BILE SALT-STIMULATED LIPASE IN HUMAN MILK AND CARBOXYL ESTER HYDROLASE IN PANCREATIC JUICE

Are they identical enzymes?

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1. Introduction

Human milk contains a lipase that readily hydrolyzes a variety of ester substrates [1–3]. A distinguishing feature of this lipase is that for activity against emulsified long-chain triacylglycerols it requires primary bile salts [1,2]. This so-called bile salt-stimulated lipase (BSSL) is an evolutionary newcomer as a milk enzyme; so far it has been found only in milk from man and gorilla (review [4]). Recent data strongly suggest that it has important roles, both quantitative and qualitative, for the digestion of milk lipids in the intestine of the breast-fed infant ([5], review [6]).

Human pancreatic juice contains an enzyme which has been designated carboxyl ester hydrolase (CEH) [7]. This enzyme has many properties in common with BSSL, e.g., stimulation by bile salts, ability to hydrolyze retinyl esters, inhibition by organophosphates (cf. [4,6] and [7,8]). It is the only enzyme in human pancreatic juice with activity against cholesteryl esters [8]. BSSL crossreacts immunochemically with a protein in human pancreatic juice [9]. We have now compared immunochemical, molecular and kinetic properties of bile salt-stimulated lipase and carboxyl ester hydrolase. We conclude that the 2 enzymes are very similar but not identical.

2. Materials and methods

BSSL was purified from human milk [3] and CEH from human pancreatic juice [7]. Antisera to the purified enzymes were raised in rabbits. Immunodiffusions were performed according to [10]. Agar plates were prepared with 1.5% agarose in a 0.05 M veronal buffer (pH 8.6), containing 1 M NaCl. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis was according to (11) with 7% polyacrylamide in the gels. Staining and destaining were according to [12].

Determination of the rates of hydrolysis of emulsified long-chain triacylglycerols [3] and cholesteryl esters [13] and synthesis of cholesteryl esters [14] were performed as described. Details of the experiments are given in legends to tables and figures.

3. Results

3.1. *Immunochemical and electrophoretic studies*

An antiserum to purified bile salt-stimulated lipase gave a single precipitin line against purified BSSL, human whey proteins, human pancreatic juice and purified CEH (fig.1a). Furthermore, the purified enzyme proteins gave fusing precipitin lines against an antiserum to CEH (fig.1b).

The molecular sizes of the 2 enzymes were compared by SDS–polyacrylamide gel electrophoresis. A difference in electrophoretic mobility was observed with CEH having the faster migration. This difference became evident when the 2 enzymes were mixed prior to the electrophoresis (fig.2). Using CEH with an M_r of 100 000 [7] as one of the standard proteins the M_r for BSSL was 107 000 calculated from calibrated gels.

3.2. *Specific activities and inhibition studies*

The specific activities of CEH and BSSL against 3 different substrates were virtually identical (table 1).

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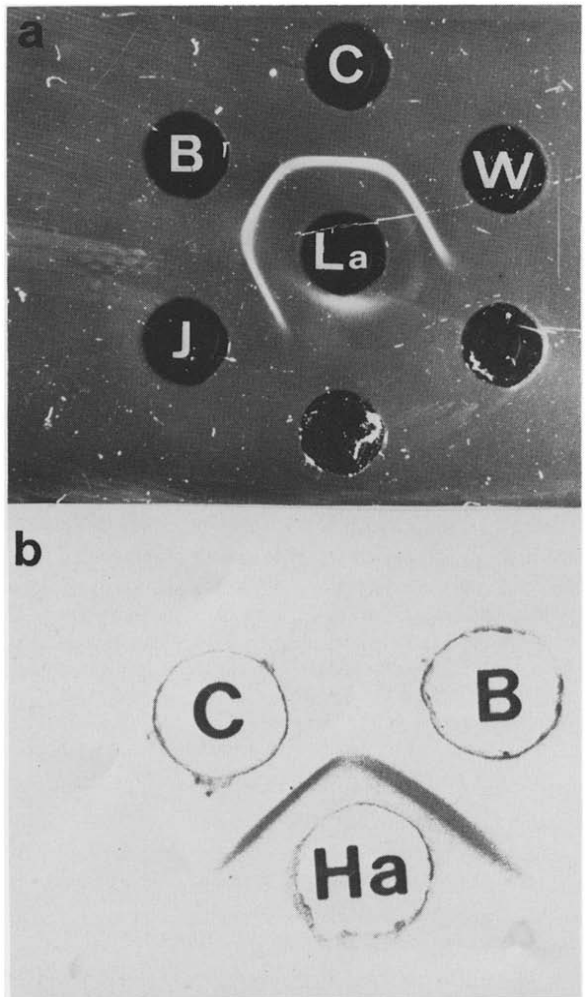

