Heat Inactivation of Bile Salt-Stimulated Lipase Activity in Human Milk and Colostrum

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ABSTRACT

Time-temperature relationships for heat-inactivation of bile salt-stimulated lipase activity in human milk and colostrum were systematically measured using a pH-stat assay procedure with trolley as substrate. The enzyme was not affected in either menstruum at 45°C for 40 min. The enzyme was destroyed almost instantaneously at 60°C, and was slightly more heat-sensitive in colostrum than in milk. The bile salt-stimulated lipase(s) in human milk was more heat sensitive than lipase in bovine milk.

Human milk contains two lipases, one with the classical properties of serum-stimulated lipase and a second lipase whose activity is stimulated by bile salts (8). Bile salt-stimulated (BSS) lipase appears to account for most of the lipolytic activity in human milk. Human milk contains enough of this lipase, and the enzyme’s turnover rate is sufficiently rapid, to hydrolyze the milk triglycerides almost completely in 20 min (11). Olivercrona and Hernell (11) concluded that the BSS lipase activity in human milk is essential for proper fat absorption by neonates, particularly low birth weight and pre-term infants. In contrast, although cow’s milk lipase might be important in milk fat synthesis (12), no specific desirable function is associated with its presence in milk. Indeed, serum-stimulated lipase activity in bovine milk leads to development of a “lipolyzed” flavor in the unpasteurized product, but the enzyme is inactivated by all commercial pasteurization treatments.

Pasteurization is recommended for pooled and/or banked human milk and colostrum to destroy any pathogenic bacteria present (1). Ideally this heat treatment should not affect the natural anti-infective agents or other important constituents present. The most commonly used pasteurization condition has been 62.5°C for 30 min (3,4), duplicating the low temperature-long time (LT-LT) procedure for pasteurizing bovine milk. When human milk is given this heat treatment, lactoferrin and the IgA and IgM levels are reduced but the lysozyme level is stable and the milk remains effective against in vitro growth of Escherichia coli (3,4).

Since a specific function has been suggested for the BSS lipolytic activity in human milk, a systematic study of the sensitivity of the enzyme to various heat treatments is needed. The present study will explore the time-temperature relationships for heat inactivation of the BSS lipase in pooled human milk and colostrum.

EXPERIMENTAL PROCEDURES

Human milk and colostrum samples were collected by experienced personnel using an Egnell (Cary, IL) breast pump between the hours of 9 AM and 12 noon. The entire lacteal secretion of one breast was collected from each donor and included only milk from mammary glands with no visible evidence of mastitis or other abnormalities. Milk samples were from donors 4 weeks postpartum; colostrum samples were defined as the lacteal secretion collected within 48 h postpartum. All samples were immersed in ice water and transported to Texas A&M University within 2 h of collection. Samples were then pooled with each pool representing at least 6 women and containing at least 350 ml of milk or colostrum. Samples were maintained at 4 ± 2°C for a maximum of 24 h before heat treatment. Bovine milk samples were obtained from mixed whole milk from the Texas A&M University dairy herd. Milk and colostrum samples were heated to temperatures between 45 and 60°C for times varying from 15 sec to 40 min. Heating was accomplished by placing the samples between two 0.91-mm thick 303 stainless steel plates separated by a silicone gasket 2 mm thick (Fig. 1). The plates measured 12.6 × 34.7 cm, and were held together by bolts placed 3 cm on center. A 0.58-mm diameter thermistor was suspended 1 cm from the bottom of the heating chamber. The apparatus was immersed to within 1 cm of the top in a stirred water bath and allowed to equilibrate to the heating temperature. The water bath was maintained at the set temperature ±0.1°C. Forty milliliter portions of milk or colostrum were injected between the plates and timing was started when the temperature of the sample was within 0.5°C of the holding temperature. Approximately 30 sec was necessary to heat cold milk to 50°C. This time could be shortened to 10-12 sec when the milk was pre-warmed to approximately 30°C. At the
TABLE 1. Mean values and standard deviations (S.D.) for bile-stimulated lipase activity in individual and pooled samples of human milk and colostrum.

<table>
<thead>
<tr>
<th></th>
<th>Milk</th>
<th>Colostrum</th>
</tr>
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<tbody>
<tr>
<td>Sample</td>
<td>n</td>
<td>μeq NaOH/min/ml</td>
</tr>
<tr>
<td>Individual</td>
<td>26</td>
<td>7.85</td>
</tr>
<tr>
<td>Pooled</td>
<td>5</td>
<td>6.71</td>
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Figure 1. Heating chamber used for inactivating the lipase activity in human milk and colostrum.

end of the holding period, a 3-ml portion was taken using a syringe. The syringe was immediately immersed in ice water.

