ISOELECTROFOCUSING PATTERN OF 2-α-L, 3-α-L AND 4-α-L FUCOSYLTRANSFERASES FROM HUMAN MILK AND SERUM

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1. Introduction

α-L-Fucosyltransferases have been detected in soluble form in human milk [1,2], serum [3,4] and as membrane-bound enzymes in human red cell ghosts [5], lymphocytes and platelets [6], stomach mucosa and submaxillary glands [7], bone marrow [8] and kidney [9].

The *H*-gene-specified enzyme, 2-α-L-fucosyltransferase [10] and the *Le*-gene-specified enzyme, 4-α-L-fucosyltransferase [11,12] are direct products of *H* and *Le* blood group genes, respectively. Both enzymes catalyze the transfer of L-fucose in α-linkage to the C-2 position of terminal non-reducing β-D-galactosyl residue and to the C-4 position of sub-terminal *N*-acetyl-D-glucosaminyl residue, respectively.

The 3-α-L-fucosyltransferase catalyses the transfer of L-fucose in a α-linkage to C-3 position of subterminal *N*-acetyl-D-glucosamine or D-glucose residue [1,3,7]. It has not been described as being like a specified blood group enzyme, although it is involved in the synthesis of related substances.

The three enzymes have been described in milk [1,13,14]. While the 2- and 3-α-L-fucosyltransferases have been found in sera [3,4,15], the 4-α-L-fucosyltransferase has not yet been detected in adult human sera from individuals carrying the *Le* gene [3]. The 2-α-L-fucosyltransferase activity failed to appear in Bombay, Ah, Bh, Hz phenotypes [3,5,16]. The 3-α-L-fucosyltransferase activity was identified in all individuals. In [17,18] fucosyltransferase activities were reported to increase in cancer patients; the elevated activities could be an indicator of malignancy.

Here we report the electrofocusing properties of these transferases from human milk and sera. It was of interest to determine if the soluble transferases having the same specificity (2 or 3-α-L-fucosyltransferase activity) present in different molecular forms in 2 compartments of the organism.

2. Materials and methods

2.1. *Enzyme substrates*

GDP-L-[U-^{14}C]fucose was purchased from the Radiochemical Centre (Amersham) (118 Ci/mol) and from New England Nuclear (230 Ci/mol) and used in 2% ethanolic solution. Phenyl-β-D-galactopyranoside was obtained from Koch-Light and lacto-N-biose I from Sefochem (Israel). *N*-Acetyllactosamine and 2′-fucosyl-*N*-acetyllactosamine, chemically synthesized, were a gift from Professor Sinäy (Orléans).

2.2. *Sources of fucosyltransferases*

Fresh human milk was purchased from Lactarium (Ecole de Puériculture, boulevard Brune, Paris). Milk was defatted by centrifugation 30 min, 10 000 × *g*, 4°C, filtered on a G-50 Sephadex column pre-equilibrated with 7.7 mM NaCl and then lyophilised. Another lyophilised preparation, gift of H. Schenkel Brunner, was obtained according to [14].

Sera were stored at −20°C until use, then precipitated at 40% saturation of ammonium sulfate and desalted by gel filtration on a G-50 Sephadex column equilibrated in 0.05 mM Tris–HCl buffer (pH 7.2).

2.3. *Isoelectric focusing*

Isoelectric focusing was performed by the standard method [19], either on an LKB 8101 (110 ml) apparatus or, in one case, on a laboratory-made column (170 ml).

The gradient (sucrose: 4–48%, 2% ampholytes

(pH 3.5–10)) was prefocused for 60 h at 4°C. Lyophilised samples (20 mg) were solubilised in a small fraction of the gradient and then introduced into the column. Either crude extracts or ammonium sulfate precipitated samples (2–3 ml) were loaded after adjustment of their density. Electrofocusing was continued for 15–20 h with a starting power of 1 W (~500 V, ~2 mA). The final power was 3 W with 1000 V as a maximum voltage. After collecting fractions of 2 ml or 4 ml into bovine albumin (final conc. 0.5%), pH was measured at 4°C. Each fraction was filtered on G-50 Sephadex columns pre-equilibrated in Tris–HCl, 0.01 M (pH 7.2) buffer or 7.7 mM NaCl for serum and 0.05 M Tris–HCl, (pH 7.2), 1 mM glutathione, or 7.7 mM NaCl for milk. Fractions were then concentrated in batch with G-25 Sephadex (coarse) or lyophilised. Lyophilised fractions were solubilised in 400 μl distilled water before use.

