α_1-Antitrypsin and antichymotrypsin in human milk: origin, concentrations, and stability[1–3]

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ABSTRACT

Background: The protease inhibitors α_1-antitrypsin and antichymotrypsin are present in human milk, but little is known about their roles in protein digestion during infancy. It has been hypothesized that α_1-antitrypsin and antichymotrypsin may modulate digestion in the infant gut.

Objective: We determined whether the mammary gland expresses α_1-antitrypsin and antichymotrypsin, measured α_1-antitrypsin and antichymotrypsin throughout lactation, assessed the resistance of α_1-antitrypsin to proteolysis, and determined the potential of α_1-antitrypsin to affect the survival of other milk proteins.

Design: A pool of complementary DNA from the human mammary gland was analyzed with polymerase chain reaction to detect genes for α_1-antitrypsin and antichymotrypsin. α_1-Antitrypsin and antichymotrypsin concentrations were measured in milk samples obtained longitudinally (days 4–47) from 8 women. An in vitro model of infant digestion was used to assess the digestive stability of α_1-antitrypsin against pepsin and pancreatin. Lactoferrin, with α_1-antitrypsin present, was digested by pancreatin, and the digested proteins were separated.

Results: α_1-Antitrypsin and antichymotrypsin concentrations were high in early milk and decreased throughout lactation. Polymerase chain reaction products were detected for both genes. After in vitro digestion, much of the α_1-antitrypsin was still intact, whereas many other milk proteins were digested. Much of the lactoferrin was still intact after digestion, but only when α_1-antitrypsin was added.

Conclusions: The results suggest that α_1-antitrypsin and antichymotrypsin are produced by the mammary gland and are present in milk in relatively high amounts in early lactation. α_1-Antitrypsin may survive digestion and may affect the survival of other proteins. *Am J Clin Nutr* 2002;76:828–33.

KEY WORDS α_1-Antitrypsin, antichymotrypsin, human milk, milk proteins, mammary gland, infant digestion, breast milk, protease inhibitors, breast-feeding

INTRODUCTION

Little is currently known about the physiologic relevance of protease inhibitors in human milk. α_1-Antitrypsin and antichymotrypsin have been identified in human milk (1, 2). α_1-Antitrypsin belongs to the family of serpins, or serine proteinase inhibitors. The molecular mass of α_1-antitrypsin is ≈52 kDa, and it contains ≈15% carbohydrate (3). Reported concentrations of α_1-antitrypsin in human milk range from 0.1 to 0.4 g/L in early lactation, with a subsequent decrease as lactation progresses (2, 4, 5). Although the binding affinity of α_1-antitrypsin appears to be highest for human neutrophil elastase, it also has affinity for the pancreatic proteases chymotrypsin and trypsin (6). Antichymotrypsin is also a serpin, with a molecular mass of ≈68 kDa, and glycosylation accounts for 26% of its mass (7). Reported concentrations of antichymotrypsin in human milk range from 0.4 to 0.7 g/L during early lactation, with a decline as lactation progresses (1, 8). Milk antichymotrypsin appears to differ from the serum form in terms of certain characteristics, such as lower motility during gel electrophoresis (1, 8). α_1-Antitrypsin and antichymotrypsin exhibit some homology. A comparison of their amino acid sequences showed 42% homology between them (9), and the introns of both enzymes are also in identical locations.

A few hypotheses have been suggested regarding the role of protease inhibitors for both the mother and infant. Udall et al (10) postulated that endogenous proteases from the intestine could enter the portal circulation and induce inflammation in the liver, and that milk α_1-antitrypsin might inactivate some of those proteases and protect the infant liver. However, the high concentrations of serum and liver α_1-antitrypsin should also help protect the hepatocytes from potential protease damage. Another possibility is that protease inhibitors affect local proteolytic activity within the mammary gland during colostrum formation (1). Protease inhibitors might also inactivate proteases that are potentially secreted by macrophages found in breast milk (8).

