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# Purification of the *N*-Acetylglucosaminide α(1-3/4)Fucosyltransferase of Human Milk

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**The *N*-acetylglucosaminide α(1-3/4)fucosyltransferase has been purified 1.8 x $10^6$-fold from human milk by ion-exchange chromatography, affinity chromatography on GDP-agarose and HPLC. The α(1-3/4)fucosyltransferase behaves in gel filtration-HPLC as a molecule of $M_r$ 98 000, and differs from the α(1-3)fucosyltransferase which behaves like a molecule of about $M_r$ 47 000. The enzyme is a glycoprotein, and the purified preparation appears in SDS polyacrylamide gel electrophoresis as a band of $M_r$ 44 000. The results present the first purification of human milk α(1-3/4)fucosyltransferase to apparent homogeneity, and suggest that the α(1-3/4)- and α(1-3)fucosyltransferases of human milk differ in their native molecular sizes, the former being a dimer of two subunits.**

Cell surface carbohydrates undergo many structural changes during embryogenesis and in the process of cancer development [1-3]. One of the most striking changes affects the expression of fucose residues bound by α(1-3)-linkage to *N*-acetylglucosamine residues of glycoproteins and glycolipids. This structure is an integral part of the antigen determinants called X and SSEA-1, as well as of some sialylated and repetitive structures forming distinct antigen determinants defined by monoclonal antibodies [2, 4]. In the early embryo the SSEA-1 antigen follows a developmentally regulated pattern of expression, and some evidence indicates the involvement of this structure in the process of compaction [5]. In an experimental mouse metastasis model on the other hand, the re-expression of this determinant on lectin-resistant variant cells appears to be associated with a reduced metastasizing potential [6].

The biosynthesis of the structures containing fucose residues bound by α(1-3)-linkage to *N*-acetylglucosamine residues is catalyzed by the corresponding α(1-3)fucosyltransferase (galactoside 3-fucosyltransferase, EC 2.4.1.152). This enzyme has been characterized from different cell types such as mouse melanoma [7], Chinese hamster ovary [8] and mouse teratocarcinoma cells [9]. While these enzymes only catalyze the biosynthesis of α(1-3)-

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bound fucose residues, human milk is reported to contain an enzyme that has the capability to catalyze the biosynthesis of fucose residues bound both by α(1-3)-and α(1-4)-linkages to *N*-acetylglucosamine and glucose (galactoside 3(4)-L-fucosyltransferase, EC 2.4.1.65) [10]. The expression of the the α(1-4)fucosyltransferase activity in the milk is correlated with the Lewis blood group of the individual, since in the Lewis negative phenotype this activity is missing [11]. However, human milk also contains another α(1-3)fucosyltransferase that does not have α(1-4)fucosyltransferase activity and that also differs from the α(1-3/4)fucosyltransferase in only using *N*-acetylglucosamine but not glucose residues as acceptors [12]. It has been suggested that the α(1-3/4)fucosyltransferase is the gene product of the Lewis gene, or alternatively, the gene product may be a modifier of the fucosyltransferase [10, 12].

The purification of human milk α(1-3/4)fucosyltransferase has been reported [10], but we have observed, using the more sensitive detection methods of silver staining and immunoblot analysis, that this preparation is still heterogeneous. Furthermore, the previous report did not take into account the co-purification of the α(1-3/4)- and the α(1-3)fucosyltransferases during affinity chromatography [13]. In this paper, we describe the purification of the α(1-3/4)fucosyltransferase of human milk to apparent homogeneity.

## Materials and Methods

### *Materials*

Human milk was obtained from the "Institut Pasteur" (Lille, France) and was stored frozen until use. Unlabelled GDP-fucose was a gift from Professor W. M. Watkins (Clinical Research Centre, Harrow, United Kingdom) and Ms. D. Monnom (Université Libre de Bruxelles, Brussels, Belgium). Rabbit antiserum to human milk fucosyltransferase and samples of fucosyltransferase were donated by Dr. J.-P. Prieels (Université Libre de Bruxelles, Brussels, Belgium).

