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Dictyostelium cytosolic fucosyltransferase synthesizes H type 1 trisaccharide in vitro

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Abstract A fucosyltransferase activity has been detected using lacto-N-biose I as acceptor in the lower eukaryote *Dictyostelium discoideum*. This transferase requires divalent cations and is inhibited by *N*-ethylmaleimide and detergent treatment. Apparent calculated K_m values for GDP-Fuc and lacto-N-biose I are 1.27 μM and 2.80 mM, respectively. The activity is quantitatively recovered in the supernatant after centrifugation at 100 000 × *g* for 1 h. The reaction product, as determined by gel permeation chromatography, sensitivity to fucosidases, and analysis of partially methylated derivatives, is Fucα1-2Galβ1-3GlcNAc (H type 1 trisaccharide).

Key words: Glycosyltransferase; Cytosol; Carbohydrate antigen; Permethylation; (*Dictyostelium discoideum*)

1. Introduction

Glycosyltransferases have been mostly reported as membrane-bound enzymes involved in the biosynthesis of the saccharide chains of glycoproteins and glycolipids [1]. Glycosyltransferases found in biological fluids reach the extracellular compartment from the Golgi lumen through the secretory pathway, and proteolytic cleavage eventually makes them rid of the membrane anchor. On the other hand, few cytosolic glycosyltransferases have recently been demonstrated [2]. Very recently, a fucosyltransferase has been purified from the cytosol of the slime mold *Dictyostelium discoideum* [3]. The enzyme was originally discovered by its ability to fucosylate the FP21 glycoprotein [4], that was proved to be mostly cytosolic [5]. The enzyme was also found to use lipid-bound acceptors in vitro, provided they contain a terminal Galβ1-3GlcNAc disaccharide sequence [3,4]. To date, it is not known whether the enzyme utilizes simple sugar acceptors, as do mammalian fucosyltransferases, and there is no direct evidence of the linkage position and sugar substitution in the reaction product. For addressing these issues, we have characterized the activity using lacto-N-biose I as acceptor and prepared enough reaction product, radiolabeled on three different positions of the molecule, in order to establish unambiguously its structure.

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Abbreviations: Lewis a trisaccharide, Galβ1-3[Fucα1-4]GlcNAc; H type 1 trisaccharide, Fucα1-2Galβ1-3GlcNAc; lacto-N-biose I, Galβ1-3GlcNAc; LacNAc, Galβ1-4GlcNAc; GDP-Fuc, GDP-β-L-fucose; NEM, *N*-ethylmaleimide; TLCK, N^{α}-*p*-tosyl-L-lysine chloromethyl ketone; PMSF, phenylmethylsulfonyl fluoride; HPTLC, high-performance thin-layer chromatography; DMSO, dimethylsulfoxide; Dα1,2FucT, *Dictyostelium* cytosolic α1,2fucosyltransferase; Hα1,2FucT, human blood group H α1,2fucosyltransferase.

2. Materials and methods

2.1. Materials

Lacto-N-biose I, LacNAc, GlcNAc, GDP-β-L-fucose, L-Fuc, Galβ1-6GlcNAc, α1-2fucosyllactose, Galβ1-6Gal, Galβ1-3Gal1-OMe, Galβ1-4Gal, Triton X-100, *N*-ethylmaleimide, antipain, chymostatin, leupeptin, pepstatin, aprotinin, TLCK, PMSF, and sucrose were from Sigma. Anhydrous dimethylsulfoxide (water < 0.005%), dry sodium hydride, and iodomethane were from Aldrich. GDP-[1-^{3}H]Fuc was from Du Pont New England Nuclear; UDP-[6-^{3}H]Gal and [1-^{3}H]GlcNAc were from Amersham. α-Fucosidases (from bovine epididymis and almond meal) were from Oxford Glycosystem. HPTLC plates, solvents, and common chemicals were from Merck.

