

α 1,3-Fucosyltransferase 9 (FUT9; Fuc-TIX) preferentially fucosylates the distal GlcNAc residue of polylactosamine chain while the other four α 1,3FUT members preferentially fucosylate the inner GlcNAc residue

Shoko Nishihara^a, Hiroko Iwasaki^a, Mika Kaneko^a, Akira Tawada^b, Masato Ito^c,
Hisashi Narimatsu^{a,*}

^a Division of Cell Biology, Institute of Life Science, Soka University, 1-236, Tangi-cho, Hachioji-shi, Tokyo 192-8577, Japan

^b Tokyo Research Institute, Seikagaku Corporation, 3-1253 Tatsumo Higashiyamato-shi, Tokyo 207-0021, Japan

^c Department of Bioengineering, Faculty of Engineering, Soka University, 1-236 Tangi-cho, Hachioji-shi, Tokyo 192-8577, Japan

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Abstract We analyzed the substrate specificity of six human α 1,3-fucosyltransferases (α 1,3FUTs) for the 2-aminobenzamide (2AB)-labelled polylactosamine acceptor, Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc-2AB (3LN-2AB). FUT9 preferentially fucosylated the distal GlcNAc residue of the polylactosamine chain while the other four α 1,3FUT members, FUT3, FUT4, FUT5 and FUT6, preferentially fucosylated the inner GlcNAc residue. This indicated that FUT9 exhibits more efficient activity for the synthesis of Lewis x carbohydrate epitope (Le^x; CD15; stage-specific embryonal antigen-1 (SSEA-1)). In contrast, the other four members synthesize more effectively the internal Le^x epitope. FUT7 could not transfer a fucose to an acceptor which is non-sialylated.

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Key words: Fucosyltransferase 9; Polylactosamine; Lewis x; Stage-specific embryonal antigen-1; CD15

1. Introduction

The Lewis x (Le^x) carbohydrate epitope on the polylactosamine chain is detected as stage-specific embryonal antigen-1 (SSEA-1) at the morulae of the mouse embryo [1] and considered to play an important role in cell-cell interactions [2–4]. Glycolipid having the dimeric Le^x or trimeric Le^x structure accumulates in human lung, liver and colon cancer [5–8]. Dimeric or trimeric Le^x is also observed during human embryogenesis [9]. These Le^x-related structures have been regarded as onco-fetal antigens.

α 1,3-Fucosyltransferases (α 1,3FUTs) transfer a fucose (Fuc) to *N*-acetylglucosamine (GlcNAc) of a type 2 chain (Gal β 1-4GlcNAc) with an α 1,3-linkage and comprise an α 1,3FUT family. We have recently cloned a new member of the α 1,3FUT family, FUT9 (Fuc-TIX) [10,11], in addition to the five known members, FUT3 (Fuc-TIII), FUT4 (Fuc-TIV),

FUT5 (Fuc-TV), FUT6 (Fuc-TVI) and FUT7 (Fuc-TVII) [12–20]. FUT3, FUT4, FUT5, FUT6 and FUT9 can synthesize the Le^x epitope while FUT7 can not. FUT9 is unable to synthesize the sialyl Le^x epitope, while the other five α 1,3FUTs can [10,11,21].

The FUT9 gene does not cross-hybridize with the other five α 1,3FUT genes. On a phylogenetic tree of α 1,3FUT members including vertebrate and *Caenorhabditis elegans* types, there are four clusters of vertebrate α 1,3FUT genes corresponding to the FUT3-FUT5-FUT6, FUT4, FUT7 and FUT9 gene subfamilies [10]. The FUT9 gene subfamily diverged first from the ancestral gene among the α 1,3FUT subfamilies.

Mouse has three functional α 1,3Fut genes, Fut4, Fut7 and Fut9, corresponding to human FUT4, FUT7 and FUT9 genes [11,22,23]. Of the three functional mouse α 1,3Futs, only Fut9 has a very highly conserved amino acid sequence between human and mouse and the level of conservation is equal to that of α -actin, suggesting a strong selective pressure for the preservation of the FUT9 (Fut9) sequence during evolution [10].

In the present study, we analyzed the FUT activity of recombinant enzymes directed by the six α 1,3FUT genes for a neutral polylactosamine acceptor. FUT9 exhibited a completely different specificity of Fuc transfer to GlcNAc residues from the other four α 1,3FUTs.

2. Materials and methods

2.1. Preparation of oligosaccharide acceptor substrate

The structure of polylactosamine (Fig. 1a) was prepared as an acceptor substrate by the method described by Tawada et al. [24]. Briefly, purified keratan sulfate (KS) (Seikagaku Corporation, Tokyo, Japan) was partially desulfated by the methanol-HCl method [25] and digested with keratanase II (Seikagaku Corporation, Tokyo, Japan). KS-derived oligosaccharides were desulfated completely and applied to gel filtration columns of cellulofine GCL-90-sf and cellulofine GCL-25-sf to obtain the following two oligosaccharides, Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc (3LN) and Gal β 1-4GlcNAc β 1-3Gal β 1-4GlcNAc (2LN). Both oligosaccharides, whose structures were determined by NMR spectroscopy and mass spectroscopy, were fluorescently labelled with 2-aminobenzamide (2AB) according to the manual of the Signal 2AB glycan labelling kit (Oxford GlycoScience, UK).

