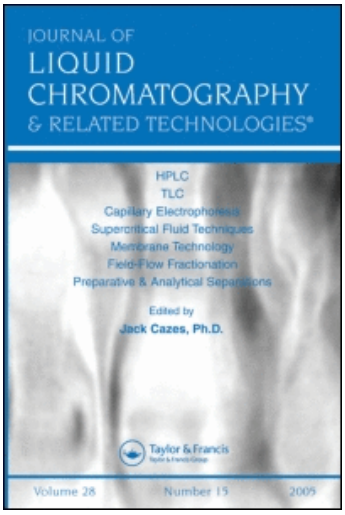

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James S. Swan[a]; Maryam Azadpur[a]; Ashok J. Bharucha[a]; Michael A. Krafczyk[a]
[a] Department of Chemistry, Bucknell University Lewisburg, Pennsylvania

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SEPARATION OF PROTEINS IN HUMAN MILK

JAMES S. SWAN, MARYAM AZADPUR,
ASHOK J. BHARUCHA AND
MICHAEL A. KRAFCZYK
Department of Chemistry
Bucknell University
Lewisburg, Pennsylvania 17837

ABSTRACT

A separation of proteins in human milk whey using high performance ion exchange chromatography is reported. Three chromatographic peaks were identified on the basis of retention times as bile salt-stimulated lipase (BSSL), lactoferrin, and α-lactalbumin. Implications for the purification of BSSL are discussed and suggestions for future purification procedures are made.

INTRODUCTION

Newborn mammals are sustained exclusively on their mother's milk. Each animal species has its own unique milk composition, presumably adapted to best meet the requirements of its own offspring. Maternal milk was also the primary source of human infant nutrition until

humans learned to domesticate cattle a few thousand years ago (1). Breast feeding by human mothers has been regaining its stature in recent years, fueled partly by the "all natural" trend as well as by scientific evidence that human milk contains numerous digestive enzymes, growth factors, and immunoglobulins that are beneficial for optimal infant development (1-5). Unfortunately, although bovine milk has been extensively studied, much remains to be learned about the composition of human milk (2, 6-10).

One protein of interest in human milk is the enzyme bile salt-stimulated lipase (BSSL, EC 3.1.1.3). This important digestive enzyme is found in milk from higher primates (11) and from dogs and cats (12). Abnormal activity of BSSL has been suggested as a contributing factor in the development of breast milk jaundice in infants (13,14). A thorough study of any such protein can require relatively large amounts of pure material, and so a fast and convenient purification method would be very desirable.

In published classical purifications of BSSL the contaminating proteins most difficult to remove have usually been identified as α-lactalbumin and lactoferrin (15,16); we, therefore, monitored the resolution of BSSL from these other two milk proteins in our separation.

EXPERIMENTAL

Materials

Tris(hydroxymethyl)aminomethane (reagent grade), lactoferrin (from human milk), and α-lactalbumin (from human milk) were all obtained from Sigma Chemical Company (St. Louis, Missouri). Sodium chloride (ACS-certified) and hydrochloric acid (ACS-reagent) were products of Fisher Scientific Company (Pittsburgh, Pennsylvania). Water was purified by passage through a Milli-RO4 system, followed by a final cleanup through a Milli-Q system (Millipore Corporation, Bedford, MA). Bile salt-stimulated lipase was purified by the method of Wang and Johnson (16).

Sample Preparation

Gloves were worn while working with the human samples, and all materials that directly contacted the milk were autoclaved before washing or disposal. Human milk whey was prepared by first centrifuging human milk in a Sorvall RC2-B superspeed refrigerated centrifuge (rotor type SS-34) at 4°C for 45 minutes at 18,000 rpm (relative centrifugal force = 39,079). The cream was removed and the pH of the resulting skim milk was adjusted to 4.6 using dilute HCl. The skim milk was then heated for 30 minutes at 40°C, and the precipitated casein proteins were removed by centrifugation at 18,000 rpm for 30 minutes. The supernatant (milk whey) was filtered through a 0.2 μm filter before analysis.

The total protein concentration in the milk whey was approximately 10 mg/mL.