2.2. Cells and subcellular fractionation

Dictyostelium strain AX2 cells were grown at 23°C in a rotary shaker in axenic medium [6]. At a culture density of 5–8 × 10^6 cells/ml, cells were collected by pelleting at 1300 rpm for 4 min, at 4°C. Cell pellet was resuspended with 0.25 M sucrose-TKM buffer (50 mM Tris, 25 mM KCl, 5 mM $MgCl_2$, pH 8.0) by gently pipetting, and spun as above; washing procedure was repeated three times. It was then resuspended to a cell density of 0.5 × 10^9 cells/ml in 0.25 M sucrose-TKM buffer containing protease inhibitors to a final concentration of 20 μg/ml each antipain, chymostatin, leupeptin, and pepstatin, 0.2 TIU aprotinin, 37 μg/ml TLCK, and 1 mM PMSF. The cell suspension (4–6 ml in typical experiments) was lysed by forced passage through a polycarbonate filter with a 3 μm nominal pore diameter (Nucleopore, 25 mm filter diameter), and the lysate obtained was referred to as the homogenate. It was spun at 2000 × *g* for 6 min, at 4°C, obtaining a supernatant referred to as the postnuclear fraction. This fraction was centrifuged at 100 000 × *g* for 1 h at 4°C, obtaining a pellet and a supernatant. The latter was referred to as the cytosolic fraction; the pellet was resuspended to a protein concentration of 10–30 mg/ml with 0.25 M sucrose-TKM buffer containing the same protease inhibitors as in the filtration buffer, and referred to as the membrane fraction. Protein content was determined by the procedure of Lowry et al. [7]. COS-7 cells were cultured in Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin, 100 μg/ml streptomycin, and 2 mM L-Glu.

2.3. Enzyme assays

Dα1,2FucT was determined in a reaction mixture containing, in a final volume of 20 μl, 0.1 M Tris-HCl buffer, pH 7.4, 10 mM $MnCl_2$, 5 mM ATP, 6 μM donor GDP-[^{3}H]Fuc (spec. act. 500 mCi/mmol), 10 mM acceptor lacto-N-biose I, and 2.5–7.5 mg/ml enzyme protein, depending on the cellular fraction used. Incubation was performed at 23°C for 60 min. Enzyme sources for kinetic experiments were aliquots of a cytosolic fraction thawed after being frozen for less than 1 week. Fuc-TIII and human blood group H α1-2fucosyltransferase were assayed upon transfection and expression of the pcDNA I-α(1,3/1,4)FT and pcDNA I-α(1,2)FT plasmids, respectively, in COS-7 cells, using the cell homogenate as the enzyme source. The two plasmids were constructed by subcloning the *Xho*I inserts of pCDM7-α(1,3/1,4)FT [8] and pCDM7-α(1,2)FT [9], respectively, in the corresponding site of pcDNA I. Transfections were carried out with the DEAE dextran procedure [8]. 70 h after transfection, COS-7 cells were collected by trypsinization, pelleted, washed three times with cold phosphate-buffered saline and resuspended with 0.1 M Tris-HCl buffer, pH 7.4 to a density of 10^7 cells/ml and kept frozen at −20°C. Aliquots of the frozen suspension were thawed and diluted to a density of 10^6 cells/ml in the same buffer containing 1 mg/ml Triton X-100 and kept for 30 min on ice before using as the enzyme

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source. Fuc-TIII activity was determined in a reaction mixture containing, in a final volume of 20 μl, 0.1 M Tris-HCl buffer, pH 7.4, 10 mM $MnCl_2$, 0.5 mg/ml Triton X-100, 40 μM donor GDP-[^{3}H]Fuc (spec. act. 100 mCi/mmol), 10 mM acceptor lacto-N-biose I, and 0.25 mg/ml enzyme protein, corresponding to about 4×10^2 cells per reaction. Incubation was performed at 37°C for 60 min. H type 1 trisaccharide was prepared in a reaction mixture containing, in a final volume of 0.1 ml, 0.1 M Tris-HCl buffer, pH 6.5, 0.5 mg/ml Triton X-100, 18 μM donor GDP-[^{3}H]Fuc (spec. act. 500 mCi/mmol), 40 mM acceptor lacto-N-biose I, and 6.4 mg/ml enzyme protein, corresponding to about 5×10^4 cells. Incubation was carried out at 37°C for 90 min. Inhibition studies with NEM were carried out as reported by Stroup et al. [10]. Reaction product was assayed by Dowex 1×8 anion exchange columns and radioactivity incorporation determined by liquid scintillation counting as reported [8]. Blanks were prepared by omitting the acceptor in the reaction mixture and the incorporation values subtracted for enzyme activity calculations.

