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GLYCOSYLTRANSFERASES OF THE HUMAN CERVICAL EPITHELIUM

I. CHARACTERIZATION OF A β-GALACTOSIDE α-2-L-FUCOSYLTRANSFERASE AND THE IDENTIFICATION OF A β-*N*-ACETYLGLUCOSAMINIDE α-3-L-FUCOSYLTRANSFERASE

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Using phenyl β-D-galactoside as an acceptor, α-2-L-fucosyltransferase activity was identified in human cervical epithelium with pH optima at 6.0 and 7.2. The different response to *p*-chloromercuribenzoate, and ability to utilise asialofetuin as an acceptor, suggests the presence of two fucosyltransferases. The acid form is probably involved in glycoprotein synthesis in vivo. At pH 6.0, fucosyltransferase has a temperature optimum of 25°C, requires the presence of Triton X-100 and either manganese or magnesium for maximal activity, and has K_m values for GDP-L-[^{14}C]fucose and phenyl β-D-galactoside of $32.1 \cdot 10^{-6}$ M and $8.2 \cdot 10^{-3}$ M, respectively. Guanosine nucleotides are potent inhibitors of the fucosyltransferase reaction; GDP is a competitive inhibitor while, depending on its concentration, GTP can either inhibit or activate the reaction. The α-L-fucosidase present in cervical tissue has negligible activity towards the enzyme product, phenyl-α-2-L-[^{14}C]fucosyl-β-D-galactoside. The use of high and low molecular weight acceptors indicates the presence of a β-*N*-acetylglucosaminide α-3-L-fucosyltransferase and an *N*-acetylgalactosaminide fucosyltransferase.

Introduction

The H antigenic structure, Fucα(1 → 2)Gal, is found in many epithelial glycoproteins of individuals possessing the secretor (Se) gene [1]. The H gene specified, α-2-L-fucosyltransferase which synthesizes this structure, has been found in soluble form in human serum [2,3] and milk [4], and in particulate form in stomach mucosa and submaxillary gland [5]; in addition it has been identified in ovine brain [6] and rat intestinal mucosa [7]. Recently, the enzyme from porcine submaxillary gland has been purified to homogeneity and fully characterised [8,9]. Two further fucosyltransferases involved in the synthesis of epithelial glycoproteins include an *N*-acetyl-D-glucosaminide α-3-L-fucosyltransferase and the Le gene specified *N*-acetyl-D-glucosaminide α-4-L-fucosyltransferase [10].

During the normal menstrual cycle, human cervical mucus demonstrates an hormonally-dependent cyclic variation in its rheological properties [11]. The mucus glycoprotein responsible for the viscoelastic properties of cervical mucus [12] shows a variation in the proportion of the terminal saccharide, L-fucose, during the cycle [13,14]. Structural alterations associated with these compositional changes have not been described. Published data is not available on the oligosaccharide structure of human cervical mucus glycoprotein, but studies by Hatcher et al. [15] and Nasir-ud-Din et al. [16] using bonnet monkey (*Macaca radiata*) cervical mucin have demonstrated the presence of L-fucose in the following structures: Fucα(1 → 2)Gal, Fucα(1 → 6)Gal and Fucα(1 → 3)-GalNAc. Fucosyltransferases which synthesize the last

two structures have not yet been identified.

As part of a study of the control of L-fucose metabolism in human cervical mucus glycoprotein, an investigation into the occurrence and properties of fucosyltransferases in the human cervical epithelium was undertaken. A knowledge of the specificity of these enzymes augments more direct forms of physico-chemical analysis which may be used to obtain structural information about the mucus glycoprotein and allows the study at the enzyme level of potential, cyclic, structure-function relationships involving the terminal saccharide, L-fucose. This paper describes the characterization of a β-galactoside α-2-L-fucosyltransferase and the identification of a β-*N*-acetyl-D-glucosaminide α-3-L-fucosyltransferase.

