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Human milk fucosyltransferase and α-L-fucosidase activities change during the course of lactation

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Human milk is rich in fucosylated oligosaccharides and other glycoconjugates, some of which affect interactions between pathogens and host cell surface. Two types of enzymes, fucosyltransferase and α-L-fucosidase, involved in the biosynthesis and degradation of these compounds, were analyzed in random samples of human milk from healthy donors. In mature milk (days 52 to 78), the individual variability for fucosyltransferase was twice that of fucosidase. The activities of both enzymes were observed to change over the course of lactation from day 3 to day 370. α-Fucosyltransferase activity was greatest during the first 30 days of lactation and declined significantly between 30 and 100 days. From day 100, fucosyltransferase activity remained nearly constant. Fucosidase activity was high at the onset of lactation followed by a precipitous decline, reaching a nadir by the second week. After the fourth week, the α-L-fucosidase activity increased in direct proportion to the length of lactation. The possible relationship of these enzymes to the fucosyloligosaccharide content of human milk are discussed. (J. Nutr. Biochem. 6:582–587, 1995.)

Keywords: human milk; fucosyltransferase; α-L-fucosidase; lactation

Introduction

Human milk is unique with regard to the content and complexity of its glycoconjugates, especially oligosaccharides; many of these oligosaccharides are fucosylated.[1] Some fucosyloligosaccharides are potent inhibitors of bacterial adhesion to epithelial surfaces and increase resistance to enteric bacterial toxins in vivo. For example, we found that neutral fucosyloligosaccharide(s) of human milk protect against the stable toxin of *Escherichia coli* in vivo and in vitro and that fucosyloligosaccharides inhibit the binding of invasive strains of *Campylobacter jejuni* to target cells in vitro.[2,3] The oligosaccharide composition of human milk is known to vary as a function of several parameters including maternal blood group type, secretor status, and stage of lactation. The enzymes involved in the biosynthesis and degradation of fucosyloligosaccharides are fucosyltransferase and α-L-fucosidase.

The structural variety of fucosyl residues in oligosaccharides is determined by a family of fucosyltransferases (EC 2.4.1.65), which have been detected in a number of sources, including human milk. Their purification, properties, and substrate specificity have been described.[4,5] The major fucosyltransferase of milk is reported to be the α-3/4-fucosyltransferase, which has been cloned and is referred to as Lewis type fucosyltransferase III.[6] To our knowledge, there is no information available concerning the levels of fucosyltransferase activity of human milk among healthy individuals and changes in this activity during the course of lactation.

α-L-Fucosidase (EC 3.2.1.51) can hydrolyze fucose from fucosyloligosaccharides, changing their structure and biological activity.[7,8] Fucosidase is a ubiquitous lysosomal glycosidase found in a wide variety of organisms.[9,10] The importance of fucosidase in mammalian metabolism is ev-

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idenced by the lysosomal storage disease, fucosidosis. This disease is characterized by low α-L-fucosidase activity and an accumulation of fucoglycoconjugates in the brain and visceral tissues.[11] The activities of multiple forms of this enzyme can be detected at various stages of gestation in chorion villi, amniotic fluid, and some fetal organs.[12,13] Thus, fucosidase is widely distributed in numerous human tissues and biological fluids; however, the activity of this enzyme has not been described in human milk despite its potential involvement in the degradation of milk fucosyloligosaccharides.

Thus, both fucosyltransferase and fucosidase may reflect processes that control the fucosyloligosaccharide composition of human milk. The objectives of the present study were to determine the activity and variability of fucosyltransferase and fucosidase in the milk of a population of healthy donors over the course of lactation.

Methods and materials

GDP-L-[^{14}C]-fucose (283 mCi/mmol) was purchased from Dupont/New England Nuclear (Boston, MA USA). Unlabeled GDP-L-fucose, lactose, and 4-methylumbelliferyl α-L-fucopyranoside were purchased from Sigma Chemical (St. Louis, MO USA). BCA protein assay reagent and AG 1-X8 anion exchange resin (100 to 200 mesh, acetate form) were obtained from Pierce (Rockford, IL USA) and Bio-Rad (Richmond, CA USA), respectively.

Milk samples

Human milk samples, obtained from the Instituto Nacional de la Nutricion in Mexico and stored at −80°C until analysis, were provided by participants in the Breast Milk Study HD13021 performed by Drs. Larry K. Pickering and Ardythe Morrow (Eastern Virginia Medical School, Norfolk, VA USA) and Dr. Guillermo Ruiz-Palacios (Mexico).

Fifty samples of 1 mL each from 50 donors, taken during days 52 to 78 of lactation, were used to assess the variation in fucosyltransferase and fucosidase activities in a random population during a similar period of lactation.