2.4. Reaction product characterization

Quenched reaction mixtures were thawed, pooled, chromatographed on Dowex columns as above, and lyophilized. The obtained material was resuspended in water and 0.1 ml aliquots (corresponding to about 200000 cpm of product) were loaded on a Biogel P2 (Bio Rad) column (0.7 cm×50 cm) prepared and equilibrated in degassed water according to the manufacturer's recommendations. The column was run with water at a flow rate of about 0.07 ml/min. Total elution volume and fraction size were 20 and 0.25 ml, respectively. Sample elution was monitored by liquid scintillation counting. Collected fractions were lyophilized, resuspended in a small volume of water, eventually submitted to enzymatic treatment, and analyzed by HPTLC on silica gel plates. Plates were developed with *n*-propanol/ethylacetate/water/25% NH_4OH (6:1:3:1, v/v) for 2 h. Radioactive spots were detected by fluorography, and unlabelled reference standard were detected by anisaldehyde spray reagent (glacial acetic acid/98% sulfuric acid/4-methoxybenzaldehyde, 47:1:2, v/v). Fucosidase treatments were carried out on 5000–25000 cpm reaction product (10 μM), as follows. Bovine epididymis α-fucosidase digestion: 100 mM sodium citrate/phosphate, pH 6.0 and 0.4 U/ml enzyme; incubation was for 24 h at 37°C. Almond meal α-fucosidase digestion: 50 mM sodium acetate pH 5.0 and 0.6 mU/ml enzyme. Incubation was for 70 h at 37°C.

[*Gal*-^{3}H]lacto-N-biose I and [*GlcNAc*-^{3}H]lacto-N-biose I were synthesized enzymatically by coupling radioactive UDP-Gal to unlabelled GlcNAc or unlabelled UDP-Gal to radioactive GlcNAc, respectively, using a crude β1-3galactosyltransferase preparation (M. Trinchera and S. Bozzaro, manuscript in preparation), and purified by Biogel P2 chromatography. [*Gal*-^{3}H]Dα1,2FucT product was obtained in a reaction mixture containing, in a final volume of 0.3 ml, 0.05 M Tris-HCl buffer, pH 7.4, 10 mM $MgCl_2$, 10 mM Fuc, 5 mM ATP, 30 μM unlabelled GDP-Fuc, 0.4 mM [*Gal*-^{3}H]lacto-N-biose I (spec. act. 1.0 Ci/mmol) and 10 mg/ml cytosolic protein. [*GlcNAc*-^{3}H]Dα1,2FucT product was obtained under identical conditions, except that the reaction volume was 0.4 ml, and [*GlcNAc*-^{3}H]lacto-N-biose I was the acceptor (spec. activity 0.5 Ci/mmol). After 24 h at 23°C, an equal volume of a freshly prepared reaction mixture, containing all reagents except the acceptors, was added to each mixture, and allowed to react at 23°C for additional 24 h. [*GlcNAc*-^{3}H]Lewis a trisaccharide was obtained in a reaction mixture containing, in a final volume of 0.5 ml, 0.05 M Tris-HCl buffer, pH 7.4, 10 mM $MnCl_2$, 10 mM Fuc, 5 mM ATP, 0.5 mg/ml Triton X-100, 75 μM unlabelled GDP-Fuc, 0.12 mM [*GlcNAc*-^{3}H]lacto-N-biose I (spec. act. 0.5 Ci/mmol) and 40 mg/ml Fuc-TIII protein, as transfected-COS-7 cell homogenate (see Section 2.3). Incubation was for 22 h at 37°C. Trisaccharide products were purified by Dowex and Biogel P2 chromatography as described for the (*Fuc*-^{3}H)-labelled trisaccharides. Individual column fractions were lyophilized, resuspended in 20 μl of water, and analyzed by HPTLC. Fractions containing the products were pooled, lyophilized, and the material resuspended with 10 μl of water and spotted on a 5 cm line of an HPTLC plate. After developing, the area corresponding to the proper trisaccharide product was scraped off and silica gel eluted with water. The procedure yielded 1540000 and 760000 cpm of [*Gal*-^{3}H]- and [*GlcNAc*-^{3}H]Dα1,2FucT products, respectively, and 1910000 cpm of [*GlcNAc*-^{3}H]Lewis a. Permethylation was performed in a 1 ml screw cap reaction vial (Pierce) using 200000 cpm of radioactive samples or 500 nmol of unlabelled standard saccharides, each dissolved in 50 μl anhydrous DMSO. An equal volume of methylsulfinyl carbanion, prepared according to Dell et al. [11], was added to each vial under a stream of nitrogen. After few minutes, 50 μl iodomethane were added, and the mixture allowed to react for 30 min at room temperature. After stopping the reaction with 150 μl of water, the mixture was loaded onto a Sep-Pak C-18 cartridge previously equilibrated with water, and washed with 3 ml each of water, 50% aqueous acetonitrile, and acetonitrile. Over 85% of loaded radioactivity was recovered in the 50% aqueous acetonitrile fractions, which were dried in a rotary evaporator, resuspended in water, made 2 M trifluoroacetic acid (60 μl final volume), and kept at 100°C for 4 h [12]. Hydrolyzates were freeze dried, resuspended in 10–20 μl acetone and analyzed by HPTLC using two solvent systems: acetone/4.5 M NH_4OH (500:9, v/v), and *n*-propanol/ethyl acetate/water/25% NH_4OH (6:1:3:1, v/v). Reference standard partially methylated sugars were obtained by parallel treatment of the following compounds: Galβ1-6Gal (2,3,4-tri-*O*-methylgalactose and 2,3,4,6-tetra-*O*-methylgalactose), α1-2fucosyllactose (3,4,6-tri-*O*-methylgalactose), Galβ1-3Galβ1-O-Me (2,4,6-tri-*O*-methylgalactose), Galβ1-4Gal (2,3,6-tri-*O*-methylgalactose), lacto-N-biose I (4,6-di-*O*-methylglucosamine), [*GlcNAc*-^{3}H]Lewis a trisaccharide (6-mono-*O*-methylglucosamine). Unlabelled partially methylated hexoses were detected by molybdate spray reagent (35 mM ammonium molybdate and 5 mM cerium sulfate in 6% sulfuric acid).