2.2. Establishment of *Namalwa* cells transfected stably with each of six α 1,3FUT genes

In the previous studies, the DNA fragments encoding the full-length open reading frames of the six human α 1,3FUT genes, FUT3, FUT4,

*Corresponding author. Fax: (81)-426-91-9315.

E-mail: hisashi@scc1.t.soka.ac.jp

Abbreviations: 2AB, 2-aminobenzamide; CNS, central nervous system; FUT, fucosyltransferase; GlcNAc, *N*-acetylglucosamine; gu, glucose unit; KS, keratan sulfate; Le^x, Lewis x; LN, *N*-acetylglucosamine, Gal β 1-4GlcNAc; PBL, peripheral blood leukocyte; SSEA-1, stage-specific embryonal antigen-1

FUT5, *FUT6*, *FUT7* and *FUT9*, were subcloned in a pAMo vector for expression in *Namalwa* cells [10,26,27]. Stable transformant cells were then selected using geneticin (1.2 mg/ml) (G418, Sigma).

2.3. Assay of the activity of six α 1,3FUTs towards poly lactosamine

Stable transformant cells were sonicated in 20 mM HEPES buffer (pH 7.2) containing 1% Triton X-100. Each cell lysate, containing 60 μ g protein, was used for α 1,3FUT activity assay in 50 mM cacodylate buffer (pH 6.8), 5 mM ATP, 10 mM L-Fuc, 75 mM guanosine diphosphate Fuc, 25 mM $MnCl_2$ and 15 mM acceptor substrate, 3LN-2AB. After incubation at 37°C for 2 h, the enzyme reactions were terminated by boiling for 3 min followed by adding of water. After centrifugation of the reaction mixtures, each supernatant was filtrated and subjected to reverse-phase high performance liquid chromatography (HPLC) analysis on a TSKgel ODS-80Ts QA column (4.6 \times 250 mm; Tosoh, Tokyo, Japan) and eluted with a 20 mM ammonium acetate buffer (pH 4.0) containing 7% methanol at a flow rate of 1.0 ml/min at 50°C, with monitoring by a fluorescence spectrophotometer (JASCO FP-920; Nihon Bunkoh, Tokyo, Japan).

2.4. Purification of fucosylated poly lactosamine

On HPLC analysis using a TSKgel ODS-80Ts QA column, the fraction containing each fucosylated 3LN-2AB was pooled, dried up and subjected to digestion by exo-glycosidases.

2.5. Digestion of fucosylated poly lactosamine by Jack bean β -galactosidase

Jack bean β -galactosidase digestion of each fucosylated 3LN-2AB was carried out in a 50 mM sodium citrate buffer (pH 3.5) containing 300 mU/ml of Jack bean β -galactosidase (Oxford GlycoScience, UK). After incubation at 37°C for 15 h, each digest was concentrated to dryness, applied to a normal-phase GlycoSep N HPLC column (4.6 \times 250 mm; Oxford GlycoScience, UK) and eluted with 250 mM ammonium formate buffer (pH 4.4) containing 65% acetonitrile at a flow rate of 1.0 ml/min at 30°C, with monitoring by a fluorescence spectrophotometer. 2AB-labelled glucose homopolymer standard was purchased from Oxford GlycoScience (UK).

2.6. Sequential digestion of fucosylated poly lactosamine by exo-glycosidases

Sequential digestion with exo-glycosidases was carried out in a 50 mM sodium citrate buffer (pH 5.0) containing an appropriate concentration of each exo-glycosidase, i.e. 0.2 mU/ml of almond meal α 1,3/4-fucosidase (Oxford GlycoScience, UK), 600 mU/ml of Jack bean β -galactosidase or 10 U/ml of Jack bean β -N-acetylhexosaminidase (Oxford GlycoScience, UK). After incubation at 37°C for 15 h, each digest was boiled for 5 min and then centrifuged. An appropriate amount of each supernatant was subjected to reverse-phase HPLC analysis using a TSKgel ODS-80Ts QA column under the conditions described above. The remaining supernatant was used for the next step, digestion by other exo-glycosidases.