The following materials were purchased from the sources in parentheses: *N*-acetyllactosamine, 2'-fucosyllactose, lacto-*N*-biose I and lacto-*N*-fucopentaose I and II (BioCarb, Lund, Sweden), methyl α-D-mannose (Merck, Darmstadt, W. Germany), delipidated bovine serum albumin, GMP and GDP-agarose (Sigma, St. Louis, MO, U.S.A.), Iodobeads (Pierce, Rockville, IL, U.S.A.), AG 1-X8 ion-exchange resin (100–200 mesh, chloride form) (Bio-Rad, Richmond, CA, U.S.A.), TSK CM-3SW HPLC-column (7.5 x 150 mm) (Pharmacia-LKB, Uppsala, Sweden), Superose 12 HPLC-column (1 x 30 cm), molecular-weight marker proteins for HPLC (thyroglobulin 669 000, ferritin 440 000, catalase 232 000, aldolase 158 000, bovine serum albumin 67 000, ovalbumin 43 000, chymotrypsinogen 25 000 and ribonuclease 13 700) and molecular-weight marker proteins for polyacrylamide gel electrophoresis (phosphorylase b 94 000, bovine serum albumin 67 000, ovalbumin 43 000, carbonic anhydrase 30 000, soybean trypsin inhibitor 20 100, lysozyme 14 400) (Pharmacia, Sweden), $^{125}$I-labelled molecular-weight marker proteins (phosphorylase b 93 000, bovine serum albumin 69 000, ovalbumin 46 000, carbonic anhydrase 30 000, soybean trypsin inhibitor 20 000) and $^{125}$I-labelled protein A (Amersham International, Amersham, U.K.), GDP-[$^{14}$C]fucose (222 mCi/mmol) (New England Nuclear, Boston, MA, U.S.A.).

*Fucosyltransferase Assay*

The reaction mixture (100 µl) contained 5 µmol of 3-(*N*-morpholino)propanesulfonic acid (Mops)/NaOH buffer pH 7.5, 0.5 µmol of $MnCl_2$, 10 µmol of NaCl, 1 nmol of GDP-fucose (19 500 cpm.), 100 µg of delipidated bovine serum albumin and 24 µmol of lactose. Fucosyltransferase (200 µU) was added into positive controls. Control assays without acceptor were performed to correct for endogenous acceptor activity. After 15 min at 37°C the reaction was stopped by the addition of 1 ml of cold water. The mixture was applied to a 1 ml column of AG 1-X8 in a Pasteur pipette. The column was washed with 1 ml of water, and the combined effluents containing the fucosylated product were collected in a scintillation vial for counting [14, 15]. An amount of 300-400 cpm. of radioactivity was observed for control samples incubated without fucosyltransferase, and this activity was subtracted from the sample radioactivities. One unit of activity was defined as the amount of enzyme that transfers 1 µmol of fucose/min under the standard assay conditions.

Assays for specific isoenzymes: α(1-3)fucosyltransferase activity was assayed as described above using 300 nmol of 2'-fucosyllactose or 150 nmol of *N*-acetyllactosamine instead of lactose. The α(1-4)fucosyltransferase activity was measured using 27 nmol of lacto-*N*-fucopentaose I or 26 nmol of lacto-*N*-biose I.

*Enzyme Purification Procedure*

A total of 72.5 l of human milk was processed through steps 1–4 in three aliquots of 2.5, 28 and 42 l. All operations except HPLC were performed at 4°C. Plasticware was used whenever possible.

*Step 1: Delipidation.* The thawed milk was pooled and centrifuged in a Beckman 6B-L centrifuge at 5 000 x *g* for 30 min. The fat was discarded and the milk serum was centrifuged again at 5 000 x *g* for 60 min. The small floating lipid particles were removed by aspiration.

*Step 2: Ion-exchange Chromatography on SP-Sephadex.* The delipidated milk was mixed with dry SP-Sephadex C-50 (2 g of SP-Sephadex/l of milk) and gently stirred overnight. After adsorption the resin was washed with three volumes of water, and poured into a column (10 cm diameter) filled to half of its volume with SP-Sephadex C-50 equilibrated with 10 mM sodium cacodylate buffer pH 6.5 containing 5 mM $MgCl_2$, 2% glycerol and 0.05% sodium azide. The column was eluted with a linear gradient of 0-600 mM NaCl in the equilibration buffer at a flow rate of 6 ml/min. The total volume of the gradient was ten column volumes. Fractions with fucosyltransferase activity were pooled.

*Step 3: Affinity chromatography on GDP-agarose.* The fucosyltransferase pool was dialysed overnight against 50 mM sodium cacodylate buffer pH 7.2 containing 100 mM NaCl and 25% (v/v) glycerol, and applied to a guanosine 5'-diphosphate-agarose column (2.5 x 7 cm, 1.3 µmole ligand/ml gel) equilibrated with the dialysis buffer. The column was washed with two volumes of the same buffer and eluted with 50 mM sodium cacodylate buffer pH 7.2 containing 0.5 mM GMP, 0.8 M NaCl, 25% glycerol and 0.05% sodium azide.