Materials and Methods

GDP-L-[U-^{14}C]fucose (4.26 GBq mmol^{-1}) was supplied by the Radiochemical Centre, Amersham, U.K. Agarose-ε-amino-caproyl-fucopyranosylamine (Lot AF38) was from Miles-Yeda, Israel. Bovine epididymal α-L-fucosidase, mono- and disaccharides, nucleotides and fetuin were from Sigma. The linkage isomers of galactosyl-*N*-acetylglucosamine were gifts from Dr. A. Gauhe and Professor R. Jeanloz. Human α_1 acid glycoprotein was purchased from Miles Laboratories.

Preparation of acceptors. Asialofetuin and asialo α_1 acid glycoprotein were prepared by hydrolysis of the parent glycoprotein with 0.025 M H_2SO_4 acid at 80°C for 1 h. After exhaustive dialysis against distilled water the protein solution was lyophilized. Hydrolysis removed more than 98% of the expected sialic acid, as monitored using the periodate-thiobarbituric acid method [17], exposing 0.24 and 0.36 μmol terminal galactose residues/mg asialofetuin and asialo α_1 acid glycoprotein, respectively (calculated assuming that each mol of *N*-acetylneuraminic acid hydrolysed was linked to 1 mol sub-terminal galactose). Agalacto-asialofetuin was prepared by a limited Smith degradation of asialofetuin [18]. GLC analysis revealed that more than 99% of the expected galactose had been removed, exposing 0.16 μmol terminal *N*-acetylglucosamine residues/mg glycoprotein.

Preparation of tissue homogenate. Cytologically normal cervical tissue was obtained from premenopausal women at routine hysterectomy. Immediately after excision, the cervix was cut from the uterus and the region between the internal and external os dissected out. After washing with 0.15 M NaCl to remove any mucus or blood, the epithelium was dissected from the underlying tissue, adjusted to 10% w/v with 0.25 M sucrose, pH 7.0, and homogenised by 4 or 5 strokes in a glass in glass, hand-held homogeniser. All manipulations were carried out at the temperature of melting ice. The homogenate was centrifuged at 2 500 × *g* for 15 min at 4°C, and the supernatant stored at −20°C until subsequent assay.

Fucosyltransferase assay. The standard incubation mixture contained phenyl β-D-galactoside, GDP-L-[^{14}C]fucose, cation, ATP, detergent, buffer and enzyme in a final volume of 80 μl as shown in Fig. 1. A control incubation lacking exogenous acceptor was included for all assays. The assay reaction products were separated by ion-exchange or paper chromtography.

Ion-exchange chromatography. The reaction mixture was diluted with 1 ml distilled water and passed down a column of 0.8 ml Dowex AG-1X2 (100–200 mesh, Cl^- form) contained in a Pasteur pipette. The column was washed with 1 ml distilled water and the combined eluates added to 15 ml Permablend II scintillant (Packard) and counted on a Beckman LS250 scintillation counter. The difference between the total counts of the complete assay mixture and the control was equivalent to the counts incorporated into the exogenous acceptor.

Descending paper chromatography. Absolute alcohol (80 μl) was added to the assay mixture which was mixed and centrifuged at 2 000 × *g* for 10 min at room temperature. An aliquot of the supernatant was chromatographed on Whatman No 1 for 6 h in a solvent system of ethyl acetate/pyridine/water (10 : 4 : 3, v/v). Reference standards of L-fucose and lactose were chromatographed and located with alkaline silver nitrate [19]. Radioactive areas were measured after cutting the paper into strips which were added to 15 ml Permablend III scintillant (Packard). If the L-[^{14}C]fucosylated product had a similar mobility to GDP-L-[^{14}C]fucose the assay mixture was first treated with Dowex AG-1X2 and lyophilized. The residue was dissolved in 50% aqueous ethanol and treated as above.