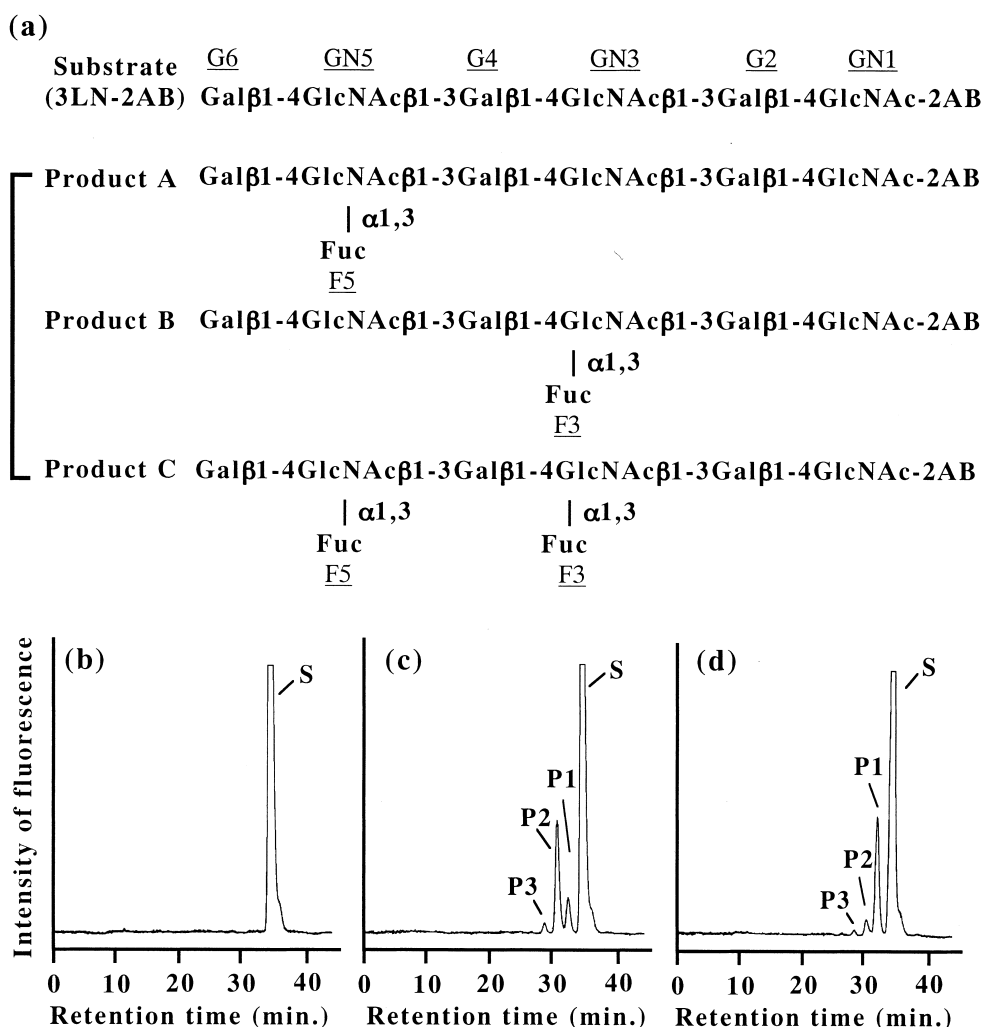


Fig. 1. A unique specificity of FUT9 for 2AB-labelled poly lactosamine acceptor. (a) Top: 3LN-2AB, an oligosaccharide structure of acceptor substrate used in the present study. Each saccharide residue of the poly lactosamine chain is numbered. Below: three expected products of the enzyme reaction are presented as product A, B and C. (b–d) Reverse-phase HPLC analysis of the reaction products after incubation with α 1,3FUTs. (b) mock reaction, (c) FUT4, (d) FUT9. S, 3LN-2AB; P1, product 1; P2, product 2; P3, product 3.

2.7. Assay of the activity of FUT4, FUT6 and FUT9 towards 3LN-2AB and monofucosylated 3LN-2AB

The enzymatic reactions were performed as described above, except that 1 mM purified monofucosylated 3LN-2AB, product A and product B, was also used as acceptor substrate (Fig. 1a).

3. Results

3.1. A unique specificity of FUT9 for 2AB-labelled polylectosamine acceptor

Representative HPLC profiles of reaction products of the six human α 1,3FUTs are shown in Fig. 1b–d. FUT7 could not transfer a Fuc to the 2AB-labelled polylectosamine ac-