Another possible role of milk protease inhibitors could be to increase the survival of other milk proteins via partial inhibition of pancreatic proteases. Some of the advantages of breast-feeding have been attributed to biologically active proteins unique to human milk; these proteins need to remain intact in the gastrointestinal tract to retain their bioactivity. Examples of such milk proteins are lactoferrin and lysozyme. Lactoferrin facilitates the absorption of iron and lysozyme maintains a bacteriostatic gastrointestinal environment (11). In infants, low secretion of

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hydrochloric acid in the stomach results in a pH of ≈3.5–5, far higher than the optimal pH for pepsin (12). As a result, digestion in the duodenum plays a key role in infants. Although it is unlikely that α_1-antitrypsin and antichymotrypsin completely inhibit pancreatic proteolytic activity, they may allow enough of these milk proteins to survive and influence infant development.

SUBJECTS AND METHODS

In this longitudinal study, breast-milk samples were provided by 8 mothers from day 4 to day 47 postpartum. All of the subjects provided written, informed consent, and the study protocol was approved by the University of California, Davis, Human Subjects Committee.

All reagents were purchased from Sigma Chemicals (St Louis). Human serum α_1-antitrypsin and antichymotrypsin were obtained from Athena Biochemicals (Athens, GA) and ICN Biomedicals (Costa Mesa, CA), respectively, because neither of the milk forms are commercially available. Human lactoferrin (iron-saturated) was purchased from Sigma Chemicals. We obtained rabbit anti-human α_1-antitrypsin from Sigma Chemicals and rabbit anti-human antichymotrypsin from Accurate Chemicals (Westbury, NY). Goat anti-α_1-antitrypsin conjugated to horseradish peroxidase (HRP) was purchased from ICN Biomedicals and sheep anti-antichymotrypsin conjugated to HRP was obtained from Biodesign International (Kennebuck, ME). Donkey anti-rabbit IgG conjugated to HRP was purchased from Amersham Pharmacia Biotech (Piscataway, NJ).

Whey isolation by casein precipitation

Whey was isolated by first adjusting the pH of each milk sample to 4.3 with the use of 1 mol HCl/L. Calcium chloride was added to a final concentration of 60 mmol/L. The samples were incubated at room temperature for 20 min and then at 4 °C for 45 min. Then the samples were spun in an ultracentrifuge at 4 °C for 1 h at 240 000 × *g*.

Immunoassays

The concentrations of standards for the assays ranged from 1.25 to 20 mg α_1-antitrypsin/L and from 5 to 40 mg antichymotrypsin/L diluted in phosphate-buffered saline (pH 7.4) and 0.05 % Tween-20 polyabsorbate (PBST; ICI Americas Inc, Wilmington, DE). A serum sample and a mature milk sample not included in the data analysis were used as interassay controls. Nunc Immuno-plate Maxisorp 96-well plates (Nalge Nunc International, Naperville, IL) were coated for 16 h at 4 °C with a 1:10 000 dilution of rabbit anti-human α_1-antitrypsin or a 1:2500 dilution of rabbit anti-human antichymotrypsin in 0.05 mol sodium bicarbonate/L (pH 9.6). The plates were washed 3 times with PBST and were subsequently incubated with sample for 1 h at room temperature while rocking. The plates were washed again 3 times with PBST and then incubated with a 1:50 000 dilution of goat anti-human α_1-antitrypsin conjugated to HRP or a 1:250 dilution of anti-human antichymotrypsin conjugated to HRP for 1 h at room temperature. The plates were washed 3 times with PBST, and bound antibody was detected with the HRP- and hydrogen peroxide-catalyzed reaction of 3,3′,5,5′ tetramethylbenzidine. The reaction was stopped with sulfuric acid (2 mol/L), and the plates were read on a Labsystems Multiscan Ascent microtiter plate reader (Labsystems Inc, Franklin, MA) at 450 nm, with 620 nm as a reference filter.

Lowry assay

Whey samples were diluted 1:100 in 0.15 mol NaCl/L. The standards consisted of bovine serum albumin (dissolved in 0.15 mol NaCl/L) in concentrations ranging from 0.025 to 0.20 g/L. A modification of the Lowry assay (13) was used, as described by Peterson (14). The same interassay controls that were used in the immunoassays were used for this assay.