Enzymic hydrolysis of phenyl-L-[^{14}C]fucosyl-β-

D-*galactoside*. The area on the paper chromatogram containing phenyl-L-[^{14}C]fucosyl-β-D-galactoside was eluted with distilled water by descending paper chromatography and the eluate lyophilized giving 58 pmol (15 000 cpm) product. The residue was dissolved in 1 ml distilled water containing 20 μmol $CaCl_2$ and 500 μmol $(NH_4)_2SO_4$ and incubated with 0.05 unit bovine epididymal α-L-fucosidase for 1 h at 37°C. The reaction mixture was heated at 100°C for 1 min and desalted by passage down a column (1.5 × 7.0 cm) of Biodeminerolit (BDH) mixed bed ion-exchange resin (H^+ and OH^- form). The column was washed with 25 ml distilled water and the eluates combined, lyophilized, dissolved in 50% aqueous ethanol and chromatographed on Whatman No. 1 as above.

*Alkaline treatment of phenyl-*L*-[^{14}C]fucosyl-β-*D*-galactoside*. Phenyl-L-[^{14}C]fucosyl-β-D-galactoside (81 pmol, 21 00 cpm) was incubated with 500 μl 0.5 M NaOH at 100°C for 10 min, cooled and titrated to neutrality. The reaction mixture was desalted by treatment with Biodeminerolit, lyophilized, dissolved in 50% aqueous ethanol and subjected to paper chromatography as above.

Treatment of homogenate with agarose-ε-amino-caproyl-fucopyranosylamine. Pooled tissue homogenate (2.4 g protein l^{-1}) was passed down a column (0.5 × 4.0 cm) of agarose-ε-amino-caproyl-fucopyranosylamine, equilibrated with 10 mM NaH_2PO_4 to remove α-L-fucosidase [20]. The eluate and a control homogenate were assayed for α-L-fucosidase and total protein.

Assay of α-L-*fucosidase*. The activity of α-L-fucosidase was assayed using 4-methylumbelliferyl-α-L-fucopyranoside as described by Robinson and Thorpe [21].

Total protein. Total protein was measured by the method of Lowry et al. [22] using bovine serum albumin as a standard.

Results

Under the standard assay conditions, with phenyl β-D-galactoside as the exogenous acceptor, two well defined peaks of fucosyltransferase activity were identified, at pH 6.0 and 7.2 (Fig. 1). Activity at each pH could be differentiated by the temperature optimum, response to *p*-chloromercuribenzoate, and

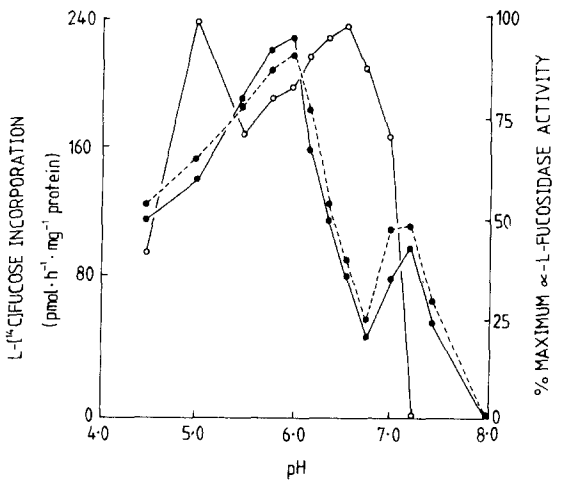

Fig. 1. Effect of pH on the incorporation of L-[^{14}C]fucose into phenyl β-D-galactoside in a control homogenate (●——●) and an homogenate treated with agarose-ε-amino-caproyl-fucopyranosylamine (● - - - - - - ●). The activity of α-L-fucosidase in the control homogenate is also shown (○——○) and is expressed as the percent of its maximum activity at pH 5.0. The activity of α-L-fucosidase in the affinity-treated homogenate was less than 1% of its control counterpart at each pH value. The standard fucosyltransferase assay mixture contained in a final volume of 80 μl: GDP-L-[^{14}C]fucose, 0.53 nmol (137 500 cpm), phenyl β-D-galactoside, 6.4 μmol; ATP, 0.162 μmol; Triton X-100, 500 μl · l^{-1}; $MnCl_2$, 0.8 μmol; cacodylate/HCl buffer pH 6.0, 4.05 μmol; and cervical tissue homogenate containing up to 3.0 g · l^{-1} protein, 20 μl. Incubation was at 37°C for 5 h. Other buffers used were as follows: pH 4.0–4.5, sodium acetate; pH 5.0–7.0, cacodylate/HCl; and pH 7.2–8.0, Tris-HCl. Each buffer at 4.05 μmol/assay.