Chromatographic Equipment

The HPLC used for this work was an SSI model GS 400 gradient system from Scientific Systems, Inc. (State College, Pennsylvania,). Samples were injected with a Rheodyne model 7125 injection valve equipped with a 20 µL sample loop (Rheodyne, Inc., Cotati, CA). An ISCO model V^4 variable wavelength detector was used at 254 nm and 0.02 AUFS (Isco, Inc., Lincoln, NE), and chromatograms were recorded with a Spectra-Physics model 4290 computing integrator (Spectra-Physics, San Jose, CA). The separation was accomplished on a SynChropak AX300 weak anion exchange column 250mm x 4.6mm I.D. (SynChrom, Inc., Lafayette, IN). Column temperature was maintained at 35°C with an SSI model 505 Column Oven (Scientific Systems, Inc.)

Chromatographic Conditions

A binary gradient was employed where mobile phase A was 100 mM Tris-HCl, pH 7.9, and mobile phase B was 100 mM Tris-HCl with 300 mM NaCl, pH 7.9. A linear gradient was used with %B increasing from 0% to 100% in 20 minutes, and the flow rate was 0.5 mL/min.

RESULTS AND DISCUSSION

After optimization of mobile phase conditions the separation illustrated in Figure 1 was achieved. Peaks

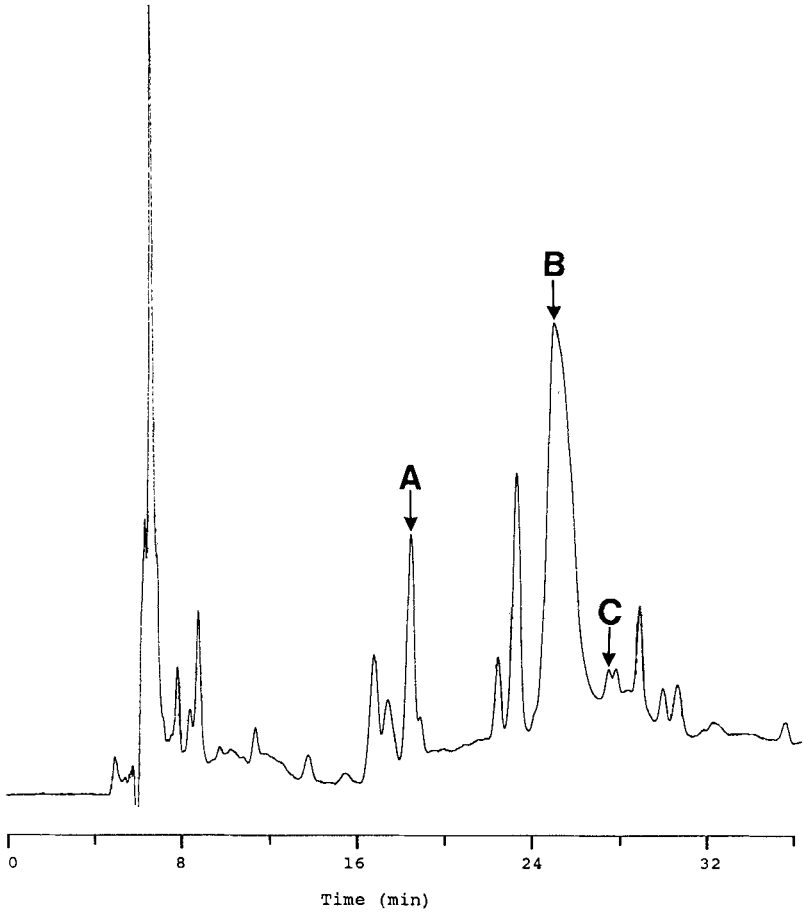

FIGURE 1. Chromatogram of human milk whey. A = lactoferrin, B = α-lactalbumin, C = BSSL. Conditions are given in text.

corresponding to BSSL, α-lactalbumin, and lactoferrin were tentatively identified based on the retention times of authentic protein samples. The long retention time for BSSL (approximately 27 min) was not unexpected since this protein is known to contain a high

percentage of acidic amino acids and has an isoelectric point of 3.7 (17).