In vitro digestion in buffer and human milk

The procedure developed by Rudloff and Lönnerdal (15) was used, with some modifications. The α_1-antitrypsin was prepared at a concentration of 1 g/L in PBS and was diluted 1:1 in either PBS or thawed, pooled mature human milk, resulting in a final concentration of 0.5 g/L. Hydrochloric acid (1 mol/L) was added to all samples to adjust the pH to 4.5, and 2.5 μL 2% pepsin in 0.01 mol HCl/L (3100 U/mg solid) was added; samples were then placed in a shaking incubator for 30 min at 37 °C. The pH was then restored to 7.0 by dropwise addition of 1 mol $NaHCO_3$/L. Subsequently, 2.5 μL 0.4% pancreatin in 0.1 mol $NaHCO_3$/L were added to each sample. The samples were then incubated for 1 h at 37 °C, and the reaction was halted by diluting the samples 1:1 in sample buffer and boiling for 3 min.

Digestion of lactoferrin and α_1-antitrypsin

Samples (1 mL) containing 1 g human lactoferrin/L, 0.1 g pancreatin/L, and either 0.10, 0.25, or 0.50 g α_1-antitrypsin/L or 0.50 g human albumin/L in PBS were incubated for 1 h at 37 °C. A sample with 1 g lactoferrin/L only was used as an undigested lactoferrin control, and the sample with albumin was used as a control to show that the effects of α_1-antitrypsin are a result of its inhibitory activity, not the addition of extra protein. The reaction was halted by diluting the samples 1:1 in sample buffer and boiling for 3 min.

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis

For sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), α_1-antitrypsin samples were diluted 1:1 in sample buffer consisting of 1.25 mol tris-HCl/L (pH 6.8), 10% SDS, 10% sucrose, and 0.05% bromophenol blue. α_1-Antitrypsin (≈2 μg/well) was loaded on a 12% minigel (BioRad, Hercules, CA). Gels were run for 35 min at 200 V in tris-glycine buffer consisting of 25 mmol tris-HCl/L (pH 8.2), 0.19 mol glycine/L, and 0.1% SDS. For detection of proteins, gels were stained for 6 h in Coomassie Brilliant Blue R. Destaining with acetic acid:ethanol:water (10:25:65) was halted when the background was clear.

Western blot analysis

After separation by SDS-PAGE, proteins were electroblotted onto a nitrocellulose membrane in tris-glycine buffer (25 mmol tris/L and 0.19 mol glycine/L, pH 8.2) at a constant current of 60 mA for 45 min at 4 °C. The membrane was blocked overnight at 4 °C in a 4% bovine serum albumin solution. After blocking, the membrane was washed 3 times (5 min/wash) in PBST (PBS, pH 7.4 and 0.1% Tween-20) to reduce nonspecific binding of antiserum to the membrane. The membrane was incubated in a 1:5000 dilution of rabbit anti-α_1-antitrypsin for 1 h at room temperature, followed by 3 washes in PBST and incubation in a 1:5000 dilution of donkey anti-rabbit IgG conjugated to HRP for 1 h at room temperature. The membrane was washed 3 times in PBST, and bound antibody was detected with a chemiluminescent system (ECL