substrate specificity, thus suggesting the presence of two enzymes. At pH 6.0 the temperature optimum for the incorporation of L-[^{14}C]fucose into phenyl β-D-galactoside was 25°C, while at pH 7.2 it was 37°C. Although both acid and neutral forms of the enzyme were almost totally inhibited by 3 mM *N*-ethylmaleimide, a differential effect was seen in the presence of an alternative thiol blocking reagent, *p*-chloromercuribenzoate. At a *p*-chloromercuribenzoate concentration of 50 μM there was total loss of enzyme activity at pH 6.0, while at pH 7.2 almost 30% of the original enzyme activity remained. Although asialofetuin served as an acceptor substrate at pH 6.0, no incorporation of L-[^{14}C]fucose could be demonstrated at pH 7.2. Finally, the presence of

two distinct forms of the enzyme was inferred by the observation that the ratio of acid to neutral fucosyltransferase activity was highly variable in different cervical biopsies. In eight individual biopsies this ratio varied between 1 : 1 and 13 : 1. In view of its probable significance in glycoprotein synthesis in vivo, further characterization of fucosyltransferase activity was restricted to the form demonstrating activity at pH 6.0.

Relation between fucosyltransferase and α-L-fucosidase activity

Using 4-methylumbelliferyl-α-L-fucopyranoside as a substrate, levels of α-L-fucosidase in tissue homogenates were typically between 0.4–1.0 mU mg^{-1} protein. The effect of endogenous α-L-fucosidase on the activity of fucosyltransferase at pH 6.0 was investigated by incubating the enzyme product, phenyl-L-[^{14}C]fucosyl-β-D-galactoside (21 000 cpm), with tissue homogenate and monitoring its hydrolysis. The standard assay was scaled up 10-times and incubated at 37°C for 2 h, when the fucosyltransferase reaction was completely inhibited with 5 mM GDP by addition of the solid reagent and an aliquot removed for the assay of phenyl-L-[^{14}C]-fucosyl-β-D-galactoside and α-L-fucosidase. At a concentration of 5 mM, GDP had no effect on the activity of α-L-fucosidase. After a 24 h incubation period more than 80% of the original α-L-fucosidase activity remained but hydrolysis of phenyl-L-[^{14}C]-fucosyl-β-D-galactoside was less than 7%.

The effect of endogenous α-L-fucosidase on the pH curve of fucosyltransferase was studied by comparing pH profiles of fucosyltransferase in a pooled control homogenate and the same homogenate treated with the affinity resin agarose-ε-aminocaproyl-fucopyranosylamine (Fig. 1). The two pH profiles of fucosyltransferase activity displayed the same optima, while the specific activity at each pH was essentially the same. Incorporation of L-[^{14}C]-fucose into endogenous acceptor could not be demonstrated in either the control or affinity resin-treated homogenate. At each pH investigated the affinity-treated homogenate retained less than 1% of the α-L-fucosidase activity of the control. The effect of endogenous α-L-fucosidase on the measurement of fucosyltransferase activity, using phenyl β-D-galactoside as an acceptor, can therefore be considered minimal.

Optimum conditions for the assay of fucosyltransferase activity

The rate of L-[^{14}C]fucose incorporation into phenyl β-D-galactoside at pH 6.0 was constant for up to 5 h at 37°C; after 24 h approx. 60% of the original enzyme activity remained. Enzyme activity was proportional to the protein concentration to at least a final concentration of 0.75 g · l^{-1}. Fucosyltransferase demonstrated a partial requirement for Triton X-100 and either manganese or magnesium. Optimum stimulation of enzyme activity (230% of the control reaction rate) was seen at a Triton X-100 concentration of 500 μl · l^{-1}, above this concentration there was progressive inhibition of activity. A well defined optimum manganese concentration of 10 mM produced a 95% increase in the fucosyltransferase reaction rate.