2.4. *Assays of fucosyltransferase activities*

The 2-α-L-fucosyltransferase activity was tested on phenyl-β-D-galactoside as substrate [20]. The reaction mixture was: 30 μl sample; phenyl-β-D-galactoside 32 mM in 0.05 mM MES buffer, 0.96 μmol; Tris–HCl, (pH 7.2), 3.6 μmol; $MgCl_2$, 0.6 μmol; ATP, 0.6 μmol; GDP [^{14}C]fuc, 2 nmol; in 85 μl final vol. After incubation for 72 h at 37°C, the mixture was submitted to descending chromatography, carried out on Whatman 40 paper, in ethyl acetate–pyridine–water (10:4:3, by vol.) for 6 h.

The 3-α-L-fucosyltransferase activity was tested on *N*-acetyllactosamine or 2′-fucosyl-*N*-acetyllactosamine as acceptor, while the 4-α-L-fucosyltransferase was tested on lacto-N-Biose I. The incubation mixture contained the same reagents as above, except the phenyl-β-D-galactoside which was substituted by lacto-N-Biose I. The final volume was 60 μl. After incubation at 37°C for 72 h, samples were electrophoresed in 0.1 M (pH 3.5) ammonium formate buffer (Whatman 40 paper; 2000 V, 2 h). The neutral substances remaining at the origin were separated by descending chromatography in pyridine–ethyl acetate–acetic acid–water (5:5:1:3, by vol.). Specific products were characterised by their migration with regard to standard sugars (lactose or fucose), and compared to oligosaccharides of established structures.

The fucosidasic activity was tested in the same conditions as for transferase; the acceptor substrate was substituted by 5 μl [^{14}C]Fuc–Gal–phenyl or [^{14}C]-Fuc–Gal β1 → 3 GlNac (6000 cpm and 7500 cpm, respectively) and GDP [^{14}C]fuc by distilled water. These two ^{14}C-labelled substrates were obtained by elution.

3. Results and discussion

Electrofocusing studies were carried out on sera from different blood groups. An example of the isoelectrofocusing pattern for an AB serum is shown in fig.1.

The 2-α-L-fucosyltransferase activity tested with phenyl-β-D-galactoside as substrate focused at an average of pH 4.7 for the 5 sera studied. Lyophilisation of samples, instead of concentration with G-25 Sephadex increased clearly the yield of recovery of 2-α-L-fucosyltransferase activity (~0.5–30%). With this improvement, it was repeatedly found that only one peak of enzyme activity exists, at acidic pH. The *H*-gene-speci-