As can be seen in Figure 1, this high performance ion exchange (HPIE) method separates BSSL from a multitude of other milk proteins. We are currently scaling up this separation to facilitate peak collection, although based on the appearance of the chromatogram it is likely that the BSSL obtained directly from the HPIE column will not be highly pure, and a multidimensional procedure may be necessary for the production of homogeneous enzyme. Since BSSL is known to have an affinity for heparin (15,16), a potentially fruitful approach would be to couple a heparin-based affinity column with our HPIE procedure. This purification scheme is currently being explored.

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REFERENCES

1. Ogra, P. L. and Greene, H. L., Human Milk and Breast Feeding: an Update on the State of the Art, Pediatr. Res., 16, 266, 1982.

2. Hamosh, M., Berkow, B., Bitman, J., Freed, L., Happ, B., Jones, J. B., Mehta, N. R., Wood, D. L., and Hamosh, P., Handling and Storage of Human Milk Specimens for Research, J. Pediatr. Gastroenterol. Nutr., 3, 284, 1984.

3. Heitlinger, L. A., Enzymes in Mother's Milk and Their Possible Role in Digestion, J. Pediatr. Gastroenterol. Nutr., 2(suppl. 1), S113, 1983.

4. Read, L. C., Upton, F. M., Francis, G. L., Wallace, J. C., Dahlenberg, G. W., and Ballard, F. J., Changes in the Growth-Promoting Activity of Human Milk during Lactation, Pediatr. Res., 18, 133, 1984.

5. Weaver, E. A., Rudloff, H. E., Goldblum, R. M., Davis, C. P., and Goldman, A. S., Secretion of Immunoglobulin A by Human Milk Leukocytes Initiated by Surface Membrane Stimuli, J. Immunol., 132, 684, 1984.

6. Gupta, B. B., Determination of Native and Denatured Milk Proteins by High-Performance Size Exclusion Chromatography, J. Chromatogr., 282, 463, 1983.

7. Andrews, A. T., Taylor, M. D., and Owen, A. J., Rapid Analysis of Bovine Milk Proteins by Fast Protein Liquid Chromatography, J. Chromatogr., 348, 177, 1985.

8. Chaplin, L. C., Hydrophobic Interaction Fast Protein Liquid Chromatography of Milk Proteins, J. Chromatogr. 363, 329, 1986.

9. Montgomery, P. A., Patton, S., Huston, G. E., and Josephson, R. V., Gel Electrophoretic Analysis of Proteins in Human Milk and Colostrum, Comp. Biochem. Physiol., 86 B, 635, 1987.

10. Sanchez-Pozo, A., Morales, J. L., Izquierdo, A., Martinez-Valverde, A., and Gil, A., Protein Composition of Human Milk in Relation to Mother's Weight and Socioeconomic Status, Hum. Nutr. Clin. Nutr. 41, 115, 1987.

11. Freudenberg, E., A Lipase in the Milk of the Gorilla, Experientia, 22, 317, 1966.

12. Freed, L. M., York, C. M., Hamosh, M., Mehta, N. R., Sturman, J. A., Oftedal, O. T., and Hamosh, P., Bile Salt-Stimulated Lipase: the Enzyme is Present in Non-Primate Milk, Hum. Lactation 2: [Proc. Int. Workshop Matern. Environ. Factors Hum. Lactation], Hamosh, M. and Goldman, A. S., eds., Plenum, New York, 1986, p. 595.

13. Poland, R. L., Schultz, G. E., and Garg, G., High Milk Lipase Activity Associated with Breast Milk Jaundice, Pediatr. Res., 14, 1328, 1980.

14. Mehta, N. R., Jones, J. J., and Hamosh, M., Lipases in Preterm Human Milk: Ontogeny and Physiologic Significance, J. Pediatr. Gastroenterol. Nutr., 1, 317, 1982.

15. Blackberg, L. and Hernell, O., The Bile Salt-Stimulated Lipase in Human Milk, Eur. J. Biochem., 116, 221, 1981.

16. Wang, C. S. and Johnson, K., Purification of Human Milk Bile Salt-Activated Lipase, Anal. Biochem., 133, 457, 1983.

17. Wang, C. S., Human Milk Bile Salt-activated Lipase, J. Biol. Chem. 256, 10198, 1981.