*Step 4: Cation-exchange HPLC.* The activity eluted from the affinity column was pooled and dialysed overnight against 20 mM sodium phosphate buffer pH 6.8 containing 10% glycerol

and 0.05% sodium azide, and concentrated in an Amicon ultrafiltration apparatus using a PM 10 filter. The concentrated sample was applied to a TSK CM-3SW HPLC-column (7.5 x 150 mm) and eluted with a gradient of 0-500 mM NaCl in the same buffer at a flow rate of 0.5 ml/min. The fractions containing fucosyltransferase were dialysed against 50 mM sodium cacodylate buffer pH 7.2 containing 0.1 M NaCl, 50% glycerol and 0.05% sodium azide, and stored in aliquots at –20°C.

*Step 5: Superose HPLC.* Dithiothreitol was added to a final concentration of 5 mM into aliquots (100 µl) of the fucosyltransferase, and the samples were dialysed against 50 mM Mops buffer pH 7.5 containing 0.1 M NaCl, 5 mM $MnCl_2$ and 2.5% lactose. The reduced samples were subsequently chromatographed on a Superose 12 HPLC-column (1 x 30 cm) equilibrated with 50 mM Mops buffer pH 7.5 containing 0.1 M NaCl and 10% glycerol. Elution was performed with the same buffer at a flow rate of 0.5 ml/min.

*Polyacrylamide Gel Electrophoresis and Isoelectric Focusing*

Electrophoresis in 10% polyacrylamide gels in the presence of SDS was performed as described by Laemmli [16]. The sample buffer consisted of 3 vol of water and 1 vol of 0.08 M Tris/HCl pH 8.0 containing 20% sucrose, 8% SDS, 4 mM EDTA and 0.01% bromophenol blue. Dithiothreitol was added to a final concentration of 10 mM, and the samples were heated at 98°C for 10 min and applied to the gel. Gels were stained with silver [17], and staining stopped with 5% acetic acid. Immunoblotting was performed as described by Towbin *et al.* [18] and isoelectric focusing and two-dimensional gel electrophoresis according to the method of O'Farrell [19].

*Preparation of Antibodies*

Mouse monoclonal antibodies were produced after *in vivo* immunization according to the method of Galfrè and Milstein [20]. Mice were immunized and boosted intraperitoneally with 200 µl of an emulsion containing up to a total of 400 µg of partially purified fucosyltransferase preparations (from the TSK CM-3SW HPLC column step, or of the purified preparation of Prieels *et al.* [10]). In other experiments mice were immunized with fucosyltransferase preparations (1.5 µg) adsorbed to pieces of nitrocellulose that were inserted by surgery close to the inguinal lymph nodes [21]. Direct intrasplenic immunization [22] was performed with 0.5 µg of the fucosyltransferase previously purified by Prieels *et al.*, and five days later the spleen was taken for fusion. *In vitro* immunization with fucosyltransferase preparations was carried out as described [23].

For the production of polyclonal antisera, Swiss female rabbits were immunized with multiple subcutaneous injections (100 µg antigen with complete Freund's adjuvant followed by the same amount of antigen with incomplete adjuvant six weeks later) according to the method of Vaitukaitis [24]. Three weeks later 150 µg of the antigen were injected intraperitoneally without adjuvant.

Antibody production was screened with standard methodology of radio-immunoassay [25], ELISA [26], and dot immunobinding assay [27]. Antibody inhibition of the fucosyltransferase was assayed as described before [10].

**Table 1**. Purification of fucosyltransferase from human milk.
The purification was performed from a total of 72.5 l of human milk which was processed up to the TSK CM-3SW step in three aliquots (2.5, 28 and 42 l). Only part of the material from the TSK CM-3SW step was used for the final purification by HPLC on the Superose column; the values in the Table were calculated to indicate total theoretical yield. Lactose was used as acceptor for fucosyltransferase.

| Step | Total protein (mg) | Specific activity (mU/mg) | Yield (%) | Purification (-fold) |
|---|---|---|---|---|
| Whole milk (72.5 l) | 1 250 000 | 0.00380 | 100 | 1 |
| SP-Sephadex C-50 | 864 | 3.63 | 66 | 956 |
| GDP-Agarose | 53.3 | 36.4 | 41 | 9590 |
| TSK CM-3SW HPLC | 3.92 | 394 | 33 | 104 000 |
| Superose 12 HPLC | 0.105 | 6780 | 15 | 1 790 000 |

*Lectin Affinity Chromatography*

Aliquots of fucosyltransferase were applied to 2 ml columns of concanavalin A- or lentil lectin-Sepharose equilibrated with 20 mM sodium cacodylate buffer pH 7.0 containing 2% glycerol and 0.05% sodium azide. After washing, elution was performed with the same buffer supplemented with 0.5 M methyl α-D-mannose. This sugar does not inhibit the fucosyltransferase activities.