Six to eight 1-mL milk samples were obtained from each of 12 donors to sample different times throughout the course of lactation. These 90 samples represented nine different periods of lactation: 0 to 5 days of lactation (mean = 3.5 days, n = 8); 6 to 10 days (mean = 8, n = 7); 11 to 15 days (mean = 13, n = 9); 17 to 25 days (mean = 21, n = 13); 26 to 31 days (mean = 28, n = 9); 85 to 111 days (mean = 94, n = 10); 170 to 200 days (mean = 186, n = 12); 247 to 310 days (mean = 278, n = 13); and more than 355 days of lactation (mean = 368, n = 9). The average enzyme activities of each group (mean ± SEM) was plotted against the mean duration of lactation.

Each sample was centrifuged at 4°C at 3,000g for 30 min. The cream, which contained insignificant activities of these enzymes, was removed, and the enzyme activities were determined in the skimmed milk.

Fucosyltransferase assay

The reaction mixture (100 μL) contained 5 μmol of 3-(N-morpholino) propansulfonic acid (MOPS)/NaOH buffer, pH 7.5, 0.5 μmol of $MnCl_2$, 10 μmol of NaCl, 1 nmol of GDP-L-fucose (0.1 nmol of GDP-L-[^{14}C]-fucose, ~57,000 cpm and 0.9 nmol of unlabeled GDP-L-fucose), and 24 μmol of lactose.

The reaction mixture containing skim milk as a source of fucosyltransferase was used as a positive control. After 1 hr of incubation at 37°C, the reaction was terminated by the addition of 1 mL of cold water with incubation on ice. The mixture was immediately applied to a 1 mL column with AG 1-X8 resin in pipette tips. The column was washed with 1 mL of water, and the combined aqueous effluents containing the fucosylated products were collected in scintillation vials and counted after the addition of a 5 mL liquid scintillation cocktail (Ready Safe, Beckman, Fullerton CA USA). Samples incubated without any source of fucosyltransferase served as negative controls; their counts were subtracted as background from the counts of the samples with enzyme. The reaction rate was linear for at least 2 hr.

Fucosyltransferase activity was expressed as picomoles of total fucosylated products per hour per milliliter of milk and per milligram of total milk protein.

α-L-Fucosidase assay

The reaction mixture (100 μL) contained 50 μL of skim milk sample previously diluted 10 fold with distilled water (protein concentration about 1 mg/mL) and 50 μL of 2 mmol/L 4-methylumbelliferyl α-L-fucopyranoside dissolved in citrate (0.1 mol/L)-phosphate (0.2 mol/L) buffer, pH 5.0 (McIlvaine buffer). After 1 hr of incubation at 37°C, the enzymatic reaction was stopped by 2 mL of 0.25 mol/L glycine-NaOH buffer, pH 10.4. Enzymatically liberated 4-methylumbelliferone was measured by fluorescence (excitation, 365 nm; emission, 480 nm; SPC 500 C spectrofluorometer; Aminco Instruments, Urbana, IL USA). The reaction rate was linear for at least 2 hr. The fucosidase activity was expressed as nanomoles of 4-methylumbelliferyl α-L-fucopyranoside hydrolyzed per hour per milliliter of milk or per milligram of protein.

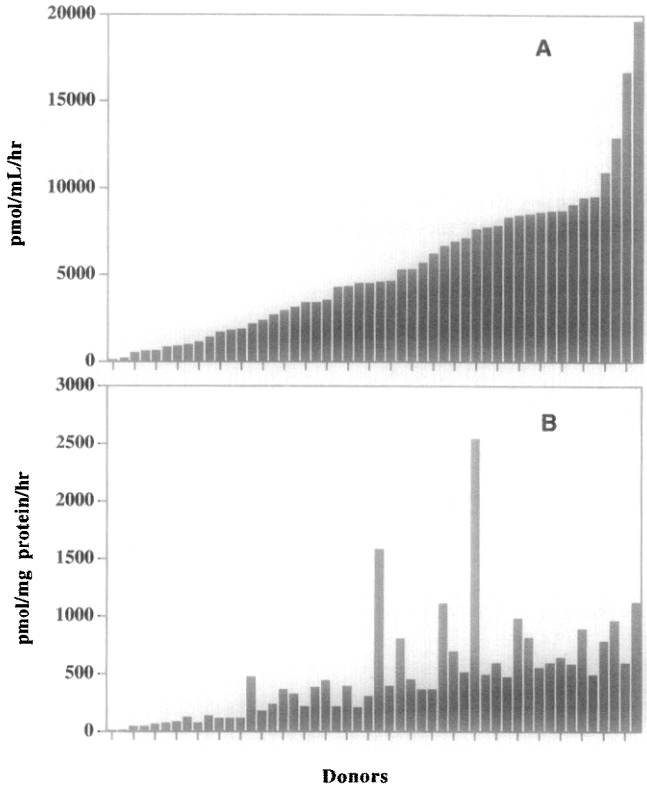

Figure 1 Fucosyltransferase activities in mature human milk (days 52 to 78) of 50 donors: (A) pmol/mL/hr; (B) pmol/mg of protein/hr.