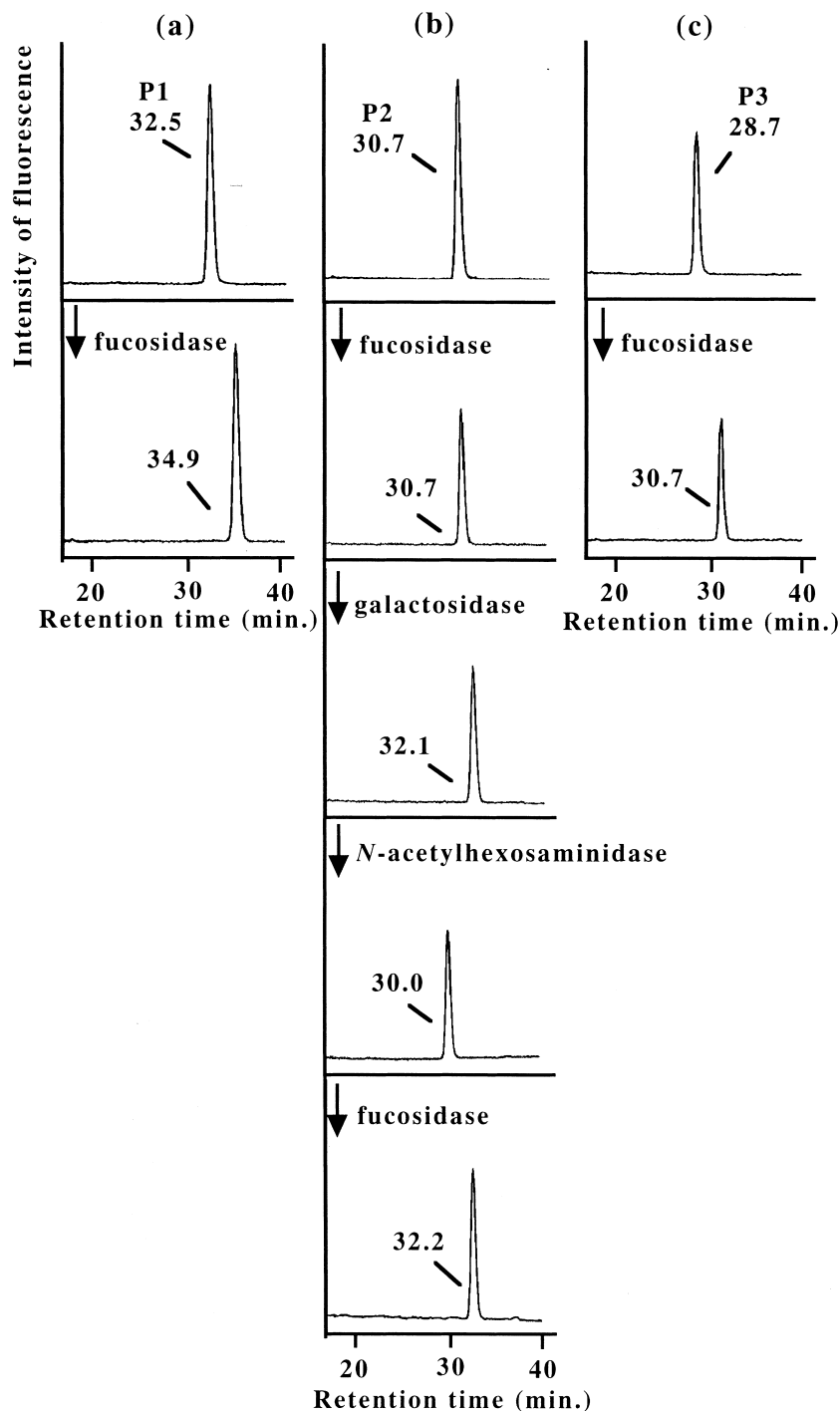


Fig. 2. Reverse-phase HPLC analysis of the products, product 1 (a), product 2 (b) and product 3 (c), before and after sequential digestion with almond meal α -fucosidase, Jack bean β -galactosidase and β -N-acetylhexosaminidase. Product 1 (a) was digested by α -fucosidase to obtain a product whose r.t. was equal to that of 3LN-2AB. Product 2 (b) could not be digested by α -fucosidase and was then digested sequentially with Jack bean β -galactosidase, β -N-acetylhexosaminidase and α -fucosidase to obtain a product whose r.t. was equal to that of 2LN-2AB. Product 3 (c) was digested by α -fucosidase to obtain a product whose r.t. was equal to that of product 2.

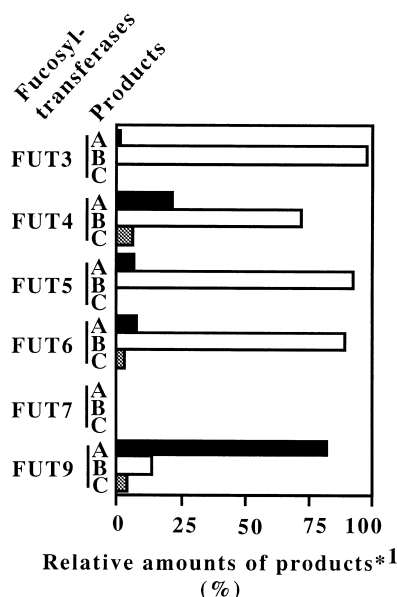


Fig. 3. Relative amounts of fucosylated products, product A (P1), product B (P2) and product C (P3), obtained from enzyme reaction with each α 1,3FUT, i.e. FUT3, FUT4, FUT5, FUT7 and FUT9, towards the polylactosamine substrate, 3LN-2AB. ^{*1}The total amount of the three products in each enzymatic reaction is 100%.

ceptor, 3LN-2AB. The other five α 1,3FUTs gave three different products, product 1 (P1), product 2 (P2) and product 3 (P3). FUT9 gave a unique HPLC profile. It produced P1 as a main peak (Fig. 1d). In contrast, the other four FUTs, i.e. FUT3, FUT4, FUT5 and FUT6, showed similar HPLC profiles, synthesizing P2 as a main product (Fig. 1c).