Stability

Upon freezing the tissue homogenate to −20°C, up to 30% of the fucosyltransferase activity at pH 6.0 could be lost, but thereafter freezing and thawing had no significant effect on enzyme activity which was stable for at least 3 months.

Effect of ATP

ATP was routinely included in the assay to inhibit hydrolysis of GDP-L-[^{14}C]fucose by endogenous

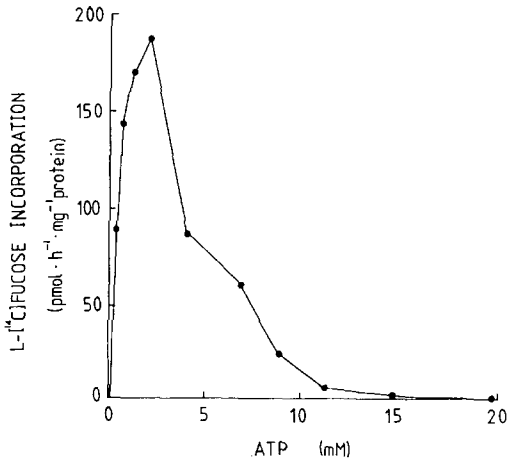

Fig. 2. Effect of ATP on the activity of cervical α-2-L-fucosyltransferase. Assay conditions are as described in Fig. 1, while the reaction products were separated on Dowex AG-1X2 (Cl^- form) prior to liquid scintillation counting.

nucleotide pyrophosphatase activity [2]. The effect of ATP on the fucosyltransferase reaction at pH 6.0 was monitored between 2.0 μM and 20 mM ATP. Optimum fucosyltransferase activity was seen at an ATP concentration of 2.0 mM (Fig. 2).

Product identification

The disaccharide product of the fucosyltransferase reaction at pH 6.0 showed a single band of radioactivity (R_{Lac} 4.0, R_{Fuc} 1.6) similar to that reported for phenyl-α-2-L-fucosyl-β-D-galactoside [2]. Treatment of phenyl-L-[^{14}C]fucosyl-β-D-galactoside (58 pmol, 15 000 cpm) with bovine epididymal α-L-fucosidase resulted in the release of more than 90% of the ^{14}C-label. This product co-chromatographed with authentic L-fucose, confirming the presence of an α anomeric linkage between L-fucose and galactose. No release of ^{14}C-label was detected when the enzyme product (81 pmol, 21 000 cpm) was treated with 0.5 M NaOH at 100°C for 10 min, thus substantiating the presence of a 1 → 2 linkage between L-[^{14}C]fucose and galactose which is stable under these conditions [23].

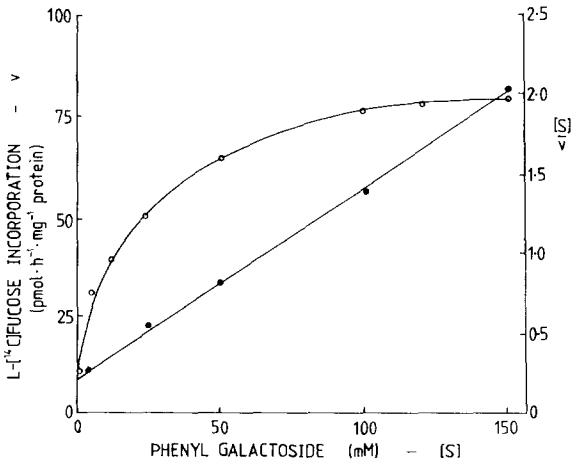

Fig. 3. Effect of substrate (phenyl β-D-galactoside) concentration, [S], on the activity, v, of cervical α-2-L-fucosyltransferase (o———o). Also shown is a Hanes-Woolf plot of [S]/v against [S] (•———•). Assay conditions are as described in Fig. 1, while assay products were separated on Dowex AG-1X2 (Cl^- form) prior to liquid scintillation counting.