Fig.1. Immunodiffusion studies of BSSL and CEH. The respective wells contained 20 μl pure CEH (C), pure BSSL (B), human whey (W), human pancreatic juice (J) and antisera prepared against CEH (Ha) or BSSL (La). The purified enzyme preparations contained 0.5–0.7 mg/ml.

The activity against an emulsion of long-chain triacylglycerol was the highest; 84 μmol/min × mg protein for CEH and 87 for BSSL. As to the hydrolysis or synthesis of cholesteryl esters the activities were much lower, 0.8 and 3.5–3.7 μmol/min × mg, respectively (table 1).

Eserine is a known inhibitor of BSSL [5]. When the 2 enzymes were preincubated with different concentrations of eserine sulphate, both enzymes were completely inhibited at ~5 mM eserine (fig.3).

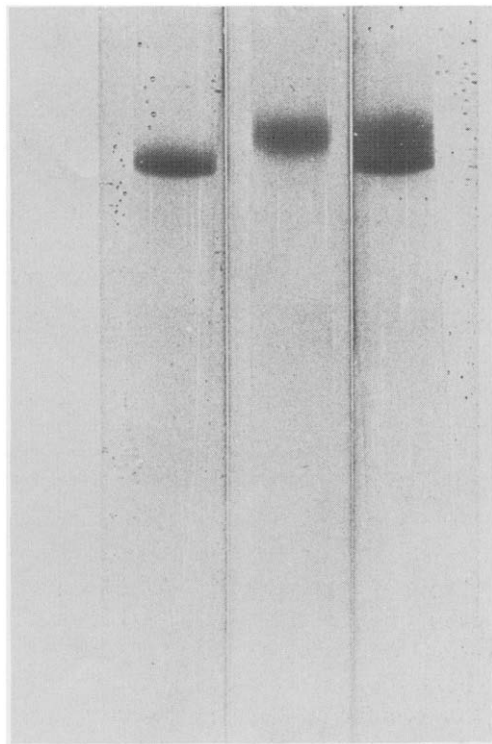

Fig.2. SDS–polyacrylamide gel electrophoresis of BSSL and CEH: 10 μg pure CEH and pure BSSL were applied to gels A and B, respectively; a mixture of 10 μg of each enzyme was applied to gel C. The cathode is at the top.

3.3. *Effect of bile salts*

Table 2 shows the effect of bile salts on the hydrolysis of cholesteryl esters and long-chain triacylglycerols by CEH and BSSL, respectively. Only primary

Table 1
Specific activities of carboxyl ester hydrolase (CEH) and bile salt-stimulated lipase (BSSL) against different substrates

Activity measured	Specific activity	
	CEH	BSSL
Long-chain triacylglycerol hydrolysis[a]	84	87
Cholesteryl ester hydrolysis[a]	0.8	0.8
Cholesteryl ester synthesis[b]	3.7	3.5

[a] Expressed as μmol fatty acid released per min and mg protein
[b] Expressed as μmol cholesteryl ester formed per min and mg protein

