THE BILE SALT-STIMULATED LIPASE IN HUMAN MILK IS AN EVOLUTIONARY NEWCOMER DERIVED FROM A NON-MILK PROTEIN

Lars BLÄCKBERG, Olle HERNELL, Thomas OLIVECRONA, Lennart DOMELLÖF+ and M. René MALINOV*

Department of Chemistry, Section on Physiological Chemistry, University of Umeå, S-901 87 Umeå, +Department of Surgery, University Hospital of Umeå, S-901 85 Umeå, Sweden and **Oregon Regional Primate Research Center, Beaverton, OR 97005, USA*

Received 17 December 1979

1. Introduction

Milk from humans [1,2] and gorillas [3] contains an inactive form of a lipase which becomes activated when the milk is mixed with intestinal contents. The activation of the enzyme is caused by interaction with certain bile salts [4]. This lipase contributes substantially to the utilization of milk lipids by the newborn [5]. For instance, heat treatment of human milk inactivates the lipase [4] and reduces fat absorption by ~1/3rd in pre-term infants [6]. This bile salt-stimulated lipase is not present in milk from rats, guinea pigs, rabbits, dogs, goats, cows, pigs or horses ([1] unpublished). We now report a search for the evolutionary origin of this lipase. Two possibilities were considered:

(i) The lipase might have evolved through a mutational change of some other milk protein;
(ii) It might be a secretory protein from some other tissue which through a change in gene regulation has become expressed also by the milk producing cells.

The results presented show that the lipase is closely related to a pancreatic protein but not to any of the other milk proteins.

2. Materials and methods

Fresh human milk was obtained from donors in Umeå. The Rhesus milk was collected in Beaverton, OR and transported frozen to Umeå. The lipase activity in human milk is retained in samples stored frozen for several months [7]. Human pancreatic juice was obtained during cannulation of the pancreatic duct of a 57 year old female patient for diagnostic radiography. Human pancreatic tissue was obtained from the same patient at operation, immediately frozen and later homogenized in 0.01 M Tris–HCl, (pH 8). The procedures are detailed in figure legends.

3. Results

Rhesus milk showed no activity against long chain triglycerides emulsified in gum arabic whether bile salts were present or not (fig.1). This is in sharp con-

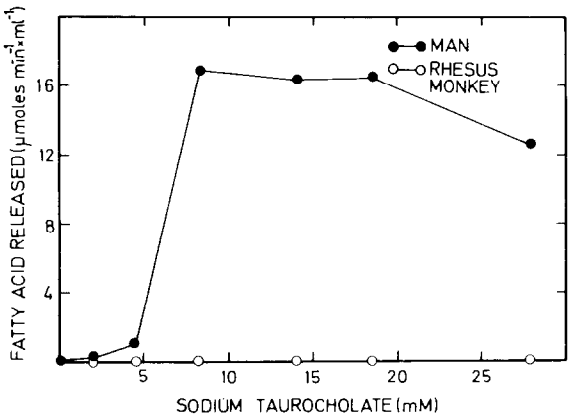

Fig.1. Effect of sodium taurocholate on lipase activity in milk from humans and from Rhesus monkey. The medium contained ^{3}H-labeled triolein emulsified in gum arabic at pH 9 as detailed elsewhere [7]. The concentration of taurocholate was varied as indicated in the figure. Human milk (10 μl) diluted 100-fold or undiluted Rhesus milk (10 μl) was added. The final volume was 200 μl. Incubation was at 37°C for 15 min.

trast to human milk which hydrolyzed this emulsion rapidly in the presence of taurocholate. Since Rhesus milk might contain inhibitory factors which masked the activity in our in vitro assay a partial purification was attempted. Figure 2 shows chromatograms of human and of Rhesus whey proteins on heparin–Sepharose. In both cases most of the proteins eluted in the breakthrough fraction or early in the salt gradient causing relatively large peaks in the first 9 fractions. Then followed peaks in fractions 10–12 and 14–16. These represent mainly serum albumin and lactoferrin, respectively [8]. Lipase activity was found in fractions 10–13 in the chromatography of human whey, but there was no activity in the fractions from Rhesus whey. Fraction 12 of human whey (corresponding to the peak of lipase activity) gave 3 bands on SDS gels (fig.3). Further purification has indicated that the uppermost band is the lipase.

