Human milk lipases.
Properties and physiological implications.
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Umeå University Medical Dissertations, No 21, 1974.
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SUMMARY IN ENGLISH

I. It was shown that human milk contains two triglyceride lipases. One is stimulated by serum (serum-stimulated lipase) and the other is stimulated by bile salts (bile salt-stimulated lipase). After centrifugation of the milk the serum-stimulated lipase is found in the cream while the bile salt-stimulated lipase almost exclusively is found in the skim milk. The bile salt-stimulated lipase is always found in high activity while the activity of serum-stimulated lipase is much lower, and also is more variable. Methods were developed to assay the activity of each lipase without significant contribution from the other. The serum-stimulated lipase was purified 9 500-fold over whole milk. The major purification step was chromatography on heparin--Sepharose. The purified lipase was free of bile salt-stimulated lipase activity. It probably contained carbohydrate and the molecular weight of the constituent polypeptide chain probably was about 60 000. It also had other properties in common with other serum--stimulated or so-called lipoprotein lipases.

II. A rabbit antiserum was raised against a serum-stimulated lipase (lipoprotein lipase) purified from bovine milk. The antiserum crossreacted with serum-stimulated lipase but not with bile salt--stimulated lipase from human milk. This gave further proof that the lipases in human milk reside in different enzyme molecules. When postheparin plasma was chromatographed on heparin-Sepharose one lipase activity was eluted with 0.7 M NaCl. This activity was not stimulated by serum and was resistant to 1 M NaCl in the incubation medium. This lipase, designated as the salt-resistant lipase, was probably identical to the so-called liver lipase described by others. At 1.5 M NaCl another triglyceride lipase activity was eluted. This activity had the properties of a serum--stimulated lipase (lipoprotein lipase). The two lipase activities showed different time-courses for their appearance in plasma after heparin injection. When postheparin plasma was mixed with the antiserum before separation on heparin-Sepharose only the lipase activity eluted at 0.7 M NaCl, i.e. the salt-resistant lipase was detected.

The results obtained show that the serum-stimulated lipase in bovine milk, human milk and human postheparin plasma have immunological

determinants in common. For a similar reduction in activity the same relative amount of antiserum was needed for the serum-stimulated lipases in human milk and in human postheparin plasma.

III. An enzyme preparation from human skim milk, enriched in bile salt-stimulated lipase activity, but devoid of serum-stimulated lipase activity, was active against both emulsified, water-insoluble substrates (trioleylglycerol and tributyrylglycerol) and a water--soluble substrate (p-nitrophenyl acetate). Thus, the substrate specificity was rather low. Bile salt was a prerequisite for activity against emulsified trioleylglycerol but not for activity against the other two substrates, although bile salt enhanced the activity also against these substrates. The enzyme was rapidly inactivated in the presence of emulsified triacylglycerols. Bile salt protected the lipase from this inactivation. During the hydrolysis of emulsified trioleylglycerol at pH 8.0 or at pH 6.5 there was a rapid release of free glycerol and glycerol and free fatty acids were the major products formed during the reaction. Bile salt caused a more than 10-fold increase of the maximal reaction rate when p-nitrophenyl acetate was the substrate.

IV. The bile salt-stimulated lipase of human milk was inactive against emulsified trioleylglycerol but became active in the presence of sodium cholate, sodium chenodeoxycholate or the taurine or glycine conjugates of these bile salts, but not in the presence of sodium deoxycholate or its taurine and glycine conjugates. Thus, there may be a specificity for primary bile salts in this activation. The lipase was stable for at least 1 hour at 37^{o}C and pH 3.5. Trypsin and chymotrypsin caused inactivation of the lipase at pH 6.5 but these inactivations were almost abolished in the presence of bile salts. High concentrations of pepsin slowly inactivated the lipase at pH 4.0. Milk contains bile salt-stimulated lipase activity in such amounts that it might account for a total hydrolysis of the milk triacylglycerols in less than 30 min under conditions similar to those supposed to be prevailing in the small intestine of the newborn. It was concluded that the bile salt-stimulated lipase in human milk is stable enough to be active in the intestine and that it is present in high enough activity to contribute significantly to the hydrolysis of the milk triacylglycerols in the intestine.