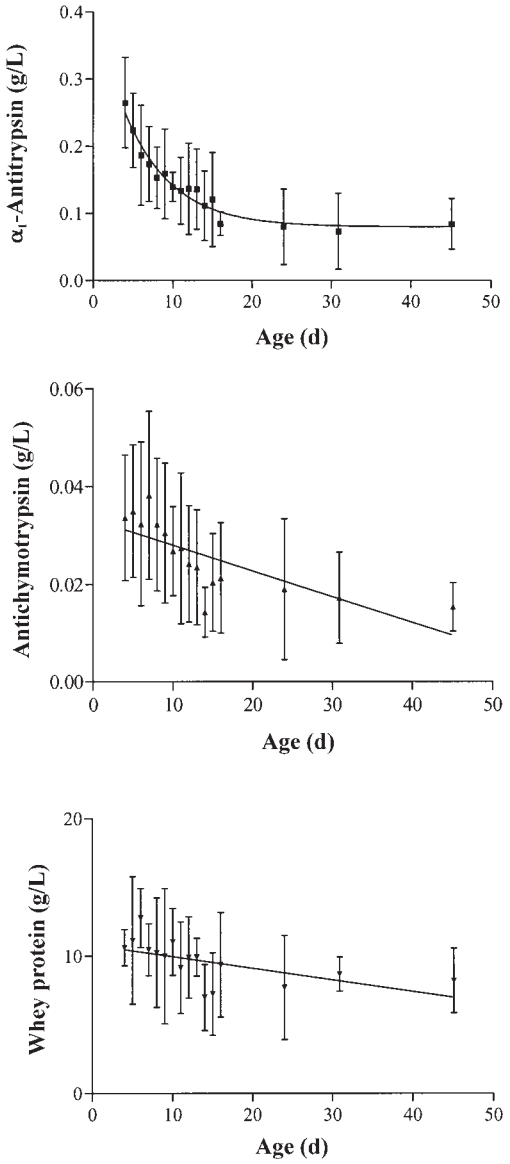

FIGURE 1. Mean (±SD) concentrations of α_1-antitrypsin, antichymotrypsin, and whey protein in breast-milk samples obtained from 8 women. The best-fit curves reflect the one-phase exponential decay model for α_1-antitrypsin concentrations and the linear regression model for antichymotrypsin and whey protein concentrations.

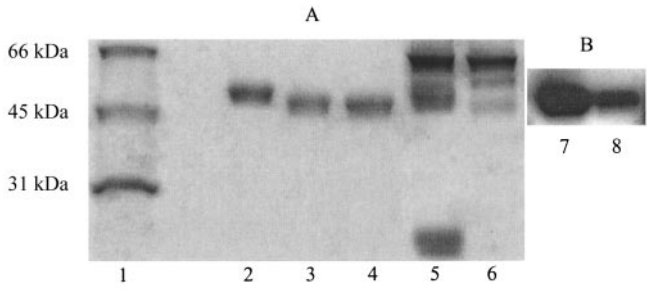

FIGURE 2. A: In vitro digestion of α_1-antitrypsin added to phosphate-buffered saline (PBS) and mature human milk as analyzed with sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) in nonreducing conditions with Coomassie staining: lane 1, broad-range markers; lane 2, α_1-antitrypsin (control, undigested) in PBS; lane 3, α_1-antitrypsin after 30 min pepsin + 60 min pancreatin digestion in PBS; lane 4, α_1-antitrypsin after 60 min pepsin + 120 min pancreatin digestion in PBS; lane 5, α_1-antitrypsin added to milk (control, undigested); and lane 6, α_1-antitrypsin added to milk after 30 min pepsin + 60 min pancreatin digestion. B: Western blot of α_1-antitrypsin added to milk: lane 7, control or undigested; lane 8, α_1-antitrypsin added to milk after 30 min pepsin + 60 min pancreatin digestion. (Lanes 7 and 8 show Western blots of the same samples shown in lanes 5 and 6 of the SDS-PAGE, respectively).

reagent) consisting of an HRP- and hydrogen peroxide– catalyzed reaction of luminol.