3. Results

3.1. Detection and subcellular distribution of fucosyltransferase activity toward lacto-N-biose I in Dictyostelium

In the presence of lacto-N-biose I, a reproducible acceptor-dependent incorporation of radioactivity into the neutral compounds unretained by Dowex columns is measurable using cell homogenate, postnuclear and cytosolic fractions. The distribution of protein and Dα1,2FucT activity in subcellular fractions is presented in Table 1. The results show that Dα1,2FucT activity is quantitatively recovered in the supernatant, while no activity is detectable in the membranes. Freeze-thawing of the membranes, detergent treatment, or inclusion in the reaction mixture of higher GDP-Fuc concentration, are ineffective in providing any detectable activity with lacto N-biose I as acceptor.

Table 1
Distribution of Dα1,2FucT activity in subcellular fractions

	Protein		Dα1,2FucT			
	mg	Recovery (%)	Specific activity	RSA	Total activity	Recovery (%)
Homogenate	40.83	100	45.06		1.84	100
Postnuclear fraction	32.50	79.6	61.59	1.37	2.00	108.6
Cytosolic fraction	16.34	40.0	127.9	2.83	2.09	113.5
Membrane fraction	15.26	37.3			undetectable	

Protein content and Dα1,2FucT activity were measured in the cell homogenate and in subcellular fractions prepared as described under Section 2. Specific activity values are expressed as pmol/mg protein per h transferred Fuc, and total activity values as pmol/h transferred Fuc, referred to 10^9 cells as starting material. Results are the means for three independent experiments. RSA, relative specific activity.

