distal position, because the second LN in the hexasaccharide LNβ1–3LNβ1–3LN and its analog in the octasaccharide LNβ1–3LNβ1–3LNβ1–3LN reacted fairly well (Table III). This view is also supported by the data showing that the third LN unit of the hexasaccharide LNβ1–3LNβ1–3LN reacted extremely slowly, but the third LN unit in the octasaccharide LNβ1–3LNβ1–3LNβ1–3LNβ1–3LN reacted fairly well. The most distal LN units of the linear polylactosamines LNβ1–3LNβ1–3LNβ1–3LNβ1–3LN and LNβ1–3LNβ1–3LNβ1–3LN were also reacted very slowly. Considered together, the data in Table III show that the Fuc-TV reactivity of the proximal LN of neutral i-type chains was consistently high, whereas the distal LN units always reacted poorly. The midchain LN of the chains possessed intermediate reactivities.

To rule out the possibility that the apparent poor distal fucosylation was due to the presence of site specific α3-fucosidases in lysates of the transfected CHO cells, a preparative experiment was performed using purified soluble recombinant Fuc-TV as the enzyme. Here, 29 mol % of the pentasaccharide LNβ1–3LNβ1–3Galβ1–OMe (Glycan 23) was converted to a monofucosylated fraction according to MALDI-TOF mass spectrometry. The monofucosylated product was isolated by high pH anion exchange chromatography on a Dionex Carbopac PA-1 column, and was identified as LNβ1–3Lexβ1–3Galβ1–OMe by 1H NMR spectroscopy (22) as well as by exoglycosidase degradation monitored by MALDI-TOF mass spectrometry (data not shown). Hence, the poor relative reactivity with distal LN units of neutral i-type polylactosamines, observed with Fuc-TV of the CHO cell lysates, is a property of the purified Fuc-TV too and does not represent an artifact caused by fucosidase action.

The Fuc-TV present in CHO cell lysates reacted well with the inner LN unit of the pentasaccharide LNβ1–3LNβ1–3Galβ1–OMe (LNβ1–3LNβ1–3Galβ1–OMe) of the reaction at the distal LN unit was much weaker, yet significant (Table III). Indeed, the Fuc-TV reactivity of LNβ1–3LNβ1–3Gal β1–OMe (at the underylered unit) was similar to that of free LNB, contrasting strongly with the dissimilarity of the reactivities of the analogous pair of LNβ1–3LNβ1–3Galβ1–OMe and free LN (cf. Tables I and II). In the branched i-type hexasaccharide LNβ1–3LNβ1–3LNβ1–3LN, the other LN units reacted poorly, but the 3-linked distal LN unit reacted faster than the LN units at the distal end of i-type chains.

**Fuc-TV Reactivities of LN Units in α3-Sialylated Polylactosamines**—The data of Table III show that all reliably assessed LN units in α3-sialylated polylactosamines reacted almost twice as rapidly as their counterparts in neutral polylactosamines. The very low reactivity of the distal, sialylated LN unit in Neu5Acα2–3LNβ1–3LN and Neu5Acα2–3LNβ1–3LNβ1–3LNβ1–3LNβ1–3LN reacted similarly to that of free LNB, contrasting strongly with the dissimilarity of the reactivities of the analogous pair of LNβ1–3LNβ1–3LNβ1–3Galβ1–OMe and free LN (cf. Tables I and II). In the branched i-type hexasaccharide LNβ1–3LNβ1–3LNβ1–3LN, the lower LN units reacted poorly, but the 3-linked distal LN unit reacted faster than the LN units at the distal end of i-type chains.

The Fuc-TV Reactivities of Partially α3-Fucosylated Polylactosamines—In prefucosylated i-type polylactosamines, the Lex determinants [Lex1–4Fucα1–3GlcNAc] exerted similar but less pronounced effects than LN groups on the Fuc-TV reactivity, except for the highly branched LN β1–3-branched LN neighbor reduced strongly the Fuc-TV reactivity of a LN unit in α3-sialylated i-type polylactosamines in the same way as in neutral polylactosamines. Some reduction of the reactivity in the Neu5Acα2–3LN was caused already by a proximal, β1–3-branched Galβ1–OMe neighbor. The low reactivity of the 1,6-linked branch, compared with the 1,3-linked main chain terminus, in the doubly sialylated i-type polylactosamine reveals the presence of a higher level of branch selectivity in Fuc-TV.