Polymerase chain reaction

The PCR Advantage cDNA kit (Gibco, Grand Island, NY) was used to perform polymerase chain reaction (PCR) on a complementary DNA (cDNA) pool from the human mammary gland (Invitrogen, Carlsbad, CA). The primers were designed by using the Primer3 output program (www-genome.wi.mit.edu/cgi-bin/primer/primer3-www.cgi). The α_1-antitrypsin forward primer, 5′-CACTGTCAACTTCGGGGACACC-3′, and the α_1-antitrypsin reverse primer, 5′-CCCTTTCTCGTCGATGGTCAG-3′, were designed to amplify a region between nucleotide positions 539 and 1142. The antichymotrypsin forward primer, 5′-GCAGACAATGATGGTCCTGGTG-3′, and the antichymotrypsin reverse primer, 5′-CATCAGGAAGGGCCTGTTGAAA-3′, were modeled to create a transcript from positions 628 to 1228. The primers for each reaction were combined with a cDNA pool from the human mammary gland and the sequences were amplified with PCR under the following conditions: 94 °C for 1 min initially, followed by 25 cycles of 94 °C for 30 s, 64 °C for 1 min, and 72 °C for 2 min.

The products were separated with electrophoresis on a 1% agarose gel and were visualized under ultraviolet light. In addition, the products were purified in a Microcon 100 (Millipore, Bedford, MA) and sequenced by Davis Sequencing (Davis, CA) with an ABI Prism 377 Sequencer (Applied Biosystems, Foster City, CA) by using the PCR dideoxy-chain-termination method. Sequence translations and analysis were performed by using CHROMAS software, version 1.45 (Technelysium Pty Ltd, Helensvale, Australia). Sequences were searched by using the BLAST program (www.ncbi.nlm.nih.gov; National Center for Biotechnology Information, Bethesda, MD; accessed September 2000) to determine sequence homology to human serum α_1-antitrypsin and antichymotrypsin.

Statistical analyses

STATVIEW (version 5.0.1) was used for the data analysis (16). A one-sample *t* test versus mean = 0 was performed to compare the decreasing concentrations of α_1-antitrypsin, antichymotrypsin, and whey protein with the between-subject variability in the milk samples. GRAPHPAD PRISM (version 3.02; 17) was used to graph the α_1-antitrypsin, antichymotrypsin, and whey protein concentrations. The best-fit curves reflect the one-phase exponential decay model for α_1-antitrypsin concentrations and the linear regression model for antichymotrypsin and whey protein concentrations.

RESULTS

The data obtained with the enzyme-linked immunosorbent assays for both α_1-antitrypsin and antichymotrypsin showed rapidly decreasing concentrations over time and high variability

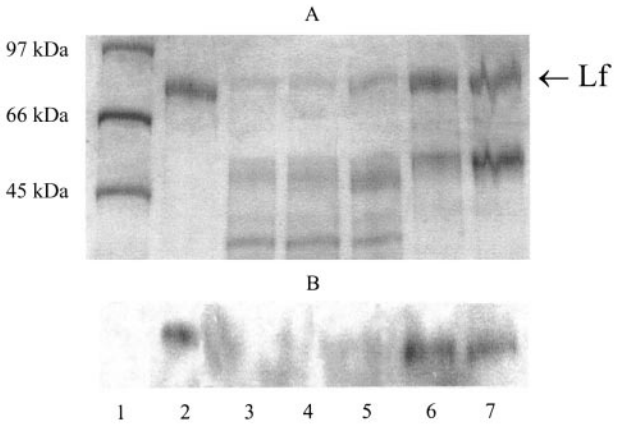

FIGURE 3. Ability of α_1-antitrypsin to protect lactoferrin from digestion, as analyzed with sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) in nonreducing conditions with Coomassie-staining (A) and Western blot (B): lane 1, broad-range markers (SDS-PAGE only); lane 2, lactoferrin only with no pancreatin or α_1-antitrypsin; lane 3, lactoferrin and pancreatin with no α_1-antitrypsin; lane 4, lactoferrin, pancreatin, and albumin; lanes 5, 6 and 7, lactoferrin, pancreatin, and 0.10, 0.25, and 0.50 g α_1-antitrypsin/L, respectively. The arrow points to lactoferrin (Lf) at ≈80 kDa.