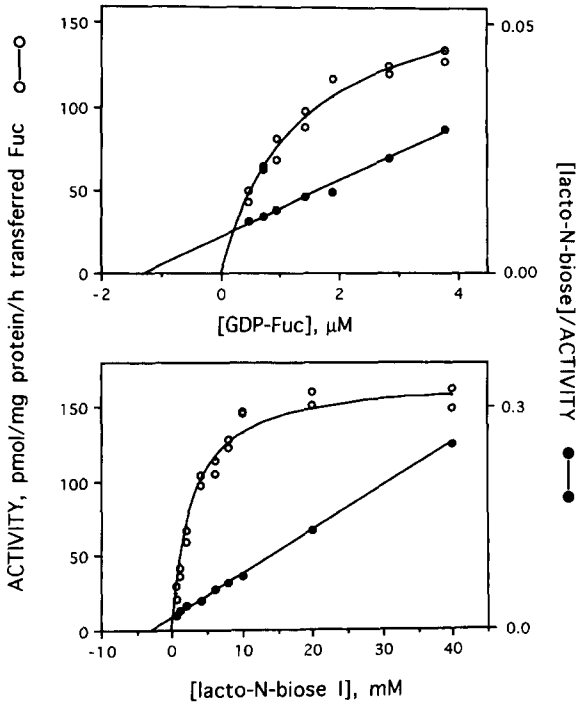

Fig. 1. Kinetic characterization of Dα1,2FucT. Enzyme activity measurements were carried out as described under Section 2. The effect of donor GDP-Fuc (upper panel) and acceptor lacto-N-biose I (lower panel) is presented. Blank values obtained in parallel reactions lacking the acceptor were subtracted from each point. Activity data (○) represent duplicate assays. The right scale (●) shows the transposition in the Hanes-Woolf plot of the activity values according to the formula: $[S]/v = 1/V_{max} \times [S] + K_m/V_{max}$, where velocity ($v$) values are the activity values expressed as pmol/mg protein per h transferred Fuc.

3.2. Characterization of the enzymatic activity

Using the cytosolic fraction as the enzyme source, we found that the fucosyltransferase activity is linear over the protein concentration range from 0.5 to 5 mg/ml and increases linearly with incubation time from 20 to 120 min. It is readily detectable in a relatively broad pH range, from 6.5 to 7.2, with the maximum being attained at pH 6.9. Maximum activity is at 23°C, while at 16 and 30°C it is 61 and 62% of the maximum, respectively, and drops to less than 30% at 4 or 37°C. Activity with lacto-N-biose I is totally inhibited by EDTA, and is strongly stimulated by divalent cations such Mn^{2+} and Mg^{2+}. Mn^{2+} stimulation is evident at a concentration of 5 mM, and then increases slowly up to 20 mM. It is also stimulated by low concentrations of ATP and to a lower extent by Fuc. Incorporation is completely inhibited by treatment with 30 mM NEM and by heating at 100°C. Detergent inhibits the activity in a concentration-dependent manner; 0.2 mg/ml Triton X-100 in the incubation mixture provides about 39 and 47% inhibition using the cytosolic or the postnuclear fractions, respectively. None of the following tested compounds is able to act as acceptor: Gal, phenyl-β-galactoside, Galβ1-6GlcNAc, lactose, Galβ1-6Gal, Xyl, Glc, GlcNAc, and GalNAc. In particular, Gal is totally ineffective at any tested concentration; a little activity is measurable with Galβ1-3Gal1-OMe and with LacNAc at high concentrations (over 60 mM).

The dependence of Dα1,2FucT on the concentrations of GDP-Fuc as donor and lacto-N-biose I as acceptor follows a typical Michaelis-Menten curve (Fig. 1). Apparent calculated K_m for the donor is 1.27 μM; apparent calculated K_m and V_{max} for the acceptor are 2.8 mM and 169.5 pmol/mg protein per h transferred Fuc, respectively.

3.3. Purification and fucosidase treatment of the reaction product

The reaction products with unlabelled lacto-N-biose I as acceptor and GDP-[^{3}H]Fuc as donor of Dα1,2FucT, Hα1,2FucT (H type 1 trisaccharide) and Fuc-TIII (Lewis a trisaccharide), obtained by Dowex chromatography, were freeze-dried and subjected to gel permeation chromatography on Biogel P2. Hα1,2FucT reaction provides two peaks which represent over 95% of the loaded radioactivity. The first peak, representing 91% of the recovered radioactivity, is assumed as the reference H type 1 trisaccharide; the second peak is eluted at the size of monosaccharides. Fuc-TIII reaction provides two peaks which represent over 95% of the loaded radioactivity. The first peak, representing 89% of the recovered radioactivity, is assumed as the reference Lewis a trisaccharide; the second peak is eluted at the size of monosaccharides. Parallel chromatography of the Dα1,2FucT reaction product provides two peaks representing 91% of loaded radioactivity. Both peaks correspond to those obtained with Hα1,2FucT and Fuc-TIII, but in this case the first peak, eluted at the same size of H type 1 and Lewis a trisaccharides, represents 71% of the recovered radioactivity. H type 1 trisaccharide, Lewis a trisaccharide, and the corresponding peak obtained with the Dα1,2FucT product, as well as the minor peaks eluted in the monosaccharide area, provide single spots when analyzed by HPTLC. Spots derived from the minor peaks appear identical by HPTLC, and comigrate with reference standard Fuc (not shown). The spot derived from the Dα1,2FucT major peak comigrates with H type 1 trisaccharide, moves faster than the Lewis a trisaccharide, and is affected by bovine epididymis α-fucosidase but not by almond meal α-fucosidase (Fig. 2). Under the conditions used (see Section 2), the H type 1 trisaccharide is also sensitive to bovine epididymis α-fucosidase, and insensitive to the almond meal enzyme, while the Lewis a trisaccharide is totally sensitive to almond meal α-fucosidase treatment, and almost insensitive to bovine epididymis α-fucosidase (not shown).