3.2. Assignment of P1, P2 and P3 to the expected fucosylated polylactosamine structures

Two possible monofucosylated products, products A and B, and one possible difucosylated product, product C, were expected to be synthesized by α 1,3FUTs (Fig. 1a). To determine the structure of P1, P2 and P3 (Fig. 1c,d), each peak of P1, P2 and P3 was purified and applied to a normal-phase HPLC column. P1 and P2 showed almost the same retention time (r.t.), 18.8 min (5.8 gu), while P3 gave a slower r.t., 24.9 min (6.8 gu) (data not shown). The addition of monosaccharide to oligosaccharide cores usually causes the r.t. to be prolonged on a normal-phase column [28]. Therefore, P3 was considered to be most likely the difucosylated product, product C, while P1 and P2 were considered to be monofucosylated products. Jack bean β -galactosidase is unable to release a non-reducing terminal galactose (Gal) residue from the Gal β 1-4(Fuc α 1-3)GlcNAc structure [29,30]. Only the purified P2 peak was shifted to a decreased r.t. position, 14.5 min (4.9 gu), by Jack bean β -galactosidase treatment. P1 and P3 were resistant to it. These results indicated that P1, P2 and P3 in Fig. 1c,d are products A, B and C in Fig. 1a, respectively.

To ascertain the above assignment, a sequential digestion with three exo-glycosidases was performed (Fig. 2). Almond meal α 1,3/4-fucosidase releases a Fuc from both Gal β 1-3(Fuc α 1-4)GlcNAc- and Gal β 1-4(Fuc α 1-3)GlcNAc- structures [31,32]. By treatment with almond meal α 1,3/4-fucosidase, the P1 and P3 peaks shifted to the positions of 3LN-2AB and P2, respectively (Fig. 2a,c). P2 was resistant to it

(Fig. 2b). The digestion with α 1,3/4-fucosidase supported the previous assignment of P1 as product A and P3 as product C (Fig. 1a). P2 was sequentially digested with three exo-glycosidases (Fig. 2b). The P2 peak shifted from 30.7 min r.t. to 32.1 min r.t. after the terminal Gal (G6) was released by Jack bean β -galactosidase treatment (Fig. 2b). It shifted from 32.1 min r.t. to 30.0 min r.t. after the GlcNAc residue (GN5) was released by Jack bean β -N-acetylhexosaminidase treatment and finally from 30.0 min r.t. to 32.2 min r.t. after the Fuc residue (F3) was released by α 1,3/4-fucosidase treatment. The final product of P2 after the three glycosidase digestions showed the same peak, 32.2 min r.t., as that of 2LN-2AB. This confirmed that P2 is a monofucosylated product possessing an internal Fuc (F3), i.e. product B. P3 was first digested with α 1,3/4-fucosidase and then subjected to sequential digestion by the three glycosidases, as done for P2. The α 1,3/4-fucosidase-digested P3 showed exactly the same profiles of sequential glycosidase digestion as P2 (data not shown). This confirmed that P3 is a difucosylated product, product C. The above assignment was also confirmed by measurement of the molecular weight of each product by mass spectroscopy.

There have been no reports of whether almond meal α 1,3/4-fucosidase can digest the internal Fuc or not. The present

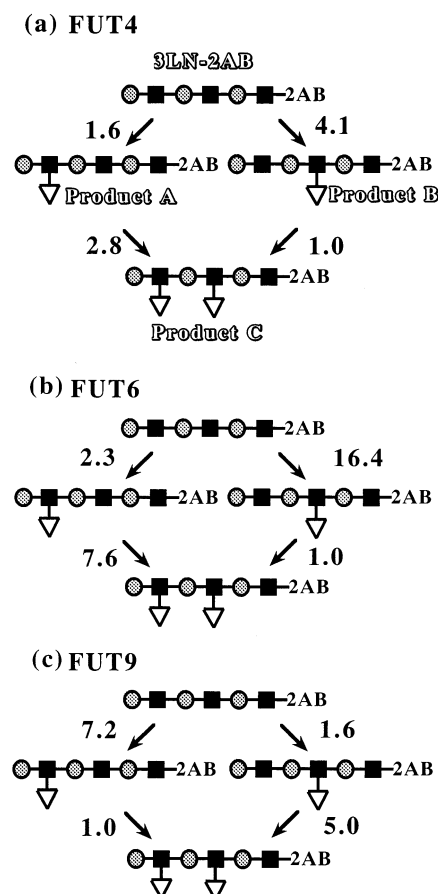


Fig. 4. Relative FUT activities through two pathways, i.e. via two monofucosylated 3LN-2AB (product A or product B), leading to the synthesis of difucosylated 3LN-2AB (product C). The weakest activity among the pathways in each enzyme reaction, FUT4 (a), FUT6 (b) and FUT9 (c), is presented as 1.0.

study demonstrated that almond meal α 1,3/4-fucosidase cannot digest the internal Fuc on polylactosamine chains.