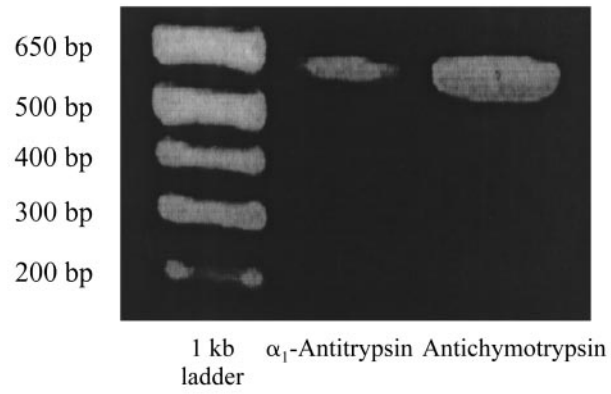

FIGURE 4. α_1-Antitrypsin and antichymotrypsin polymerase chain reaction products. The products were amplified from a mammary gland complementary DNA pool by using primers designed from known sequences of α_1-antitrypsin and antichymotrypsin complementary DNA. bp, base pairs.

between mothers (**Figure 1**). Within each mother, concentrations of both proteins decreased over time; the mean α_1-antitrypsin and antichymotrypsin concentrations declined from 0.27 and 0.034 g/L on day 4 postpartum to 0.08 and 0.021 g/L on day 16 postpartum, respectively. The interassay CVs for the serum and milk controls were 4.1% and 3.7% for α_1-antitrypsin and 2.8% and 3.9% for antichymotrypsin, respectively. Data from all 8 mothers showed negative slopes for the best-fit lines determined with one-phase exponential decay or linear regression models. The exponential decay model for α_1-antitrypsin reflects the rapid decline in milk α_1-antitrypsin concentrations within the first few days of lactation, whereas the linear regression model suggests a more steady decrease. In addition, a one-sample *t* test versus mean = 0 was conducted on the slopes of the data from each mother to determine whether the decreasing trends were independent of between-subject variability. The consistency of the decreasing trends for both α_1-antitrypsin ($P = 0.04$) and antichymotrypsin ($P = 0.0003$) was significant. Variability between mothers was particularly high; the average SD between mothers for days 4–45 was 38% and 47% of the means for α_1-antitrypsin and antichymotrypsin, respectively.

Total whey protein concentrations (Figure 1) also show a decreasing trend over time. However, this decrease is not as pronounced as those found for the protease inhibitors. Also, the trend, quantified by the slopes of the best-fit lines, was not significant when analyzed with a one-sample *t* test versus mean = 0 ($P = 0.09$). In addition, data from only 6 of the 8 mothers show negative slopes from linear regression estimations. The interassay CVs were 8.0% and 4.1% for the serum and milk controls, respectively.

The in vitro digestion experiment showed that α_1-antitrypsin is resistant to proteolysis by pancreatic enzymes in both PBS and human milk. The SDS-PAGE data show that a large amount of α_1-antitrypsin remains after digestion, even after 60 min of pepsin digestion and 120 min of pancreatin digestion (**Figure 2**). Human serum albumin was completely digested under these conditions (data not shown). The digestion of α_1-antitrypsin in milk showed that a large amount survived, especially when compared with other milk proteins that had significant degradation. The bands of some easily digested proteins were completely absent after digestion; these proteins served as internal controls and showed that they may be preferentially cleaved compared with α_1-antitrypsin. The Western blot data (Figure 2) corroborate the SDS-PAGE data and confirm that the protein at 53 kDa that resisted degradation is α_1-antitrypsin.

The addition of α_1-antitrypsin during the digestion of lactoferrin by pancreatin showed that α_1-antitrypsin has the potential to protect other milk proteins from digestion. The SDS-PAGE and Western blot data show that more lactoferrin remains intact when α_1-antitrypsin is present, particularly at the 0.25 and 0.50 g/L concentrations (**Figure 3**). The addition of 0.50 g albumin/L had no visible protective effect against the digestion of lactoferrin. Bands visible on the Coomassie-stained gel at other molecular weights are most likely peptide fragments of lactoferrin or of α_1-antitrypsin.