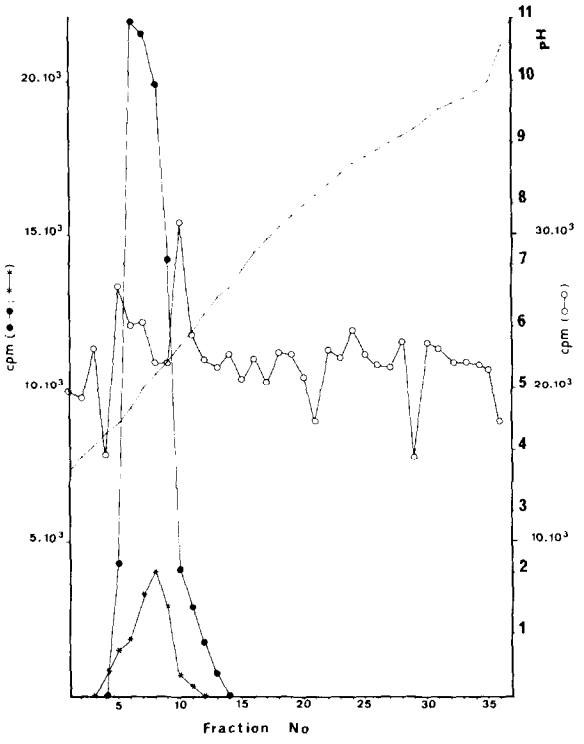

Fig.1. Isoelectrofocusing profile of AB serum. Fractions (4 ml) were lyophilised, then solubilised in 400 μl distilled H_2O and tested for 2-α-L-fucosyltransferase activity with phenyl-β-D-galactoside (●) and 3-α-L-fucosyltransferase activity with 2′-fucosyl-*N*-acetyllactosamine (∗). Enzyme activities are expressed in cpm of radiolabelled fucose incorporated into appropriate acceptor; (○) released [^{14}C]fucose.

fied enzyme from normal serum presents only one molecular form with an isoelectric point of 4.7.

The 3-α-L-fucosyltransferase was tested on *N*-acetyllactosamine for two sera and on 2′-fucosyl-*N*-acetyllactosamine for one serum (fig.1). This enzyme focused at pH 5.0 on average.

The pI-values, 4.7 for *H*-gene-specified enzyme and 5.0 for 3-α-L-fucosyltransferase are lower than the isoelectric points obtained in [21,22]: 5.1 and 5.6 for normal serum, respectively. However, the difference observed could be due to the method used: isoelectrofocusing carried out on flat bed containing Ultrodex, over pH 4–6. In our experiment (see fig.1), despite their overlapping activities, the 2 and 3-α-L-fucosyltransferases also appear as being two different enzymes in serum. Nevertheless, the isoelectrofocusing method is not efficient in separating 2- and 3-α-L-transferases from each other.

The electrofocusing pattern of 3-α-L-fucosyltransferase and 4-α-L-fucosyltransferase from 20 mg milk protein prepared as in [14] is shown in fig.2. The 3-α-L-fucosyltransferase activity appears in 3 peaks with pI-values of 6.2, 7.0 and 9.1. The patterns obtained

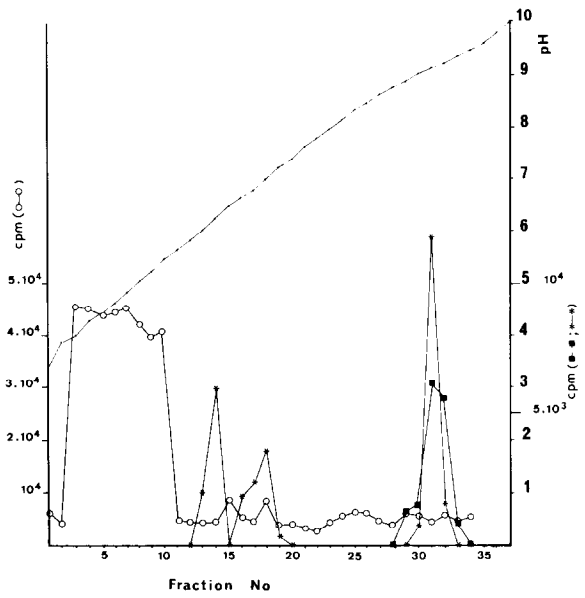

Fig.2. Isoelectric focusing profile of milk. Fractions (2 ml) were concentrated in batch with G-25 Sephadex (coarse). 3-α-L-fucosyltransferase activity was tested with *N*-acetyllactosamine (*) and 4-α-L-fucosyltransferase activity with lacto-*N*-Biose I (■). Enzyme activities are expressed in cpm of radiolabelled fucose incorporated into appropriate acceptor; (○) released [^{14}C]fucose.