3.3. Preferential activity of six α 1,3FUTs for the synthesis of Le^x , internal Le^x and dimeric Le^x structures

Relative amounts of each product, product A (Le^x), product B (internal Le^x) or product C (dimeric Le^x), synthesized by each of six α 1,3FUT enzymatic reactions are shown in Fig. 3. FUT3, FUT4, FUT5 and FUT6 preferentially fucosylated the inner GlcNAc residue, resulting in the synthesis of product B (P2; internal Le^x) as a main product. FUT7 could not synthesize any product. FUT9 preferentially fucosylated the distal GlcNAc residue on the polylactosamine chain, resulting in the synthesis of product A (P1; Le^x) as a main product. FUT4, FUT6 and FUT9 could synthesize relatively small amounts of difucosylated polylactosamine, product C (P3; dimeric Le^x).

3.4. Pathway of difucosylated polylactosamine synthesis by FUT4, FUT6 and FUT9

To determine the pathway of dimeric Le^x synthesis, α 1,3FUT activities of FUT4, FUT6 and FUT9 were assayed for monofucosylated 3LN-2AB, product A and product B. The results were summarized in Fig. 4. FUT4 and FUT6 showed higher α 1,3FUT activity levels for product A than product B to synthesize product C, that is, they again preferentially fucosylated the inner GlcNAc residue (GN3) (Fig. 4a,b). In contrast, FUT9 showed stronger α 1,3FUT activity for product B than product A to synthesize product C. Namely, FUT9 also preferentially fucosylated the distal GlcNAc residue (GN5) (Fig. 4c).

Supposing that the FUT reaction is a first-order reaction, the synthesizing rate for difucosylated product C through product A, i.e. $1.6 \times 2.8 = 4.5$ for FUT4, $2.3 \times 7.6 = 17.5$ for FUT6 and $7.2 \times 1.0 = 7.2$ for FUT9, was almost equal to that through product B, i.e. $4.1 \times 1.0 = 4.1$ for FUT4, $16.4 \times 1.0 = 16.4$ for FUT6 and $1.6 \times 5.0 = 8.0$ for FUT9, respectively. Thus, FUT4, FUT6 and FUT9 synthesize the difucosylated product through both pathways almost at the same rate.

4. Discussion

In the present study, we demonstrated that the substrate specificity of FUT9 is different from that of the other α 1,3FUTs. This is rational given that the FUT9 amino acid sequence is quite different from those of the other five α 1,3FUTs which share highly homologous sequences.

The preferential activity to transfer Fuc to the distal GlcNAc residue by FUT9 suggests that FUT9 synthesizes the Le^x epitope more efficiently in vivo than the other α 1,3FUTs. FUT9 was mainly expressed in neuronal cells in the central nervous system (CNS), stomach epithelial cells and peripheral blood leukocytes (PBLs) [10,11]. These tissues were well investigated by immunohistochemical or flow cytometrical studies for the expression of the Le^x epitope using various antibodies against Le^x . We will examine whether or not the Le^x epitopes of SSEA-1 present during embryogenesis and the CD15 antigen in the adult CNS, stomach epithelial cells and PBLs are the products of FUT9.

This is the first study clarifying the specificity towards the polylactosamine chain of all six human recombinant

α 1,3FUTs. Site specificities of recombinant FUT4 and FUT7 for polylactosamine have been reported [33]. FUT4 preferentially fucosylated inner lactosamine units on both neutral and α 2,3-sialylated polylactosamine chains, whereas FUT7 preferentially fucosylated the distal lactosamine unit on the α 2,3-sialylated polylactosamine chain. The FUT4 specificity reported by Niemela et al. is consistent with our results.

Site specificities of human milk FUT(s), which were partially purified and likely contained two α 1,3FUTs, FUT3 and FUT6 [34], for neutral and sialylated polylactosamine, have also been reported [35–37]. They prefer to transfer Fuc to an internal GlcNAc residue. Our results showing that both FUT3 and FUT6 preferentially fucosylate the internal GlcNAc residue are consistent with the human milk FUT activity.