*Other Methods*

Protein was assayed with the method of Lowry *et al.* [28] and with the method of Bradford (Bio-Rad) for low protein concentrations. Bovine serum albumin and lactoferrin were used as standards. Protein in the column effluents was monitored by absorbance at 280 nm, and GMP, GDP and GDP-fucose were detected at 254 nm. Labelling of fucosyltransferase was carried out by the Iodobead method as described by the manufacturer.

## Results

*Purification of Fucosyltransferase from Human Milk*

The results of the purification of the fucosyltransferase from human milk are summarized in Table 1. The first steps of the purification procedure were modified from the method of Prieels *et al.* [10]. The first modification involved defatting the milk prior to absorption to the

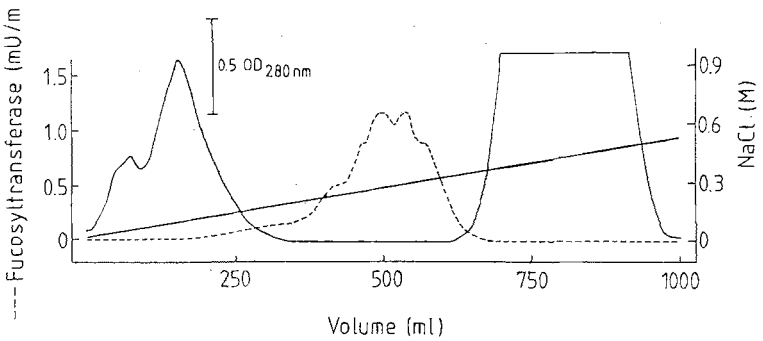


**Figure 1.** Purification of fucosyltransferase on a column of SP-Sephadex. The delipidated milk was adsorbed to SP-Sephadex and the gel suspension layered on a column of the same ion-exchanger. Elution was performed with a gradient of NaCl (0–600 mM) as described under Material and Methods. Elution of proteins was monitored by absorbance at 280 nm. The fractions containing the fucosyltransferase activity (ml 450 to 580) were taken for further purification.

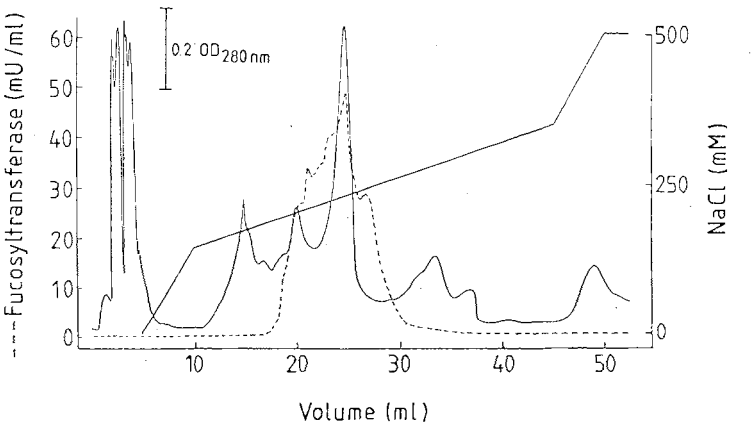


**Figure 2.** Purification of fucosyltransferase by HPLC on a column of TSK CM-3SW. The fucosyltransferase preparation obtained after affinity chromatography on GDP-agarose was subjected to chromatography as described under Material and Methods. The profile of the NaCl gradient (0–500 mM) is indicated. The effluent was monitored for proteins by absorbance at 280 nm. The fractions containing the fucosyltransferase activity (from 19-28 ml) were taken for further purification.

ion-exchanger. Essentially no fucosyltransferase activity was found in the milk fat globules, and removal of the fat facilitated the handling of the milk in subsequent steps and resulted in an improved yield in ion-exchange chromatography.

The second modification involved the elution of the fucosyltransferase from the SP-Sephadex ion-exchanger by linear gradient of salt, as opposed to the batch elution of the original procedure. As shown in Fig. 1, this resulted in a significantly improved purification, since the fucosyltransferase activity was eluted prior to the bulk of the other proteins including lactoferrin, a major contaminant of the fucosyltransferase preparation of Prieels *et al.* A minor part of the fucosyltransferase did not bind to the ion-exchanger. An increase in the incubation time or amount of SP-Sephadex did not result in the binding of the unbound fraction, which indicated that the the binding capacity of the ion-exchanger was not a limiting factor.

The substrate specificity of the unbound fucosyltransferase could not be determined because of the interference from the endogenous oligosaccharides present in the milk. However, as reported by Prieels *et al.*, the SP-Sephadex step removes the α(1-2)fucosyltransferase which does not bind to the ion-exchanger under the conditions used [10]. Both the α(1-3/4)- and the α(1-3)fucosyltransferase activities were present in the fucosyltransferase bound to SP-Sephadex.