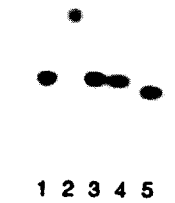

Fig. 2. HPTLC analysis and fucosidase treatment of Dα1,2FucT reaction product. Lanes: (1) purified reaction product of Dα1,2FucT; (2) bovine epididymis α-fucosidase treatment of lane 1; (3) almond meal α-fucosidase treatment of lane 1; (4) H type 1 trisaccharide (purified reaction product of Hα1,2FucT); (5) Lewis a trisaccharide (purified reaction product of Fuc-TIII). Reaction product purification and fucosidase treatments were performed as described under Section 2. HPTLC plate was developed using *n*-propanol/ethyl acetate/water/25% ammonia (6:1:3:1, v/v), as the eluting solvent system; detection was by fluorography. An additional lane, spotted with unlabelled reference standard Fuc, was cut after developing the plate and detected by anisaldehyde spray reagent.

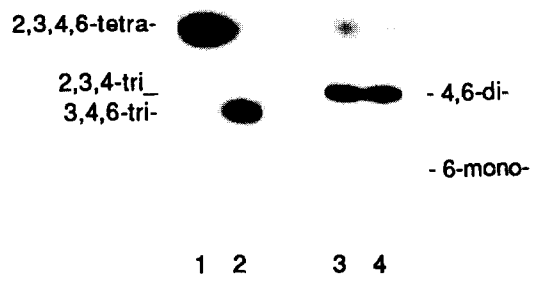

Fig. 3. HPTLC analysis of partially methylated derivatives. Lanes: (1) partially methylated galactose from unreacted [*Gal*-^{3}H]lacto-N-biose I; (2) partially methylated galactose from [*Gal*-^{3}H]Dα1,2FucT product; (3) partially methylated glucosamine from unreacted [*GlcNAc*-^{3}H]lacto-N-biose I; (4) partially methylated glucosamine from [*GlcNAc*-^{3}H]Dα1,2FucT product. Permethylation and hydrolysis were performed as described under Section 2. HPTLC plate was developed using *n*-propanol/ethyl acetate/water/25% ammonia (6:1:3:1, v/v), as the eluting solvent system; detection was by fluorography. Additional lanes, spotted with reference standard methylated galactoses and glucosamines, were cut after developing the plate and unlabelled reference standards detected by molybdate spray reagent. The position of reference 2,3,4,6-tetra-, 2,3,4-tri-, and 3,4,6-tri-*O*-methylgalactoses is indicated on the left; the position of reference 4,6-di-, and 6-mono-*O*-methylglucosamines is indicated on the right.

3.4. Analysis of partially methylated derivatives

The reaction products of Dα1,2FucT with [*Gal*-^{3}H]lacto-N-biose I or [*GlcNAc*-^{3}H]lacto-N-biose I as acceptors and unlabelled GDP-Fuc as donor, purified by Dowex chromatography, gel permeation chromatography on Biogel P2, and preparative HPTLC, are chromatographically identical to the corresponding (*Fuc*-^{3}H)-labelled product. After permethylation, hydrolysis, and analysis by HPTLC in two solvent systems, the [*Gal*-^{3}H]trisaccharide product of Dα1,2FucT provides a spot moving slower than that obtained with unreacted [*Gal*-^{3}H]lacto-N-biose I (identified as 2,3,4,6-tetra-*O*-methylgalactose) and corresponding to reference 3,4,6-tri-*O*-methylgalactose (Fig. 3). After parallel permethylation, hydrolysis, and analysis by HPTLC, the (*GlcNAc*-^{3}H)-labelled trisaccharide product of Dα1,2FucT provides a spot comigrating with that obtained with unreacted [*GlcNAc*-^{3}H]lacto-N-biose I (identified as 4,6-di-*O*-methylglucosamine), which moves faster than reference mono-*O*-methylglucosamine (Fig. 3).