**Fuc-TV Reactivities of Unconjugated Asialo N-Glycans**—The data in Table V show that the biantennary N-glycan LNβ1–2Manα1–3LNβ1–2Manα1–6Manβ1–4GlcNAc (Glycan 27) reacted poorly with Fuc-TV, resembling the trisaccharide LNβ1–2Man. By contrast, the triantennary N-glycan (Glycan 40) LNβ1–2LNβ1–4Manα1–3LNβ1–2Manα1–6Manβ1–4GlcNAc reacted at a faster rate, suggesting that the β1,4-linked, equatorial LN branch was more reactive than the β1,2-linked, axial LN branches.
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The rate of the transferase reaction at a given position of the acceptors is indicated by underlining.

| Neutral acceptor | Fuc-TV reactivity | Sialylated acceptor | Fuc-TV reactivity |
|------------------|------------------|---------------------|------------------|
| LN (1)           | 1.0              | Neu5Acα2–3′LN (14)  | 1.7              |
| GlcNAcβ1–3′LN (13) | 3.5          | Neu5Acα2–3′LNβ1–3Glcβ1–OMe (21) | 1.1          |
| LNβ1–3Glcβ1–OMe (10) | 0.39         |                      |                  |
| LNβ1–3′LN (15)   | 4.7              | Neu5Acα2–3′LNβ1–3′LN (26) | 8.3            |
| LNβ1–3′LN (15)   | 0.1              | Neu5Acα2–3′LNβ1–3′LN (26) | 0.2            |
| LNβ1–3LNβ1–OMe (17) | 6.2          |                      |                  |
| LNβ1–3LNβ1–OMe (17) | 0.1           |                      |                  |
| LNβ1–3LNβ1–3Glcβ1–OMe (23) | 4.8        | Neu5Acα2–3′LNβ1–3′LNβ1–3Glcβ1–OMe (30) | 9.2          |
| LNβ1–3LNβ1–3Glcβ1–OMe (23) | 0.3         | Neu5Acα2–3′LNβ1–3′LNβ1–3Glcβ1–OMe (31) | 0.8          |
| LNβ1–3LNβ1–3Glcβ1–OMe (34) | 3.2        | Neu5Acα2–3′LNβ1–3′LNβ1–3Glcβ1–OMe (31) | 3.9          |
| LNβ1–3LNβ1–3LN (28) | 4.3          | Neu5Acα2–3′LNβ1–3′LNβ1–3LN (38) | 0.0            |
| LNβ1–3LNβ1–3LN (28) | 1.4          | Neu5Acα2–3′LNβ1–3′LNβ1–3LN (39) | 0.3            |
| LNβ1–3LNβ1–3LN (28) | 0.06         | Neu5Acα2–3′LNβ1–3′LNβ1–3LN (38) | 1.1            |

* Initial transfer rates were measured at 5 mM acceptor concentrations and were compared with those of 5 mM LN.

**DISCUSSION**

The present experiments extend the long list of small oligosaccharides that show acceptor activity for Fuc-TV. We show here that Fuc-TV transfers to LDN (GlcNAcβ1–4GlcNAc) as efficiently as to LN, leading to a GalNAcβ1–4(Fucα1–3)GlcNAc, which possesses a rigid conformation in solution similar to that of Lex (47, 48). Both the full-length as well as the truncated recombinant Fuc-TV reacted slowly with NβNβN- diacetetyl-chitobiose, NβNβN- triacetetyl-chitotriose and NβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNβNbeta...
were compared with that of 1 mM LN. The data refer to total transfer at both
in Fig. 2, which shows their preferred sites of action at the
Km
fucosylate at the distal ends of neutral i-type polylactosamines,
for the analogous Fuc-TIV reactivities (22). Another striking
Fuc-TV and the other human Fuc-Ts working on polylac-
However, the present data reveal major differences between
bly represent a mixture of Fuc-TIII and Fuc-TVI (21, 54), are
i-type polylactosamines remains to be elucidated.
whether it, too, reacts preferentially with elongated elements of
contrast, Fuc-TIX works best with the distal LN unit of i-chains;
acceptor may be involved in the accelerated reaction. For Fuc-
a receptor LN by Murray et al. (51). Accordingly,
both K_m and k_cat effects may be responsible for the activity
differences between different acceptors observed in our exper-
iments. Several other α3-Fuc-Ts such as Fuc-TIV (22), Fuc-TIX
(49), and the α3/4Fuc-Ts of human milk (52, 53), which proba-
ably represent a mixture of Fuc-TIII and Fuc-TVI (21, 54), are
known to work well with LN units of i-type polylactosamines.
However, the present data reveal major differences between
Fuc-TV and the other human Fuc-Ts working on polylac-
tosamines. Whereas the Fuc-TV reactivities of LN units are
strongly influenced by the presence or absence of neighboring
GlcNAc, Gal, and LN units in i-type chains, this is not the case
for the analogous Fuc-TIV reactivities (22). Another striking
difference is provided by the very poor ability of Fuc-TV to
fucosylate at the distal ends of neutral i-type polylactosamines,
which is the preferred site of action of Fuc-TIX (49). The dif-
ferent site specificities of Fuc-Ts V, IV, and IX are summarized
in Fig. 2, which shows their preferred sites of action at the
composite acceptor LNβ1–3LNβ1–3LN at the reducing end of
this acceptor, and the data suggest that the tetrasac-
charide determinant LNβ1–3LN at the reducing end of the
acceptor may be involved in the accelerated reaction. For Fuc-
TV, in turn, the optimal acceptor site of the hexasaccharide
appears to be the tetrasaccharide determinant GlcNAcβ1–3LNβ1–3Gal in the middle of the acceptor. The enzyme is
known to prefer the middle LN of the hexasaccharide acceptor,
and the distal as well as the proximal LN units of the hexa-
saccharide are known to have rather similar acceptor activities
(22). Clearly, both Fuc-TIV and Fuc-TV appear to be particu-
larly well fitted to work with i-type polylactosamines. By con-
trast, Fuc-TIX works best with the distal LN unit of i-chains;
whether it, too, reacts preferentially with elongated elements of
i-type polylactosamines remains to be elucidated.