Kinetic parameters

The affinity of fucosyltransferase for the donor substrate, GDP-L-[^{14}C]fucose, was investigated by varying the concentration of the nucleotide sugar between 0.03 and 21.2 μM. A plot of $1/v$ against $1/[S]$ was linear ($P < 0.001$) with a K_m of $32.1 \cdot 10^{-6}$ M and V of 0.73 nmol · h^{-1} · mg^{-1} protein. A plot of v against [S] for the acceptor substrate, phenyl β-D-galactoside, showed that maximal velocity was obtained at a concentration of 160 mM; above this there was a concentration-dependent inhibition of fucosyltransferase activity with 45% inhibition of the control reaction rate at a phenyl β-D-galactoside concentration of 400 mM. A plot of [S]/v against [S] was linear ($P < 0.001$) and yielded a K_m of $8.2 \cdot 10^{-3}$ M and V of 67.9 pmol · h^{-1} · mg^{-1} protein (Fig. 3).

Effect of guanosine nucleotides

The effect of guanosine nucleotides at concentrations between 2 μM and 20 mM on cervical β-galactoside α-2-L-fucosyltransferase activity was studied. GMP and GDP each inhibited fucosyltransferase activity at concentrations above 20 μM; GDP was the most potent inhibitor, completely inhibiting enzyme activity at 0.6 mM. At the same concentration, GMP inhibited the fucosyltransferase reaction by 55%. The nature of GDP inhibition was investigated by studying its effect on fucosyltransferase activity at two fixed concentrations of GDP-L-[^{14}C]fucose. Graphical evaluation of the data using the method described by Dixon [24] revealed that GDP was behaving as a competitive inhibitor and had a K_i of 50 μM.

In the presence of GTP a dual effect on fucosyltransferase activity was seen. At concentrations above 20 μM, GTP inhibited the incorporation of L-[^{14}C]-fucose into phenyl β-D-galactoside, but below this concentration there was a marked stimulation of enzyme activity with 160% of the control reaction rate being seen at 2 μM GTP.

Acceptor specificity of cervical fucosyltransferase activity

The acceptor substrate specificity at pH 6.0 of cervical fucosyltransferase was investigated by studying the incorporation of L-[^{14}C]fucose into several mono- and disaccharides, and derivatives of

TABLE I

Incorporation of L-[^{14}C]fucose into low molecular weight and glycoprotein acceptors. L-[^{14}C]Fucose incorporation into various acceptors has been expressed relative to the incorporation into phenyl β-D-galactoside, arbitrarily set at 100. Fucosyltransferase assay conditions were as described in Fig. 1, with low molecular weight and glycoprotein acceptors at an assay concentration of 80 mM and 12.5 g · l^{-1}, respectively. L-[^{14}C]Fucose incorporation was determined by liquid scintillation counting after the reaction products had been treated with Dowex AG-1X2 (Cl^- form).

Acceptor	Relative incorporation of L-[^{14}C]Fucose
Phenyl β-D-galactoside	100
D-Galactose	79
N-Acetyl-D-glucosamine	0
Lactose (Galβ (1 → 4) Glc)	350
Lacto-*N*-biose I (Galβ (1 → 3) GlcNAc)	524
N-Acetyllactosamine (Galβ (1 → 4) GlcNAc)	360
N-Acetylallolactosamine (Galβ (1 → 6) GlcNAc)	86
Di-*N*-Acetylchitobiose (GlcNAcβ (1 → 4) GlcNAc)	121
Fetuin	23
Asialofetuin	57
Agalacto-asialofetuin	27
Asialo α_1 acid glycoprotein	22

fetuin and α_1 acid glycoprotein which were potential substrates for β-galactoside and β-*N*-acetylglucosaminide fucosyltransferases. The results of these incorporation studies are given in Table I.

The rate of L-[^{14}C]fucose incorporation into D-galactose, *N*-acetylallolactosamine (Galβ(1 → 6)-GlcNAc) and phenylβ-D-galactoside was very similar but enhanced incorporation into lactose (Galβ(1 → 4)-Glc), *N*-acetyllactosamine (Galβ(1 → 4)GlcNAc) and lacto-*N*-biose I (Galβ(1 → 3)GlcNAc) was observed.