In the affinity chromatography step, commercially available GDP-agarose was used, since the GDP-hexanolamine-Sepharose of the original procedure [29] was not available. This affinity matrix was found to behave similar to the GDP-hexanolamine-Sepharose, but was somewhat less efficient in purification due to the earlier elution of the fucosyltransferase from the column.

The fucosyltransferase preparation was in the next step subjected to HPLC on the cation-exchanger TSK CM-3SW. As seen in Fig. 2, the fucosyltransferase, still containing both the α(1-3/4)- and the α(1-3)fucosyltransferase activities, was eluted as a broad peak in the middle of the salt gradient.

*Separation of the α(1-3/4)- and the α(1-3)Fucosyltransferase Activities*

As the final step of purification, the fucosyltransferase preparation was subjected to HPLC on a Superose gel filtration column (Fig. 3). The yield of the fucosyltransferase was initially relatively low at this purification step. In an attempt to increase the yield, the test tubes and the column were treated with bovine serum albumin in order to reduce non-specific adsorption. However, this did not significantly increase the yield. Substitution of the sodium phosphate buffer initially used by a glycerol-containing Mops buffer increased the yield in the chromatography. Another even more important factor affecting the chromatography was pre-incubation of the sample with dithiothreitol. Omission of this step resulted in an incomplete purification of the fucosyltransferase due to the earlier elution of the main protein peak.

As seen in Fig.3, the main part of fucosyltransferase activity was eluted as a distinct peak from the Superose column. However, determination of the activity in the individual eluted fractions with different oligosaccharide acceptors indicated that the main peak of activity was followed by another component that was using mainly *N*-acetyllactosamine as accep-

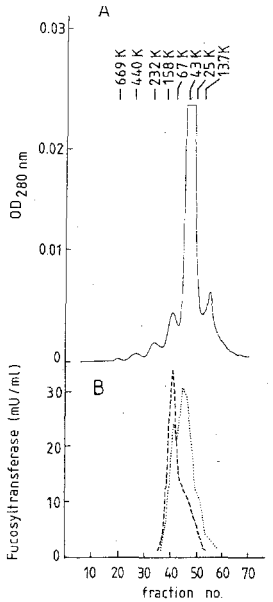


**Figure 3**. HPLC of fucosyltransferase on a column of Superose 12. An aliquot of the fucosyltransferase purified by TSK CM-3SW chromatography (0.1 mg protein) was reduced with dithiothreitol, dialysed, and subjected to chromatography as described under Material and Methods. The elution was performed with the dialysis buffer at a flow rate of 0.5 ml/min. The effluent was monitored for proteins by absorbance at 280 nm (A) and for fucosyltransferase activities (B) using lactose (– – – –) and *N*-acetyllactosamine (.....) as acceptors.

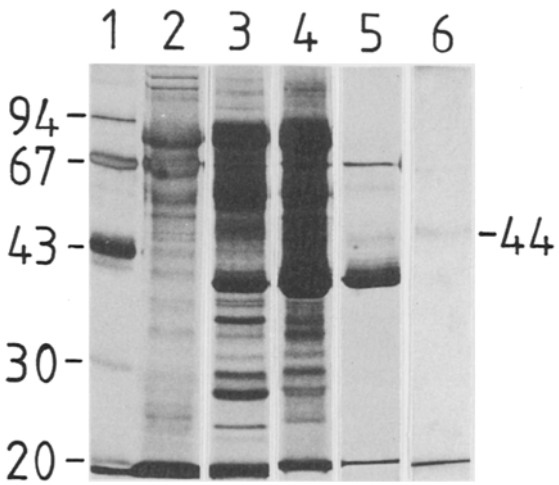


**Figure 4**. SDS-polyacrylamide gel electrophoresis of fucosyltransferase at different stages of purification. Electrophoresis was performed in 10% polyacrylamide gels and the gels were stained with silver. The positions and the $M_r$ values (x $10^{-3}$) of the molecular weight markers are indicated on the left. Lane 1, molecular weight marker proteins; lane 2, delipidated milk; lane 3, fucosyltransferase from SP-Sephadex column; lane 4, fucosyltransferase from GDP-agarose column; lane 5, fucosyltransferase from TSK CM-3SW column; lane 6; the purified fucosyltransferase after Superose HPLC (fraction 42).

tor. Table 2 shows the results of the analysis of the two fucosyltransferase activities with different oligosaccharide acceptors.