The PCR data suggest that both α_1-antitrypsin and antichymotrypsin are expressed by the human mammary gland, because their transcripts are detectable in a cDNA pool from the mammary gland. The lengths of both products are ≈600 base pairs (**Figure 4**), which matches the lengths expected from the positions of the primers. Sequence analysis using BLAST showed that 96% and 97% (504/521 and 426/439 base pairs, respectively) of the nucleotides in the products aligned with those reported in GenBank (the NIH genetic sequence database; www.ncbi.nlm.nih.gov; accessed September 2000) for liver α_1-antitrypsin (accession no. X01683) and liver antichymotrypsin (accession no. K01500), respectively.

DISCUSSION

Protease inhibitors need to be present in significant quantities to affect protein digestion in infants. Concentrations of antichymotrypsin and α_1-antitrypsin, especially α_1-antitrypsin, were particularly high during the first 21 d of lactation. As shown by the best-fit curves, concentrations of antichymotrypsin declined

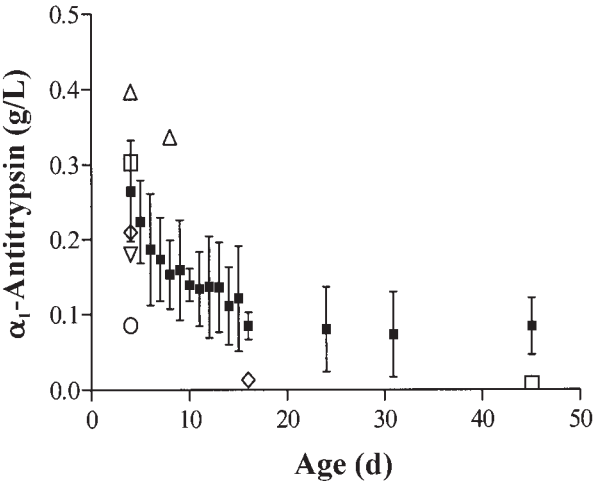

FIGURE 5. Mean (±SD) concentrations of α_1-antitrypsin in human milk, as reported in previous studies, plotted against the concentrations found in the present study: present study (■), Lindberg et al (◇;1), Totterdell et al (○; 20), McGilligan et al (□; 5), Davidson and Lönnerdal (△; 2), Urueña et al (▽; 8).

steadily from day 4 to day 16, whereas α_1-antitrypsin concentrations declined most rapidly during the first few days of lactation. The concentrations of several milk proteins, such as secretory IgA, lactoferrin, and α-lactalbumin, are also high in early milk (18); many of these proteins need to be intact to retain their bioactivity. Whey protein concentrations also declined over time, but the decreases in the protease inhibitor concentrations were more pronounced. Therefore, it is unlikely that the decreases in α_1-antitrypsin and antichymotrypsin concentrations can be fully attributed to a general decline in whey protein concentrations. The higher concentration of α_1-antitrypsin relative to antichymotrypsin suggests that α_1-antitrypsin might be the more predominant protease inhibitor, which coincides with its ability to inhibit both trypsin and chymotrypsin, the primary pancreatic proteases. In addition, antichymotrypsin has lower specificity to chymotrypsin than does α_1-antitrypsin, and antichymotrypsin does not bind to trypsin (6).

The cause and relevance of the high variability of α_1-antitrypsin and antichymotrypsin concentrations in milk among women are unknown. One source of variability during early lactation could be different maternal experiences during labor; for example, stress during labor was shown to delay lactogenesis (19). The day postpartum on which the breast begins actively secreting milk varies considerably among women, and this strongly affects protein concentrations in milk. Lindberg et al (1) did not find any physiologic problems, such as mastitis, infantile colic, or infant susceptibility to infection, linked to colostrum samples that lacked protease-inhibiting activity. Thus, it is possible that during early life, healthy infants may have pronounced variations in the amounts of milk proteins that remain intact; this applies to proteins that are prone to degradation and also to those that are inherently resistant.