Only one product of L-[^{14}C]fucosyl transfer to *N*-acetyllactosamine was detected which had an R_{Lac} (0.68) similar to the 3′ substituted trisaccharide, Galβ(1 → 4)[Fucα(1 → 3)]GlcNAc [2]. Further resolution of this product was not achieved indicating the absence of an α-2-L-fucosyltransferase which produces Fucα(1 → 2)Galβ(1 → 4)GlcNAc (R_{Lac} 0.99).

Di-*N*-Acetylchitobiose (GlcNAcβ(1 → 4)GlcNAc) also served as an acceptor indicating the presence of an *N*-acetylglucosaminide fucosyltransferase in the cervical epithelium. A single ^{14}C-labelled product migrating in the region of a trisaccharide with an R_{Lac} 0.55 and R_{Fuc} 0.19 was detected by paper chromatography. Enzymic incorporation of L-[^{14}C]-fucose into di-*N*-acetylchitobiose was almost completely inhibited by 3 mM *N*-ethylmaleimide, indicating that the product was the result of the action of an α-3-L-fucosyltransferase [25]. The K_m of di-*N*-acetylchitobiose was $3.3 \cdot 10^{-2}$ M with a V of 0.2 nmol · h^{-1} · mg^{-1} protein.

Asialofetuin showed the highest incorporation of L-[^{14}C]fucose of the glycoprotein acceptors tested. Fetuin, agalacto-asialofetuin and asialo α_1 acid glycoprotein also served as acceptors but at an efficiency approx. half that of asialofetuin.

Discussion

The study described above demonstrates the existence of an α-2-L-fucosyltransferase in the human cervical epithelium and confirms that phenylβ-D-galactoside is a specific acceptor which can be used for the assay of this enzyme. In common with other glycosyltransferases, the α-2-L-fucosyltransferase of cervical epithelium requires a divalent metal cation cofactor [26] and detergent [27] for maximum activity. The affinity of GDP-L-[^{14}C]fucose for α-2-L-fucosyltransferase (K_m $32.1 \cdot 10^{-6}$ M) is similar to the value reported for the rat intestinal enzyme [7] but is lower than that for the enzyme from porcine liver [28] or HeLa cells [29].

The bimodal pH profile of cervical α-2-L-fucosyltransferase is unusual and cannot be ascribed to the action of endogenous α-L-fucosidase degrading the substrate or products of the reaction. Maximum fucosyltransferase activity at pH 6.0 is also seen in the rat intestinal enzyme [7] but an optimum of pH 7.0–8.0 is reported for the human serum α-2-L-fucosyltransferase [25,30]. The significance of a second, minor, peak of cervical fucosyltransferase activity at pH 7.2 is not known. At this pH there is no incorporation of L-[^{14}C]fucose into asialofetuin, so it is unlikely that activity at pH 7.2 is due to the presence of the serum enzyme which has activity towards this acceptor [25]. Furthermore, absence of

activity towards this glycoprotein acceptor suggests that fucosyltransferase activity at pH 7.2 may not be significant in mucin synthesis in vivo. Other acceptors will have to be tested at this pH before physiological significance can be attributed to this activity.

Guanosine nucleotides were demonstrated to be inhibitors of cervical α-2-L-fucosyltransferase, the most effective being GDP, a product of the fucosyltransferase reaction, which was found to be a competitive inhibitor. Bella and Kim [31] have shown that GTP and GDP are competitive inhibitors of rat intestinal α-2-L-fucosyltransferase but a stimulatory effect of GTP has also been reported [28,32]. This stimulation has been ascribed to protection of GDP-L-fucose against hydrolysis by endogenous pyrophosphatase activity. The present study has demonstrated that at concentrations greater than 20 μM, GTP will inhibit cervical α-2-L-fucosyltransferase activity but below 20 μM markedly stimulates the fucosyltransferase reaction. At concentrations of GTP less than 20 μM it is unlikely that its presence as a nucleoside triphosphate is affecting the hydrolysis of GDP-fucose since an optimal concentration of 2.0 mM ATP is included in the assay to inhibit endogenous pyrophosphatase. A direct stimulation of cervical α-2-L-fucosyltransferase by GTP must therefore be considered a possibility.