The preparation of Prieels *et al.* [10], as suggested by the results of Johnson *et al.* [12, 13], represents mainly the α(1-3/4)fucosyltransferase. This enzyme prefers, of the five acceptors tested, lacto-*N*-fucopentaose I, followed by *N*-acetyllactosamine and lacto-*N*-biose I, 2'-fucosyllactose and lactose [10, 12]. Taking into account the concentrations of the substrates used, the results in Table 2 are in accordance with the identity of the main peak as the α(1-3/4)fucosyltransferase.

The second component, on the other hand, was most active with *N*-acetyllactosamine, a preferred acceptor of α(1-3)fucosyltransferase, but also showed low activity with lacto-*N*-biose I, an acceptor of α(1-4)fucosyltransferase activity. The second component was therefore indicated to represent mainly the α(1-3)fucosyltransferase [12, 13]. The low activity of the second component with 2'-fucosyllactose is in accordance with the fact that this enzyme, in contrast to the α(1-3/4)fucosyltransferase, displays little activity for transfer to glucose residues [12, 13]. The residual activity with 2'-fucosyllactose detected as well as the minor α(1-4)fucosyltransferase activity in the second component may be due to tailing of the α(1-3/4)fucosyltransferase peak, or to the presence of a low molecular weight form of this enzyme.

*Properties of the α(1-3/4)Fucosyltransferase*

Fig. 4 shows the SDS–polyacrylamide gel electrophoresis patterns of the fucosyltransferase preparations at different stages of purification. One major band, with an apparent $M_r$ of 44 000 was seen in the final preparation. A minor band at the position of $M_r$ 67 000 was also seen in some preparations, and represents an artefact frequently seen in silver-stained gels. It was not detected when the preparation was analyzed after radioactive iodination (see below). Since the elution position of the fucosyltransferase activity in the Superose chromatography (Fig. 3) corresponded to a $M_r$ of 98 000, the enzyme may be present as a dimer in its native form.

The main band before purification by Superose chromatography was a component with an apparent of $M_r$ 38 000 (Fig. 4, lane 5). This band corresponded to the main protein peak in Superose chromatography, and the elution position in gel filtration-HPLC corresponded to a $M_r$ value of 40 000, in accordance with the mobility in SDS-polyacrylamide gel electrophoresis.

In isoelectric focusing of the $^{125}$I-labelled fucosyltransferase preparation, the $M_r$ 44 000 band was separated into five components in the range of pH 7.0 to 8.0 with an additional spot at pH 5.8 (Fig. 5). The separation of the band into a series of multiple spots, as well as the diffuse appearance of the red-brown band in SDS–polyacrylamide gel electrophoresis suggested that the fucosyltransferase may be a glycoprotein. In order to test this, fucosyltransferase obtained after CM–TSK chromatography was applied to columns of Sepharose containing covalently-bound concanavalin A or lentil lectin. All fucosyltransferase activity, as measured with different oligosaccharide acceptors, was bound to both columns, which indicated that both the α(1-3/4)- and the α(1-3)fucosyltransferase were glycoproteins. It was not possible to elute the activity from concanavalin A–Sepharose even with 0.5 M methyl α-D-

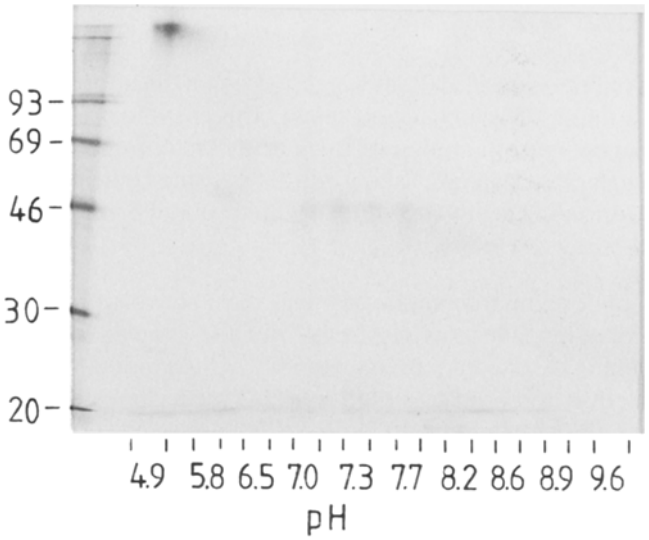


**Figure 5.** Isoelectric focusing of fucosyltransferase. The purified fucosyltransferase was labelled with $^{125}I$ and subjected to isoelectric focusing in the presence of urea followed by SDS-polyacrylamide gel electrophoresis (10% polyacrylamide) in the second dimension.