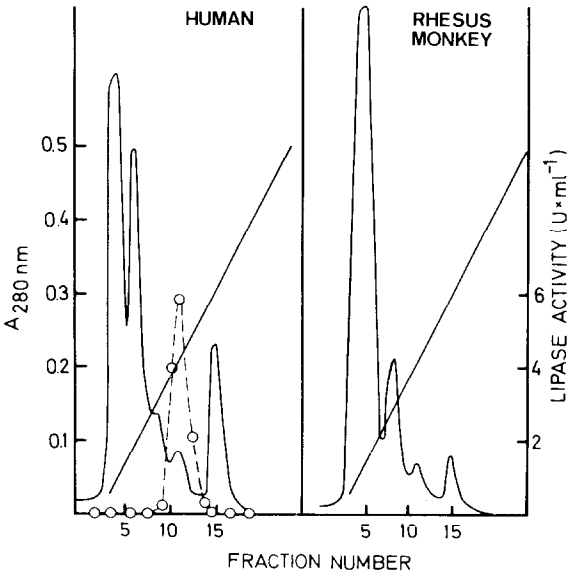

Fig.2. Chromatography of whey proteins on heparin–Sepharose. To prepare whey, the milk was centrifuged 30 min at 20 000 × *g* and the skim milk, acidified with HCl to pH 4.6, was heated at 40°C for 30 min to precipitate the caseins. The whey was dialyzed overnight against 0.05 M NaCl in 5 mM veronal–HCl buffer (pH 7.4). To a column of 1 ml settled heparin–Sepharose (prepared according to [9]) 4 ml was applied. The column was washed with 10 ml 5 mM veronal–HCl, 0.05 M NaCl (pH 7.4), then eluted with a 100 ml gradient of 0.05–1.0 M NaCl in the same buffer. Fractions (4.5 ml) were collected and their content of bile salt-stimulated lipase (o) assayed as in fig.1 with taurocholate at 10 mM. One unit (U) releases 1 μmol fatty acid/min.

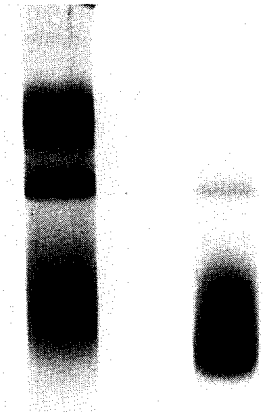

Fig.3. Polyacrylamide gel electrophoresis in sodium dodecyl sulfate (SDS) of fraction 12 from the chromatographies in fig.2. SDS and dithioerythritol were added to the samples to final conc. 1% (w/v) and 10 mM, respectively, then heated for 5 min at 90°C. Electrophoresis was according to [10] on gels with 7% acrylamide. The gels were stained with Coomassie blue and destained as in [11].

Furthermore, this component becomes labeled when the preparation is incubated with radioactive diisopropylfluorophosphate (DFP) which inactivates the lipase. It represents ~1% of the human milk proteins. This band was not seen in fractions from Rhesus whey (fig.3). The other 2 bands were present in both human and Rhesus milk. They correspond to lactoferrin (upper band) and serum albumin (lower band). An antiserum raised against the purified lipase gave only one precipitate against human milk and no precipitate against human serum (fig.4). This indicates that the antiserum was monospecific. It inhibited the activity of the lipase, demonstrating that its reactivity was indeed directed against the lipase. This antiserum gave no precipitate against Rhesus milk (fig.4). It did,

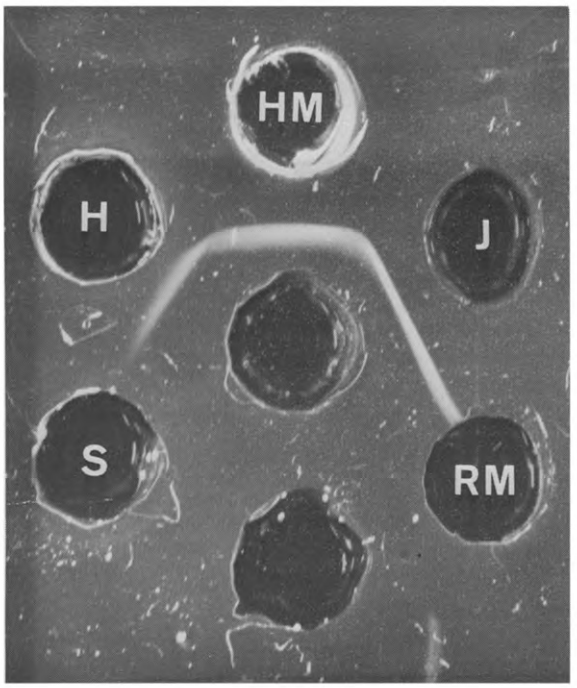

Fig.4. Immunodiffusion of human milk, Rhesus milk and of human pancreatic proteins against a rabbit antiserum prepared towards purified bile salt-stimulated lipase from human milk. The antiserum reacted with the protein in the uppermost band seen on the SDS gel of human whey in fig.3, and inhibited the bile salt-stimulated lipase activity in human whey. The gel contained 1% agarose, 1.5 M NaCl (pH 8.5). The wells contained 15 μl human serum (S), a 10% homogenate of human pancreas (H), human milk (HM), human pancreatic juice (J) and Rhesus milk (RM) as indicated. The center well contained the antiserum.