The use of mono- and disaccharide acceptors allows more specific information to be gained about the acceptor specificity of glycosyltransferases and simplifies analysis of the linkage synthesized. Higher activity was shown using *N*-acetyllactosamine and lacto-*N*-biose I as acceptors than with phenylβ-D-galactoside. The two most likely mono-L-[^{14}C]-fucosylated products with *N*-acetyllactosamine as the exogenous acceptor are Fucα(1 → 2)Galβ(1 → 4)-GlcNAc and Galβ(1 → 4)[Fucα(1 → 3)]GlcNAc. The possibility of the tetrasaccharide product being synthesized with *N*-acetyllactosamine substituted with L-[^{14}C]fucose at the C-2 of galactose and C-3 of *N*-acetylglucosamine may also exist. In the present study only one product was detected in this reaction which had a chromatographic mobility similar to the trisaccharide substituted at the C-3 of *N*-acetylglucosamine. In view of the presence of β-galactoside α-2-L-fucosyltransferase activity in the cervical epithelium (demonstrated by activity towards phenylβ-D-galactoside) it is perhaps surprising that Fucα(1 → 2)Galβ(1 → 4)GlcNAc, which is the major product of the serum fucosyltransferase reaction using *N*-acetyllactosamine as an acceptor [2], was not also identified as a product. Both serum and cervical α-2-L-fucosyltransferase demonstrate higher activity towards phenylβ-D-galactoside than *N*-acetyllactosamine, but while the serum enzyme shows very low activity towards D-galactose compared with phenylβ-D-galactoside, the activity of cervical fucosyltransferase towards these two acceptors is essentially the same.

In the present investigation only one enzyme product was identified using di-*N*-acetylchitobiose as an acceptor, although this disaccharide can act as a substrate for both α-3-L- and α-6-L-fucosyltransferase [25,33,34]. Inhibition of the cervical *N*-acetylglucosaminyl fucosyltransferase by 3 mM *N*-ethylmaleimide [25,34] indicates that di-*N*-acetylchitobiose is acting as a substrate for a cervical α-3-L-fucosyltransferase. If this activity is associated with the synthesis of cervical mucus glycoprotein it would indicate a different position of L-fucose to that reported for bonnet monkey cervical mucin [16]. Inhibition studies have also demonstrated that, in common with the human serum enzyme, cervical α-2-L-fucosyltransferase is almost totally inhibited by 3 mM *N*-ethylmaleimide.

The pattern of enzyme mediated incorporation of L-[^{14}C]fucose into high molecular weight glycoprotein acceptors (fetuin, its asialo and agalactoasialo derivatives and asialo α_1 acid glycoprotein) at pH 6.0 was not established.

Both asialofetuin and asialo α_1 acid glycoprotein can serve as acceptors for the simultaneous measurement of α-2-L- and α-3-Lfucosyltransferase [25,28,35]. The high relative activity of cervical fucosyltransferase towards asialofetuin compared with asialo α_1 acid glycoprotein may be due in part to the higher concentration of terminal *N*-acetyllactosamine structures in asialofetuin and may also reflect the resistance of tetraantennary structures such as α_1 acid glycoprotein to fucosylation by the cervical enzyme.

The *N*-ethylmaleimide inhibition of L-[^{14}C]fucose incorporation into di-*N*-acetylchitobiose indicates that agalacto-asialofetuin is not acting as a substrate for a cervical α-6-L-fucosyltransferase. Since this glycoprotein does not fulfil the substrate requirement

for a galactoside α-3-L-fucosyltransferase it is possible that L-[^{14}C]fucose incorporation is a result of the synthesis of Fucα(1 → 3)GalNAc structures associated with the *O*-glycosidically linked oligosaccharides of fetuin. Similar structures are found in bonnet monkey cervical mucin [16]. The use of alternative acceptors such as fish antifreeze glycoprotein [38] should help to decide whether or not α-*N*-acetylgalactosaminide fucosyltransferase activity is present.

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