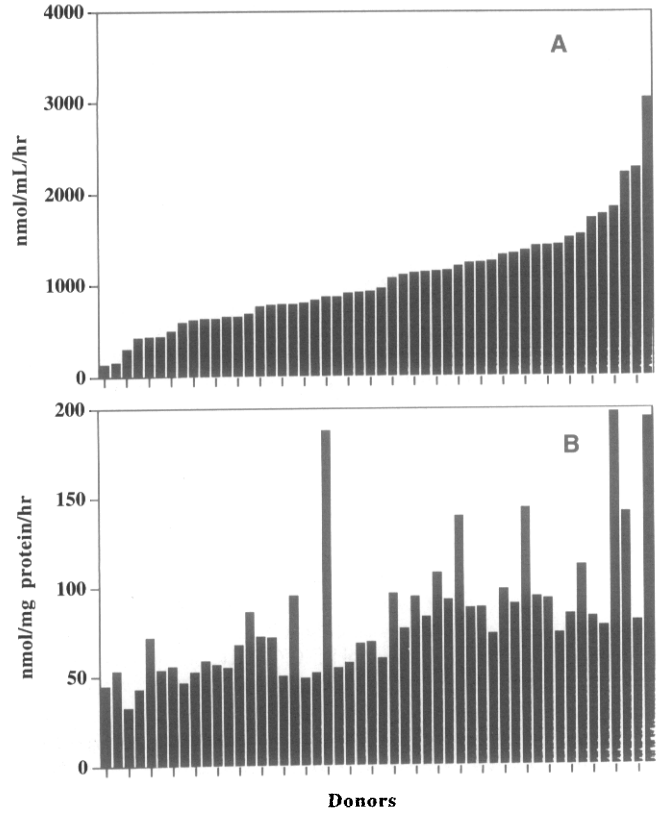

Figure 2 α-L-Fucosidase activities in mature human milk (days 52 to 78) of 50 donors: (A) nmol/mL/hr; (B) nmol/mg of protein/hr.

Protein determination

Protein was determined using a BCA protein assay (Pierce, Rockford, IL USA) modified for use in 96-well microtiter plates. To each protein sample of 50 μL, 200 μL of working reagent was added, followed by incubation at 37°C for 30 min. Absorbance at 562 nm was determined via a microtiter plate reader (BT 2000 Microkinetics Reader Spectrophotometer, Fisher Biotech, Pittsburgh, PA USA). The standard curve consisted of a series of known concentrations of bovine serum albumin.

Statistical analysis

The activity of both enzymes was evaluated for each donor as a function of the duration of lactation. The patterns of change for individuals over lactation closely resembled those described by the composite data. For the graphed aggregate data, the best curve fit was determined using linear least-squares regression. The linear correlation coefficient (r) was used to evaluate the relationship between enzyme activity and time of lactation.[14]

Results

Fucosyltransferase activity was calculated from 50 donors both in terms of milk volume and total milk protein to compensate for the changes in milk volume and milk protein during some stages of lactation *(Figure 1)*. The mean fucosyltransferase activity for this population sample was 5,360 ± 590 pmol/mL of milk/hr or 472 ± 64 pmol/mg of protein/hr. Fucosidase activity for these 50 donors is shown in *Figure 2*. The mean fucosidase activity was 1,055 ± 79 nmol/mL of milk/hr or 83 ± 5 nmol/mg of protein/hr. Both fucosyltransferase and fucosidase activities display a high degree of variability across donors. However, the relative variability, i.e., the standard error of the mean divided by the mean, was greater for fucosyltransferase (~12%) than for fucosidase (~6%).

The activities of fucosyltransferase and fucosidase change over the course of lactation. Fucosyltransferase activity is initially at its highest level early in lactation *(Figure 3A* and *3B)*. When activity is expressed in terms of protein content, there is a transient decrease on day 13, coinciding with elevated protein in these samples. Otherwise, the fucosyltransferase activity remains high until day 30 and then declines significantly between 30 and 100 days to about one third of its former value. From day 100, fucosyltransferase activity seems to stabilize, staying nearly constant for the remainder of lactation. This pattern of decline most closely resembles a power function both when the activity is expressed in terms of milk volume *(Figure 3A; r = 0.89)* or milk protein *(Figure 3B; r = 0.74)*.