4. Discussion

In this paper we present evidence that the fucosyltransferase from the cytosol of *Dictyostelium* AX2 cells is able to use lacto-N-biose I as acceptor forming the H type 1 trisaccharide product. Optimum assay conditions include neutral pH and 23°C temperature, and divalent cations are absolute requirements for activity detection. Dα1,2FucT activity is quantitatively recovered in a cytosolic fraction, with respect to the homogenate, and is undetectable in membrane fractions. Accordingly, its activity is not stimulated, but instead inhibited, by detergent treatment. In addition it has a very high affinity for GDP-Fuc and is not able to use LacNAc efficiently as substrate. These data strongly suggest that Dα1,2FucT is the same fucosyltransferase as recently purified by West et al. [4]. We also found that Dα1,2FucT is inhibited by NEM, as other fucosyltransferases [10,13]. In this regard, it has been recently suggested that NEM sensitivity is related to specific amino acid sequence in the GDP-Fuc binding domain [14]. Dα1,2FucT activity transfers Fuc only to the galactose moiety of lacto-N-biose I in an α1-2 linkage. Upon gel permeation chromatography, the (*Fuc*-^{3}H)-labelled product was found of the same size of H type 1 and Lea trisaccharides. In addition, analysis by a rapid and effective HPTLC method indicated that it comigrates with H type 1 trisaccharide and is sensitive to α-fucosidase treatment. In particular, we found that fucose is almost completely cleaved from the Dα1,2FucT product by bovine epididymis α-fucosidase under conditions that leave Lewis a trisaccharide almost unaffected. Treatment with almond meal α-fucosidase determines the opposite effect. Bovine epididymis α-fucosidase is known to cleave α1-6 fucosyl residues [15] but its strict specificity is not defined, while almond meal α-fucosidase is considered to have α1-3/4 preference [16]. In order to establish unambiguously sugar substitution and linkage position, we perform a permethylation study. For this purpose, we prepared enough Dα1,2FucT product radiolabeled either on the Gal or the GlcNAc moiety. The results clearly indicate that only the galactose residue is substituted in the product, while the GlcNAc residue is not. Since the partially methylated galactose obtained comigrates with reference 3,4,6-tri-*O*-methylgalactose, we conclude that the reaction product of Dα1,2FucT with lacto-N-biose I as acceptor is Fucα1-2Galβ1-3GlcNAc (H type 1 trisaccharide). Dα1,2FucT does not efficiently utilize Gal and β-galactosides different from lacto-N-biose I, which instead are acceptors commonly used by mammalian α1,2fucosyltransferases. Since the putative endogenous substrate of Dα1,2FucT is reported to lack hexosamines [5], we suggest that the Galβ1-3GlcNAc sequence mimics in vitro the FP21 oligosaccharide structure required for enzyme recognition, that is not defined at the present. In this regard, it is interesting to note that the secretor-type α1,2fucosyltransferases prefer type 1 chain acceptors, but can still utilize Gal, phenyl-β-galactoside, and type 2 acceptors, and are not stimulated by divalent cations [17–19]. Our data indicate that Dα1,2FucT is distinct from other α1,2 fucosyltransferases but retain the ability to synthesize the H type 1 trisaccharide in vitro, while cytosolic glycosyltransferases reported to date do not share relevant common characteristics with membrane enzymes. Other known cytosolic glycosylations involve the construction of short disaccharide sequences or the addition of single monosaccharide units, which are thought to be mainly regulatory modifications; potential roles include enzyme regulation, protein folding and stability, and intracellular transport [2]. The characteristics of Dα1,2FucT here reported, together with the information available on FP21 [3–5], suggest an even more complex role of such a cytosolic glycosylation pathway in *Dictyostelium*.

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