however, give precipitin lines with human pancreatic juice and with a homogenate of human pancreatic tissue (fig.4). These lines fused with that obtained with human milk, indicating immunological identity between the milk lipase and a secretory protein in the pancreas. The same result was obtained with pancreatic juice from 5 other patients.

4. Discussion

A bile salt-dependent lipase was known to be present in milk from humans [1] and gorillas [3] but not in lower animals. Here we show that this lipase activity is not present in milk from Rhesus monkeys. Furthermore, there was no protein in Rhesus milk which reacted with the antiserum to the lipase or which resembled it in size or chromatographic behaviour. Thus, this lipase is an evolutionary newcomer which must have appeared somewhere between the Rhesus monkey and the gorilla. The driving force for its evolution was presumably a need to improve the utilization of milk lipids in general and/or some critical component in milk; e.g., the vitamin A esters [5]. In humans the exocrine pancreatic function is not fully developed at birth [5]. The provision of a lipase with the milk ensures that there is sufficient lipolytic activity for digestion of milk lipids in the intestine of the newborn. Since the enzyme is completely without activity against the milk lipids until it becomes activated by bile salts [4] it can readily be secreted into the milk without doing any harm to the milk droplets while they are stored in the breast.

Our results suggest that the bile salt-stimulated lipase is a pancreatic protein, which in humans is secreted also by the lactating mammary gland. The presence of bile salt-stimulated lipases in the pancreas is well documented [12–16]. Thus, the appearance of such an enzyme in milk may have been caused by a change in tissue specificity of gene expression rather than by a mutational change of some other milk protein.

Acknowledgements

We are grateful to Miss Kerstin Wiklund for her skillful technical assistance, and to Mrs Karin Gladh for preparing the manuscript. This work was supported by the Swedish Medical Research Council (19X-05708), The Wennergreska Samfundet, The Swedish Baby Food Industry Fund for Nutritional Research, The Swedish Nutrition Foundation, The Swedish Society of Medical Sciences, and the Medical Faculty, University of Umeå.

References

[1] Freudenberg, E. (1953) Die Frauenmilch-lipase. S. Karger, Basel.
[2] Hernell, O. and Olivecrona, T. (1974) J. Lipid Res. 15, 367–374.
[3] Freudenberg, E. (1966) Experentia 22, 317.
[4] Hernell, O. (1975) Eur. J. Clin. Invest. 5, 267–272.

[5] Fredrikzon, B., Hernell, O., Bläckberg, L. and Olivecrona, T. (1978) Pediat. Res. 12, 1048–1052.
[6] Williamson, S., Finucane, E., Ellis, H. and Gamsu, H. R. (1978) Arch. Dis. Childhood 53, 555–563.
[7] Hernell, O. and Olivecrona, T. (1974) Biochim. Biophys. Acta 369, 234–244.
[8] Bläckberg, L. and Hernell, O. (1980) FEBS Lett. 109, 180–184.
[9] Iverius, P.-H. (1971) Biochem. J. 124, 677–683.
[10] Weber, K. and Osborn, M. (1969) J. Biol. Chem. 244, 4406–4412.
[11] Fairbanks, G., Steck, T. L. and Wallach, D. F. H. (1971) Biochemistry 10, 2606–2617.
[12] Morgan, R. G. H., Barrowman, J., Filipek Wender, H. and Borgström, B. (1968) Biochim. Biophys. Acta 167, 355–366.
[13] Hyun, J., Kothari, H., Herm, E., Mortenson, J., Treadwell, C. R. and Vahouny, G. V. (1969) J. Biol. Chem. 244, 1937–1945.
[14] Albro, P. W. and Latimer, A. D. (1974) Biochemistry 13, 1431–1439.
[15] Albro, P. W., Corbett, B. J. and Hass, J. R. (1976) Biochim. Biophys. Acta 431, 493–506.
[16] Patton, J. S., Warner, T. G. and Benson, A. A. (1977) Biochim. Biophys. Acta 482, 322–330.