Fucosidase activity during the earliest period of lactation seems to be relatively high. From 13 to 30 days, however, fucosidase activity declines to its lowest point. Between days 30 and 100, fucosidase activity starts to rise gradually, reaching its highest levels at day 370, the last available samples from each donor *(Figures 4A* and *4B)*. The pattern for increasing fucosidase activities is a linear function, both when the activity is expressed in terms of milk volume *(Figure 4A; r = 0.85)* or milk protein *(Figure 4B; r = 0.97)*.

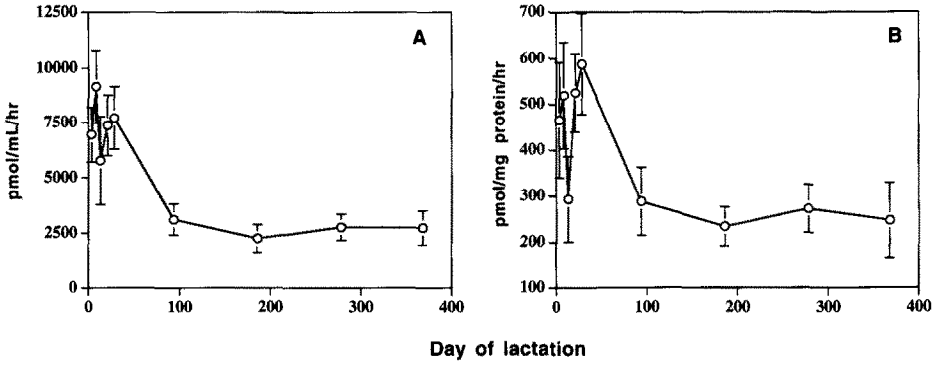

Figure 3 Fucosyltransferase activity (mean ± SEM) of human milk during lactation: (A) pmol/mL/hr; (B) pmol/mg of protein/hr.

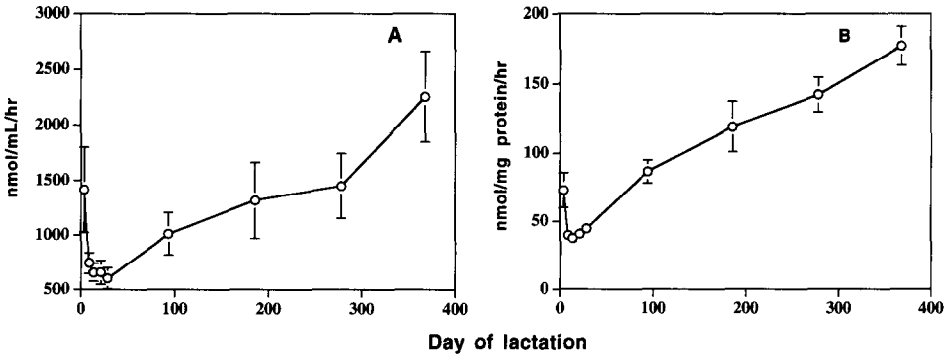

Figure 4 α-L-Fucosidase activity (mean ± SEM) of human milk during lactation: (A) nmol/mL/hr; (B) nmol/mg of protein/hr.

Discussion

The above data show that human milk contains both fucosyltransferase and fucosidase; wide individual variability in the activity of these enzymes is apparent in milk samples representing a fixed period of lactation in a random population. Such variability may reflect genetic polymorphism of these enzymes in milk. High variability has been reported in the α-1,2- and α-1,3-fucosyltransferase activities of human lymphocytes, granulocytes,[15] and serum.[16]

In human milk the dominant fucosyltransferase activity is reported to be that of the Lewis gene product, Fuc III (fucosyltransferase III), that is an α-3/4-fucosyltransferase.[6,17] In fresh milk from donors whose Lewis blood group type is $Le^{(a-b+)}$, α-2-fucosyltransferase activity is considerably lower than α-3/4-fucosyltransferase activities and is less stable in storage.[5] Furthermore, with pooled milk samples in which lactose was the single exogenous low molecular weight acceptor, we identified 3-fucosyllactose by high performance liquid chromatography as the major fucosylated product (>80% of total radioactivity; data not shown). In the measurements of fucosyltransferase activity presented herein, lactose was used as the single exogenous acceptor at a concentration (250 mmol/L) that was designed to be relatively high compared with other potential endogenous acceptors. Thus, the variation in fucosyltransferase activity observed in milk is likely to be a reflection of variation in α-3/4-fucosyltransferase.