Site-specific FUT activity for type 2 chain glycosphingolipid nLc₆ has been reported using cell lysates of various cancer cell lines [5,7,8] and Chinese hamster ovary (CHO) mutant cells [38]. The cell lysates of Colo205 cells (a colon cancer cell line) and LEC11 CHO mutant cells exhibit preferential Fuc transfer to the internal GlcNAc residue, while the lysates of PC9 and NCI-H69 cells (lung cancer cell lines) and LEC12 CHO cells prefer to transfer a Fuc to the distal GlcNAc residue. In a previous study, the *FUT3*, *FUT4* and *FUT6* genes were found to be expressed in human colorectal cancer tissues and colorectal cancer cell lines including Colo205 [39]. The *FUT9* gene was not expressed in colorectal tissues and Colo205 cells (data not shown). Recently, LEC11 cells have been reported to express the *FUT* gene orthologous to the human *FUT6* gene [40]. The preferential activity to transfer Fuc to the internal GlcNAc of Colo205 and LEC11 cell lysates is attributed to FUT3, FUT4 and FUT6, not to FUT9. PC9, NCI-H69 and LEC12 CHO cells, which exhibited preferential activity to transfer Fuc to the distal GlcNAc, may express FUT9. In a preliminary experiment, we observed that PC9 cells expressed a substantial amount of FUT9 transcripts and the PC9 cell lysates preferentially fucosylated the distal GlcNAc residue of the polylactosamine acceptor.

We tried to construct a molecular model of the polylactosamine chain based on the interaction of hydrogen bonds (data not shown). The tentative model showed a zigzag structure of the smallest repeating unit, (Gal β 1-4GlcNAc β 1-)₂. The 3-OH of the distal GlcNAc residue (GN5) was exposed to solvent, while the 3-OH of the inner GlcNAc residue (GN3) was somewhat hindered. This zigzag structure of the polylactosamine backbone is not influenced by the fucosylation of GlcNAc residue. This may be true, because we demonstrated in the present study that each α 1,3FUT showed the similar activity of Fuc transfer irrespective of whether the acceptor was non-fucosylated or monofucosylated (Fig. 4). FUT9 and the other α 1,3FUTs cooperatively synthesize the dimeric Le^x structure. Stomach mucosae and PBLs are the tissues expressing both FUT9 and FUT4 [10], in which the dimeric Le^x may be abundantly expressed.

Finally, the products fucosylated by the unique specificity of FUT9, i.e. the preferential Fuc transfer to the distal GlcNAc, should have specific biological roles differing from those of the products fucosylated by the other α 1,3FUTs.