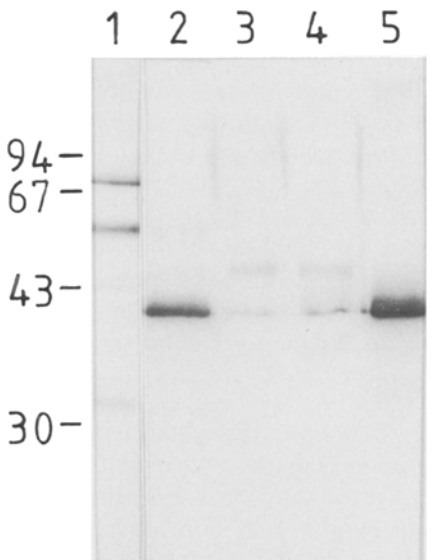


**Figure 6.** Immunoblotting of fucosyltransferase. The fucosyltransferase samples were transferred after SDS-polyacrylamide gel electrophoresis to nitrocellulose filters. Detection was carried out with a rabbit antiserum obtained by immunization with the partially purified fucosyltransferase obtained by HPLC on TSK CM-3SW, and $^{125}I$-labelled protein A followed by autoradiography. Lane 1, delipidated milk; lane 2, fucosyltransferase from TSK CM-3SW column; lane 3, Superose fractions 41–43 (fucosyltransferase); lane 4, Superose fractions 44–45; lane 5, Superose fractions 46–49 (main protein peak).

mannose, whereas from the lentil lectin-column it was eluted in a highly retarded fashion (results not shown).

Several attempts were made to produce monoclonal antibodies against fucosyltransferase. Using the minor amounts of the purified preparation available did not result in the production of antibodies. Different *in vivo* and *in vitro* immunization protocols were used with partially purified fucosyltransferase for the production of monoclonal antibodies. Clones secreting antibodies, among others to the $M_r$ 38 000 component as well as lactoferrin were obtained, but none against fucosyltransferase, suggesting a very poor immunogenicity of this protein.

Subsequently two rabbit antisera were used for immunoblotting analyses. One was produced by Prieels *et al.* and has been shown to inhibit about 80% of the fucosyltransferase activity [10]. The other antiserum was obtained by immunizing with the CM–TSK purified fucosyltransferase and inhibited about 10% of the activity. In accordance with the fact that partially purified fucosyltransferase had been used for the immunization, neither of these antisera was monospecific. Both also reacted with some other components of the milk, notably lactoferrin and the $M_r$ 38 000 protein (Fig. 6, Lanes 1 and 2). Nevertheless, both antisera recognized in the purified fucosyltransferase preparation the main $M_r$ 44 000 component. In this preparation some reactivity was also present at the position of the $M_r$ 38 000 component, apparently due to the presence of minor amounts of this immunodominant protein (Fig. 6, Lanes 4 and 5). A weak band of an apparent $M_r$ of 42 000 was sometimes also seen in the preparation and might reflect the presence of a truncated form of the transferase. Neither rabbit polyclonal antibodies against lactoferrin nor any of the monoclonal antibodies obtained, including some specific for the $M_r$ 38 000 protein, recognized the $M_r$ 44 000 or 42 000 components, which excluded that these bands could be degradation products or precursors of lactoferrin or the $M_r$ 38 000 protein.

## Discussion

The purification scheme used represents the first purification of the α(1-3/4)fucosyltransferase to apparent homogeneity. The availability of a purification method is a pre-requisite for further studies of the enzyme at the molecular level. A significant improvement as compared with the method of Prieels *et al.* [10], was the elution of the SP-Sephadex with a gradient of salt, which resulted in much better purification at this step. In addition, the use of the Superose HPLC as the final step in the purification was necessary for the removal of the major $M_r$ 38 000 contaminant of the enzyme preparation as well as to separate the α(1-3/4)- from the α(1-3)fucosyltransferase activity. The specific activity of the final preparation was 3.5 higher than that of the preparation of Prieels *et al.* [10], as expected because of the impurities detected in the latter preparation. In its purified form the fucosyltransferase was much more unstable than the preparation before the last step of purification, which interfered with the characterization of the purified enzyme.

In gel filtration-HPLC the α(1-3/4)fucosyltransferase activity was eluted at a position corresponding to an $M_r$ of 98 000. In contrast, the second fucosyltransferase component was eluted at a position corresponding to an $M_r$ of about 47 000. Results of the analysis of the acceptor

**Table 2.** Comparison of the two fucosyltransferase activities separated by Superose 12. HPLC.The α(1-3/4)- and α(1-3)fucosyltransferase activities represented by fractions 42 and 45, respectively, of the Superose chromatography (Fig. 3) were assayed under standard conditions using the amounts indicated of the different oligosaccharides.