The fucosyltransferase activity of the 50 donors shows a wider degree of variation than fucosidase, and the percentage of samples with low activity is similar to the percentage of nonsecretors expected in this population; however, these data are insufficient to directly link enzyme levels to secretor status. In longitudinal measures of fucosyltransferase, 9 of the 12 donors had similar absolute values, whereas the other 3 had appreciably lower values, again consistent with the ratio of secretors to nonsecretors. The variability of the milk fucosidase activity, although somewhat less than that of milk fucosyltransferase, was still appreciable. Such polymorphism in fucosidase activity has also been observed in human serum, leukocytes, and fibroblasts.[13,18–23]

Another polymorphism, structural in nature, was revealed by isoelectric focusing of fucosidase from several tissues and biological fluids (for review, see Johnson and Alhadeff[10]). However, the isoelectric polymorphism seen in fucosidases of kidney, liver, placenta, and blood does not relate to the polymorphism of fucosidase activity.[13,24,25] Studies on the physical features associated with the variation in human milk fucosyltransferase and fucosidase activities could reveal a basis for their distribution and properties. However, the milk fucosidase activities exhibited strong central tendencies, providing no indication of a distribution that would suggest a strong relationship between this enzyme and blood group type. Furthermore, fucosidase levels in donors followed longitudinally throughout lactation consistently had values close to those of the composite data, providing no indication of polymorphism in milk.

The milk fucosyltransferase levels measured in this study are highest early in lactation, then diminish and stabilize at a lower level later in lactation. The levels of total oligosaccharides in milk also are high early in lactation and diminish over the course of lactation.[26] However, the total oligosaccharides in milk show their greatest change over the first several days of lactation,[27] whereas the fucosyltransferase changes occur over the course of several weeks. But the concentration of the fucosyloligosaccharides over the course of lactation is not known.

The milk fucosidase levels display a precipitous drop in activity over the first week of lactation that coincides with the precipitous drop in macrophages, polymorphonuclear leukocytes, and lymphocytes reported to occur over the first week of lactation.[28] These leukocytes, as isolated from blood, contain significant amounts of lysosomal hydrolases including fucosidase.[29] Therefore, we postulate that milk leukocytes may be a major source of the fucosidase seen in early lactation. The source of the fucosidase activity that gradually increases from the fourth week and continues increasing throughout the remainder of lactation could be of alveolar origin. Preliminary studies indicate that the fucosyltransferase and fucosidase of fresh mature human milk (100 days postpartum) is indeed more closely associated with the fluid than the cellular compartment of human milk. This increase in fucosidase activity seems unrelated to any decrease in volume due to partial weaning but rather seems to be intrinsic to the lactation process.

Although we found that phases of human lactation are characterized by significant changes in fucosyltransferase and fucosidase activities, the impact of these changes on the metabolism of fucosyloligosaccharides is unclear.

Fucosyltransferases catalyze the fucosylation of different acceptors and localize in the Golgi complex,[30] and the oligosaccharides of milk are likewise thought to be synthesized in the Golgi complex of the alveolar cell.[31] The final

products of these enzymes in epithelial cells of the mammary gland include the more than 80 known fucosyloligosaccharides of human milk and undoubtedly many others. For fucosylation to occur also in the milk would require not only the fucosyltransferases and lactose known to be present, but also high concentrations of GDP-fucose to act as the donor of fucosyl residues and other co-factors.[32] Such high concentrations of nucleotide sugars have not been described in human milk.

Fucosidase is a typical lysosomal enzyme with acidic pH optimum 4.5 to 5.0. However, we have found (unpublished data) that human milk fucosidase is rather stable at the neutral pH typical for milk. Fucosidase is also more stable to heat than fucosyltransferase. Thus, conditions permissive for hydrolysis of fucosyloligosaccharides by fucosidase may exist in human milk, but direct evidence for such a process is lacking.

Although we suspect that much of the variation in fucosyltransferase and fucosidase levels among individuals is genetically based, the physiologic events in the mammary tissue, and specifically in the alveolar cell, that underlie the changes in fucosyltransferase and fucosidase activities over the course of lactation are unknown. Other factors may contribute to both types of variation, including changes in physiological and pathological states, exposure to xenobiotic agents, etc.[33] Also, the relationship between these enzymes and the fucosyloligosaccharide profile of human milk is not known. Some of these fucosyloligosaccharides are thought to have important biological activity.[34,35] Determining this relationship would allow the use of these enzymes as a measure of how various agents and conditions may affect the fucosyloligosaccharides of milk.

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