The BSS lipase activity was measured by a modification of the method of Hernell and Olivecrona (8). A pH-stat titrator (Metrohm Herisau) was used for this study. The enzyme substrate was prepared by blending 10 ml of triolein with 10 g of ice and 80 g of a 10% (w/v) aqueous gum arabic solution in a Waring blender for 10 min. Five milliliters of substrate, 0.2 ml 2.85 M NaCl and 0.1 ml of 10% aqueous sodium taurocholate were placed in a titration chamber and allowed to equilibrate to 37°C. Sodium taurocholate was omitted from the assay mixture when measuring bovine milk lipase. One milliliter of milk or colostrum was added and the pH adjusted to 8.8. The volume of 0.01 N NaOH required to maintain pH 8.8 was plotted continuously as a function of time. Enzyme activity was expressed as μeq NaOH/min/ml enzyme source.

Data were analyzed using a randomized block design with pools treated as blocks. Pools were processed and analyzed at 2-week intervals.

RESULTS

The BSS lipase activity in individual samples of human milk (Table 1) averaged 7.85 and ranged from 4.58 to 13.27 μeq NaOH/min/ml enzyme source. The pooled colostrum samples exhibited more lipase activity, and were somewhat less variable, than the pooled milk samples. The standard deviations for pooled milk and colostrum samples were 1.50 and 0.68, respectively. This is less than the standard deviations for individual milk and colostrum samples, which were 2.43 and 1.50, respectively. The differences in activity between pools of both milk and colostrum were sufficiently large to make comparisons from the replications of the heat inactivation data difficult. Therefore, the values for residual lipase activity were expressed as percentages of original activity in each unheated pool.

Results (3 pools) for heat-inactivation of BSS lipase activity in human milk and colostrum are summarized in Fig. 2 and 3. There was no inactivation of BSS in milk or colostrum at 45°C for holding times up to 40 min. Lipase activity decreased rapidly at 50°C, and the heat effect appears to be slightly greater in colostrum than in milk. Greater than 90% of the BSS lipase activity was lost when milk was heated 20 min at 53°C. However, 10 min of heating at 53°C was needed to produce comparable results in colostrum. Increasing heating time or temperature beyond this point produced an even greater reduction in BSS lipase activity with essentially instantaneous destruction of lipase activity at 60°C.

Heat inactivation data based on total loss of lipase activity for milk and colostrum are summarized in Fig. 4. A linear relationship exists between temperature and the common logarithm of time, as was shown previously for bovine lipase (9), and for denaturation of serum proteins in bovine milk (6). The time and temperature relationship for
total inactivation of bovine milk lipase activity is included for comparative purposes. This relationship provides a means of validating our heating system with commercially available systems for heating larger quantities of milk because the time and temperature relationship for inactivation of bovine milk lipase parallels very closely that published by Hetrick and Tracy (9).

**DISCUSSION**

Recent evidence suggests that the BSS lipase activity in human milk is important in fat absorption by the neonate (11), particularly for pre-term and low birth weight infants. This places an unusual importance on this enzyme, because such components in both human and bovine milks have been assumed to bear no important nutritional or physiological importance to the newborn (13). For instance, a major emphasis in the commercial processing of bovine milk is the deliberate destruction of lipase to prevent development of hydrolytic rancidity from free fatty acid accumulation during storage.

The data for pooled colostrum indicate that the BSS lipase activity was slightly more heat labile in this menstruum. The reason for this difference is not clear even though difference between the composition of human milk and colostrum are known (2,5), and the fact that lipase levels generally were higher in colostrum than in the milk samples studied.

Hernell (7), using an acetone-ether powder from human milk for an enzyme source, reported 95% destruction of the BSS lipase activity when it was heated for 40 min at 50°C. The heating apparatus was not described. This, in addition to differences in the enzyme form between the studies makes comparisons difficult. However, it is important to note that we found considerably less heat-inactivation at 50°C for 40 min than did Hernell (7). Since data collected in this study are from fresh milk, they should be more applicable in a banking situation (10).

The greater heat stability of the bovine enzyme as compared to the human enzyme is apparent at high temperature-short time combinations, which would be consistent with many commercial procedures for heat processing milk. This indicates that current commercial practices for pasteurizing bovine milk might not be applicable to a banking situation where it would be desirable to preserve lipase activity.

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**REFERENCES**