above may result from degradation or aggregation of a single enzyme occurring either in vivo after the secretion process (soluble unstable molecule) or in vitro; e.g., by sialic acid release. However, the 3 apparent forms may be 3 isozymes. A variety of mechanisms is available for generating molecular multiplicity of one enzyme. In the cell of origin a gene can be duplicated and every gene can then diverge by mutation to produce a modified version of the original enzyme. After differentiation, each isozyme form can be expressed in a certain tissue, while another form can be expressed in another tissue [23]. So the single form with a pI of 5.0 in serum, which differs from the pI of multiple molecular forms in milk, can be explained. Isozymes can, however, result from epigenic phenomena. The multiple enzymes can get an identical primary structure (encoded by the same gene), but differ in other respects, such as different subsequent modifications in different tissues. For example, if they are glycoproteins, they may vary in the nature of the carbohydrate side chains.

The 2-α-L-fucosyltransferase in milk does not show up. Its activity appears in traces in crude milk from secretor individuals, relative to the 3- and 4-α-L-fucosyltransferase activities. With phenyl-β-D-galactoside as acceptor, it is very low or hardly detectable. Lactose is a better acceptor for the 2-α-L-fucosyltransferase activity but using this substrate, the 3-α-L-fucosyltransferase activity also gave a product which is not easily separated from the H hapten. Attempts to concentrate the 2-α-L-fucosyltransferase activity failed because of its instability.

The *Le*-gene-specified 4-α-L-fucosyltransferase shows one peak with pI value of 9.1. It seems that a single form of this enzyme is present in milk. As stated, the *Le*-gene-specified enzyme is absent in serum.

In all the experiments, in milk and serum, degradation with production of fucose competes with transferase activity. The released [^{14}C]fucose can be due to either fucose hydrolase, pyrophosphatase and phosphatase activities or fucosidase activity acting directly on the products formed by each specific enzyme; it can also be due to chemical hydrolysis. However, the phenomena differ in serum or milk.

After focusing in the presence of serum, the released [^{14}C]fucose was seen all over the pH gradient. In [24] fucosidasic activity was detected in serum only between pH 4.9–6.4; activity was tested with specific substrate, the 4-methylumbelliferyl-α-L-fucoside. Our results could be induced by focusing at vari-

ous pH of all the above types of enzymes, which release [^{14}C]fucose. Chemical degradation could also arise, although the same experimental conditions for milk studies do not induce the same degradation. Nevertheless, 2- and 3-α-L-fucosyltransferase activities in serum were easily detectable.

In milk, fucose was mainly released at pH 4.0–5.5 (see fig.2). In the other part of the gradient [^{14}C]-fucose was low and corresponded to normal decomposition of GDP [^{14}C]fucose. The weak 2-α-L-fucosyltransferase activity in crude milk preparation, before focusing, is always associated with a strong fucosidasic activity. Attempts to decrease the second activity and increase the first failed; the two activities appear to be linked as is also suggested by [25]. It could be possible that these two activities are supported by the same molecular species. It is feasible that, after isoelectrofocusing, the weak and unstable 2-α-L-fucosyltransferase activity is lost, whereas fucosidase activity is preserved and focused at pH 4.0–5.5. In order to prove if fucosidase activity corresponds to the degradation of the product formed by transferase activity, [^{14}C]-Fuc α1 → 2 Gal–phenyl and [^{14}C]Fuc α1 → 2 Gal β1 → 3 GlNac were tested as substrates in all the fractions of isoelectrofocusing, under the same conditions of transferase activity; [^{14}C]fucose was not released. Consequently, we suppose that it is a GDP fucose hydrolase activity, which could be associated to the transferase activity. This hypothesis has been put forward in [25] where 2-α-L-fucosyltransferase has been purified from porcine submaxillary gland.

In summary: we have found 1 form of 2-α-L-fucosyltransferase in serum (pI 4.7); 4 forms of 3-α-L-fucosyltransferase, with 1 in serum (pI 5.0) and 3 different ones in milk (pI 6.2, 7.0, 9.1); and 1 form of 4-α-L-fucosyltransferase in milk (pI 9.1).

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