McGilligan et al (5) stated that previous studies (1, 20) reported conflicting data regarding α_1-antitrypsin concentrations in milk. This may be a result of high interindividual variability, but the most likely factor is the age of the infant when the milk was sampled. When the findings of other investigators are plotted against the concentrations found in this study (**Figure 5**), it is evident that the mothers were at various stages of lactation in those studies. The studies that reported lower concentrations tended to sample milk during later stages of lactation. Differences between the previously reported antichymotrypsin concentrations in milk may also be attributed to the time (ie, the day postpartum) when the milk was sampled. Although reported colostrum antichymotrypsin concentrations range from 0.4 to 0.7 g/L, Lindberg et al (1) found that antichymotrypsin declines by >80% between day 1 (colostrum) and day 4. This may explain why the antichymotrypsin concentrations on day 4 in this study are much lower than the concentrations found in colostrum in the other 2 studies. Antichymotrypsin concentrations after day 16 in this study generally corroborate those found by Lindberg et al (1).

The resistance of α_1-antitrypsin to digestion in human milk supports the theory that α_1-antitrypsin can survive and can effectuate its activity in the infant duodenum. In the infant, low secretion of hydrochloric acid in the stomach results in a pH of ≈3.5–5, far higher than the optimal pH condition for pepsin (12). As a result, digestion in the duodenum plays a key role in the infant for the first few months of life, whereas pepsin activity increases as the infant ages. In the current study, the conditions of the in vitro model of infant digestion (pH 4.5 during pepsin treatment) were designed to reflect the low secretion of acid. The in vitro digestion of human milk proteins shows that casein (≈27 kDa, Figure 2) is more effectively digested than is α_1-antitrypsin. The relative resistance of α_1-antitrypsin compared with that of other proteins may be an important factor, in addition to the changes in total proteolytic capacity. Human milk proteins, particularly those that are easily digested, might provide alternate substrates for the proteases and might thereby protect α_1-antitrypsin indirectly. α_1-Antitrypsin was also detected in the feces of breast-fed infants, which supports the theory that it is capable of surviving digestion in vivo, particularly during the first 3 mo of life (2). This evidence also supports the validity of the in vitro model of digestion used in the current study. The resistance of α_1-antitrypsin to digestion, combined with its comparatively high concentrations in human milk, may allow significant amounts to reach the duodenum and increase the survival of other milk proteins in the upper gastrointestinal tract of the infant, particularly during the first few weeks of life.

The ability of α_1-antitrypsin to protect lactoferrin from digestion by pancreatin in vitro suggests that it has the potential to protect milk proteins by inhibiting proteases. In our digestion experiment, we used concentrations of lactoferrin (1.4 g/L) (18) and α_1-antitrypsin that are typically found in human milk. Pepsin was not added because its activity is low in infants, and α_1-antitrypsin inhibits trypsin and chymotrypsin but not pepsin. Albumin did not prevent the degradation of lactoferrin, which suggests that α_1-antitrypsin protected lactoferrin with its inhibitory activity and did not merely serve as an alternate substrate for the proteases.

The detection of the transcripts in cDNA derived from the mammary gland supports the suggestion (8) that the protease inhibitors are produced locally in the breast. Local production in the mammary gland might suggest that protease inhibitors play a significant role in mammary function or in infant development. However, this does not eliminate the possibility that a portion of the milk α_1-antitrypsin or antichymotrypsin might originate in the serum. Milk α_1-antitrypsin concentrations are only ≈10% of the concentration found in normal

adult serum (1.3 g/L) (21), and milk antichymotrypsin concentrations are also lower than serum concentrations (0.3–0.6 g/L) (22, 23). Particularly for α_1-antitrypsin, the relative concentration found in milk as compared with serum represents a downward diffusion gradient.

The results of this study support the putative role of protease inhibitors in aiding the survival of other biologically active milk proteins in the gastrointestinal tract of infants. The specific results that provide support for this theory are the significant concentrations of α_1-antitrypsin and antichymotrypsin in breast milk, the resistance of milk α_1-antitrypsin to digestion, the ability of α_1-antitrypsin to protect lactoferrin from proteolysis, and the detection of transcripts of the protease inhibitors through PCR of mammary gland cDNA.

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