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References

- [1] Gooi, H.C., Feizi, T., Kapadia, A., Knowles, B.B., Solter, D. and Evans, M.J. (1981) *Nature* 292, 156–158.
- [2] Muramatsu, H. and Muramatsu, T. (1983) *FEBS Lett.* 163, 181–184.
- [3] Bird, J.M. and Kimber, S.J. (1984) *Dev. Biol.* 104, 449–460.
- [4] Fenderson, B.A., Zehavi, U. and Hakomori, S. (1984) *J. Exp. Med.* 160, 1591–1596.
- [5] Holmes, E.H., Ostrander, G.K. and Hakomori, S. (1985) *J. Biol. Chem.* 260, 7619–7627.
- [6] Hakomori, S., Nudelman, E., Levery, S.B. and Kannagi, R. (1984) *J. Biol. Chem.* 259, 4672–4680.
- [7] Holmes, E.H., Ostrander, G.K. and Hakomori, S. (1986) *J. Biol. Chem.* 261, 3737–3743.
- [8] Holmes, E.H. and Levery, S.B. (1989) *Arch. Biochem. Biophys.* 274, 633–647.
- [9] Fukushi, Y., Hakomori, S. and Shepard, T. (1984) *J. Exp. Med.* 160, 506–520.
- [10] Kaneko, M., Kudo, T., Iwasaki, H., Ikehara, Y., Nishihara, S., Nakagawa, S., Sasaki, K., Shiina, T., Inoko, H., Saitou, N. and Narimatsu, H. (1999) *FEBS Lett.* 452, 237–242.
- [11] Kudo, T., Ikehara, Y., Togayachi, A., Kaneko, M., Hiraga, T., Sasaki, K. and Narimatsu, H. (1998) *J. Biol. Chem.* 273, 26729–26738.
- [12] Kukowska-Latallo, J.F., Larsen, R.D., Nair, R.P. and Lowe, J.B. (1990) *Genes Dev.* 4, 1288–1303.
- [13] Weston, B.W., Smith, P.L., Kelly, R.J. and Lowe, J.B. (1992) *J. Biol. Chem.* 267, 24575–24584.
- [14] Lowe, J.B., Kukowska-Latallo, J.F., Nair, R.P., Larsen, R.D., Marks, R.M., Macher, B.A., Kelly, R.J. and Ernst, L.K. (1991) *J. Biol. Chem.* 266, 17467–17477.
- [15] Sasaki, K., Kurata, K., Funayama, K., Nagata, M., Watanabe, E., Ohta, S., Hanai, N. and Nishi, T. (1994) *J. Biol. Chem.* 269, 14730–14737.
- [16] Natsuka, S., Gersten, K.M., Zenita, K., Kannagi, R. and Lowe, J.B. (1994) *J. Biol. Chem.* 269, 16789–16794.
- [17] Goelz, S.E., Hession, C., Goff, D., Griffiths, B., Tizard, R., Newman, B., Chi Rosso, G. and Lobb, R. (1990) *Cell* 63, 1349–1356.
- [18] Weston, B.W., Nair, R.P., Larsen, R.D. and Lowe, J.B. (1992) *J. Biol. Chem.* 267, 4152–4160.
- [19] Koszdin, K.L. and Bowen, B.R. (1992) *Biochem. Biophys. Res. Commun.* 187, 152–157.
- [20] Nishihara, S., Nakazato, M., Kudo, T., Kimura, H., Ando, T. and Narimatsu, H. (1993) *Biochem. Biophys. Res. Commun.* 190, 42–46.
- [21] Kimura, H., Shinya, N., Nishihara, S., Kaneko, M., Irimura, T. and Narimatsu, H. (1997) *Biophys. Biochem. Res. Commun.* 237, 131–137.
- [22] Gersten, K.M., Natsuka, S., Trinchera, M., Petryniak, B., Kelly, R.J., Hiraiwa, N., Jenkins, N.A., Gilbert, D.J., Copeland, N.G. and Lowe, J.B. (1995) *J. Biol. Chem.* 270, 25047–25056.
- [23] Smith, P.L., Gersten, K.M., Petryniak, B., Kelly, R.J., Rogers, C., Natsuka, Y., Alford, J.A., Scheidegger, E.P., Natsuka, S. and Lowe, J.B. (1996) *J. Biol. Chem.* 271, 8250–8259.
- [24] Tawada, A. and Yoshida, K. (1998) International Patent WO98/03524.
- [25] Kantor, T.G. and Schubert, M. (1957) *J. Am. Chem. Soc.* 79, 152–153.
- [26] Kimura, H., Kudo, T., Nishihara, S., Iwasaki, H., Shinya, N., Watanabe, R., Honda, H., Takemura, F. and Narimatsu, H. (1995) *Glycoconj. J.* 12, 802–812.
- [27] Nishihara, S., Yazawa, S., Iwasaki, H., Nakazato, M., Kudo, T., Ando, T. and Narimatsu, H. (1993) *Biochem. Biophys. Res. Commun.* 196, 624–631.
- [28] Guile, G.R., Rudd, P.M., Wing, D.R., Prime, S.B. and Dwek, R.A. (1996) *Anal. Biochem.* 240, 210–226.
- [29] Arakawa, M., Ogata, S., Muramatsu, T. and Kobata, A. (1974) *J. Biochem. (Tokyo)* 75, 707–714.
- [30] Kobata, A. (1979) *Anal. Biochem.* 100, 1–14.
- [31] Yamashita, K., Tachibana, Y., Nakayama, T., Kitamura, M., Endo, Y. and Kobata, A. (1980) *J. Biol. Chem.* 255, 5635–5642.
- [32] Scudder, P., Neville, D.C., Butters, T.D., Fleet, G.W., Dwek, R.A., Rademacher, T.W. and Jacob, G.S. (1990) *J. Biol. Chem.* 265, 16472–16477.
- [33] Niemela, R., Natunen, J., Majuri, M.L., Maaheimo, H., Helin, J., Lowe, J.B., Renkonen, O. and Renkonen, R. (1998) *J. Biol. Chem.* 273, 4021–4026.
- [34] Macher, B.A., Holmes, E.H., Swiedler, S.J., Stults, C.L. and Srnka, C.A. (1991) *Glycobiology* 1, 577–584.
- [35] de Vries, T. and Van den Eijnden, D.H. (1994) *Biochemistry* 33, 9937–9944.
- [36] de Vries, T., Norberg, T., Lonn, H. and Van den Eijnden, D.H. (1993) *Eur. J. Biochem.* 216, 769–777.
- [37] Niemela, R., Natunen, J., Penttila, L., Salminen, H., Helin, J., Maaheimo, H., Costello, C.E. and Renkonen, O. (1999) *Glycobiology* 9, 517–526.
- [38] Howard, D.R., Fukuda, M., Fukuda, M.N. and Stanley, P. (1987) *J. Biol. Chem.* 262, 16830–16837.
- [39] Kudo, T., Ikehara, Y., Togayachi, A., Morozumi, K., Watanabe, M., Nakamura, M., Nishihara, S. and Narimatsu, H. (1998) *Lab. Invest.* 78, 797–811.
- [40] Zhang, A., Potvin, B., Zaiman, A., Chen, W., Kumar, R., Phillips, L. and Stanley, P. (1999) *J. Biol. Chem.* 274, 10439–10450.