| Acceptor | Amount | Fraction 42 (mU/ml) | Fraction 45 (mU/ml |
|---|---|---|---|
| Fucα1-2Galβ1-3GlcNAcβ1-3Galβ1-4Glc (lacto-*N*-fucopentaose I) | 27 nmol | 20.2 | 9.2 |
| Galβ1-4GlcNAc (N-acetyllactosamine) | 150 nmol | 23.5 | 30.0 |
| Galβ1-3GlcNAc (lacto-*N*-biose I) | 26 nmol | 8.0 | 1.2 |
| Fucα1-2Galβ1-4Glc (2'-fucosyllactose) | 300 nmol | 7.4 | 1.3 |
| Galβ1-4Glc (lactose) | 24 μmol | 34.3 | 14.0 |

specificity of the latter enzyme activity were in accordance with its identity as the α(1-3)fucosyltransferase [12, 13]. However, since some activity was also displayed against 2'-fucosyllactose and lacto-*N*-fucopentaose I, the possibility cannot be excluded that these fractions also contained some other additional fucosyltransferase activity, possibly α(1-3/4)fucosyltransferase of smaller molecular size (monomer, see below). It is of interest that a partial separation of the α(1-3/4)- and α(1-3)fucosyltransferases by chromatography on GDP-Sepharose has been reported by Johnson and Watkins [13]. Since they used a rather long affinity column for the separation, it is possible that a gel filtration effect may have contributed to the separation, in view of the different molecular sizes of the enzymes indicated by the present study.

A single major band with an apparent $M_r$ of 44 000 was consistently present in the purified fucosyltransferase. This band appears to represent the fucosyltransferase since it was enriched parallel to the α(1-3/4)fucosyltransferase activity and was not present in fractions lacking the enzyme activity. It was not possible to confirm this by non-denaturing gel electrophoresis since under these conditions the enzyme formed aggregates and hardly penetrated the separation gel. The aggregation tendency also interfered with the isoelectric focusing experiment, since much of the radioactivity remained at the position of sample application and did not enter the electrophoresis gel (Fig. 5). A tendency to aggregation has also been observed for an α(1-3)fucosyltransferase isolated from embryonal carcinoma cells [9]. The minor $M_r$ 42 000 band detected in the enzyme preparation in some immunoblotting experiments may represent another molecular weight form of the fucosyltransferase, similar to those observed for other glycosyltransferases [14, 30–32].

Like some other glycosyltransferases analyzed, human milk fucosyltransferase appears to be a glycoprotein. This is indicated by the strong binding of the activity to the lectins tested. Our preliminary experiments with peptide: *N*-glycosidase F and endo-β-*N*-acetylglucosamini-

dase F also indicate that the mobility of the $M_r$ 44 000 band increases as expected by the loss of a few thousand mass units after treatment with these enzymes (unpublished results). The separation of the band into a series of multiple bands on isoelectric focusing is also in accordance with a glycoprotein nature of this component.

In view of the suggested involvement of regulatory factors in the expression of the α(1-3/4)fucosyltransferase activity in Lewis positive and negative individuals [11], the molecular form of the enzyme is of particular interest. The difference in the apparent $M_r$ values determined for the fucosyltransferase in Superose HPLC (98 000) and SDS-polyacrylamide gel electrophoresis (44 000) suggests that the molecule may be a dimer of two identical subunits in its native form. Dimeric forms have been suggested for the A and B blood-group *N*-acetylgalactosaminyl- and galactosyltransferases [33, 34] and recently also for β(1-4)galactosyltransferase isolated from lactating rat mammary gland [35]. At present it cannot be predicted how the putatively dimeric nature of the fucosyltransferase may reflect the enzymic activity or its regulation.

Due to the co-purification of the $M_r$ 38 000 component with the fucosyltransferase activity and the difficulties encountered in finding the appropriate method for its removal, we tested whether the purified molecule could be a modifier of the enzyme. However, no difference in the total activity of the fucosyltransferase nor a difference in the relative acceptor activities of a selection of oligosaccharides tested could be found in mixing experiments (unpublished results).

As tested with several different methods for the production of antibodies, a consistent finding was that the fucosyltransferase was of very poor immunogenicity. This is in contrast to some components of the fucosyltransferase preparations, such as lactoferrin and the $M_r$ 38 000 component, which were strongly immunogenic and interfered with the production of fucosyltransferase antisera and immunoblot analysis of fucosyltransferase. The rabbit antiserum against fucosyltransferase reported before [10] also reacted with these components. Thus no antibodies are presently available and sequence information is therefore needed. Preliminary analysis with a gas-phase peptide sequencer gave no *N*-terminal sequence for the fucosyltransferase, whereas the $M_r$ 38 000 component revealed an *N*-terminal sequence that exhibited no significant homology with known protein sequences. The preparation and purification of peptide fragments from the fucosyltransferase in order to obtain the sequence information needed for further studies on the enzyme will be a matter of further studies.

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