The Fuc-TV worked well with the β-methyl glycoside of LN
and with the proximal LN unit of LNβ1–3LNβ1–3OMe, showing
that the high reactivity at proximal LN units in i-type polylac-
tosamines is not due to mutarotational freedom. Fuc-TV
worked well also with LNβ1–6Gal and LNβ1–6Man and their
α3-sialylated derivatives, implying that methylene group-gen-
erated conformational freedom between the acceptor LN and
its proximal substituents is important for a good reactivity. In
line with these observations were the good reactivities of the
O-glycans of core 2 type, LNβ1–6Galβ1–3GalNAc, and its
α3-sialylated form, as well as the poor reactivities of LNβ1–
2Man and the biantennary N-glycan (Glycan 37), where the
axial linkage between LN and the proximal Man generates
considerable steric hindrance. However, very poor reactivities
were also observed at the 6-linked arms in LNβ1–3LNβ1–6
LN and its α3-sialylated form. Hence, it appears that in the
1 branched polylactosamines, the properties of linear acceptors
may be dramatically changed. The very poor reactivity at the
6-linked arm in Neu5Acα2–3LNβ1–3(Neu5Acα2–3LNβ1–6
LN suggests that Fuc-TV is definitely not a tool of choice for
in vitro synthesis of oligovalent sialyl Lewis x polylac-
tosamines, representing branched structures that are potent
antagonists of lymphocyte 1-selectin (55–57).

A characteristic feature of Fuc-TV is its ability to react at
significant rates with two distinct, isomeric disaccharides, i.e.
LN and LNB. Our present data show that Fuc-TV transfers to
the LNB unit of the type 1 pentasaccharide LNβ1–3LNβ1–
3Galβ1–OMe almost as well as it transfers to the free LNB-
N disaccharide (cf. Tables I and III). By contrast, the enzyme
fails to transfer to the distal LN unit of the type 2 pentasaccharide
LNβ1–3LNβ1–3Galβ1–OMe nearly as well as it transfers to
unconjugated LN-disaccharide. This suggests that the GlcNAc
units of LN and LNB interact with different amino acid resi-
dues on the Fuc-TV surface. This observation lends support to
the conclusion drawn from a series of the mutagenesis and
domain shuffling experiments, establishing amino acid differ-
ences in modified Fuc-TV’s that are able to react only with
free LNB, only with free LN, or with both unconjugated
disaccharides (58–60).

ESL-1 is a glycoprotein ligand for E-selectin, and it is be-
thought to be N-glycosylated in its functional form (61). Tetra-
antennary N-glycans with a Neu5Acα2–3Lexβ1–3Lexβ1–
4Man branch have been reported to bind E-selectin (62). One of
the reported properties of Fuc-TV is its ability to convert non-
functional ESL-1 of CHO cells into a functionally active form
that is isolatable by affinity chromatography on a column of
immobilized E-selectin (63). The present data on Fuc-TV
reactivities of N-glycan acceptors suggest that the β1,4-linked,
equatorial LN branch reacted much faster than the β1,2-
linked, axial LN branches. Considered together, these sets of
observations suggest that (i) Fuc-TV may participate in \( \alpha3 \)-fucosylation of the \( \beta1,4 \)-linked (sialyl)\( LN \) and (sialyl)polyac-
tosamine branches of tri- and tetra-antennary N-glycans with some selectivity and that (ii) this may be important for generation of functional forms of ESL-1.

Acknowledgment—Gift samples of several N-glycans by Prof. G. Strecker are gratefully acknowledged.

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The Acceptor and Site Specificity of α3-Fucosyltransferase V: HIGH REACTIVITY OF THE PROXIMAL AND LOW OF THE DISTAL Gal β1–4GlcNAc UNIT IN i-TYPE POLYLACTOSAMINES

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J. Biol. Chem. 2000, 275:40057-40063.
doi: 10.1074/jbc.M007922200 originally published online September 27, 2000

Access the most updated version of this article at doi: 10.1074/jbc.M007922200

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