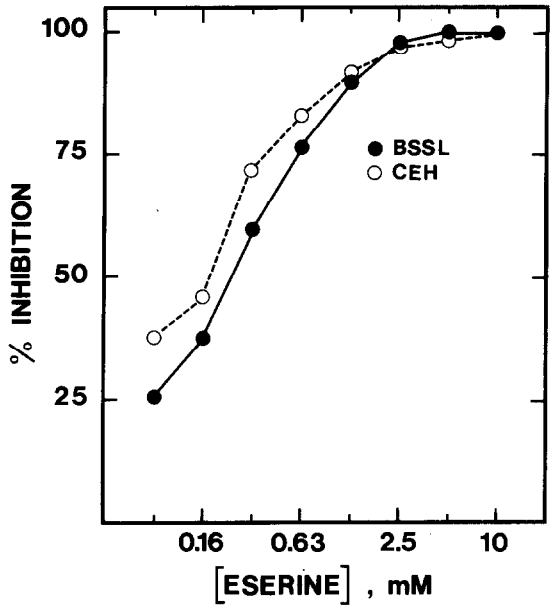

Fig.3. Inhibition of BSSL and CEH activity by eserine sulphate. BSSL (•) or CEH (○) were mixed with dilutions of eserine sulphate (0.08–10 mM) in 0.05 M veronal buffer (pH 7.4), 0.15 M NaCl. After incubation at 25°C for 30 min aliquots were taken and assayed for lipase activity with emulsified long-chain triacylglycerol as substrate [3]. Per cent inhibition was calculated in comparison with samples that had been preincubated in buffer alone.

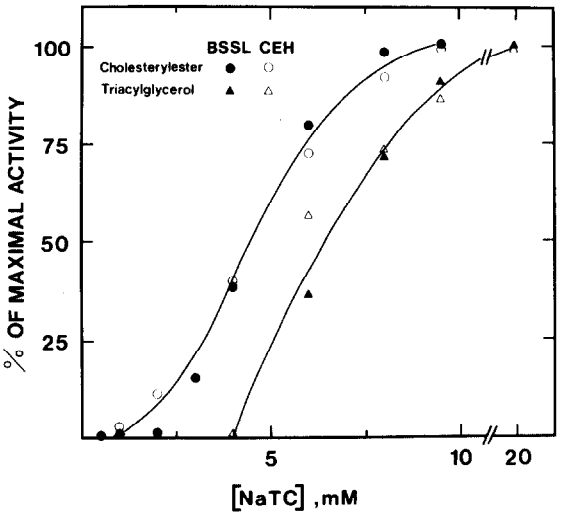

Fig.4. Effect of taurocholate on the hydrolysis rate of triacylglycerol and cholesteryl ester by BSSL and CEH. Activities were measured against emulsified long-chain triacylglycerol or cholesteryl ester in the presence of varying amounts of sodium taurocholate (NaTC). The activity is expressed as per cent of the highest activity obtained for each enzyme and substrate, respectively.

bile salts activated the enzymes. The highest activities were recorded with unconjugated cholate but also the conjugated derivates were effective with no marked difference between glycine and taurine conjugates (table 2). The concentrations of taurocholate needed for maximal activity were the same for both enzymes (fig.4). There was, however, a difference between the 2 substrates used; 50% of maximal activity was recorded using 4.4 mM bile salt when cholesteryl esters were the substrate, whereas triacylglycerol hydrolysis needed 6.5 mM for 50% of maximal activity.

Table 2
Effect of different bile salts on the activities of carboxyl ester hydrolase (CEH) and bile salt-stimulated lipase (BSSL)

Bile salt[a]	Hydroxylation	Cholesteryl ester hydrolyzing activity[b]		Triacylglycerol hydrolyzing activity[b]	
		CEH[c]	BSSL	CEH	BSSL
Cholate	3α, 7α, 12α	2.0	1.9	137	144
Taurocholate	3α, 7α, 12α	0.8	0.8	84	87
Glycocholate	3α, 7α, 12α	n.d.[d]	n.d.	98	97
Taurochenodoxycholate	3α, 7α	0.5	0.3	17	19
Taurodeoxycholate	3α, 12α	0.07	0.06	<0.5	<0.5

[a] The concentrations of the respective bile salt (sodium salts) was 10 mM
[b] Expressed as μmol fatty acid released per min and mg protein
[c] Data from [13]; [d] n.d., not determined

4. Discussion

Our observation that human pancreatic juice contains a protein that is immunochemically identical to bile salt-stimulated lipase in human milk [9] suggested that this enzyme has appeared in milk of the higher primates through expression of a gene for a pancreatic protein in the mammary gland. Amongst the lipolytic enzymes secreted with pancreatic juice carboxyl ester hydrolase seemed to be the most likely counterpart to bile salt-stimulated lipase, since these 2 enzymes have many properties in common. It was found here that the 2 enzyme proteins reacted with full immunological identity when antisera raised against one enzyme or the other were used.

The CEH has been described as an esterase. However, the activity towards emulsified long-chain triacylglycerols was high provided primary bile salts were present. In fact, this activity was virtually identical to that of BSSL. The 2 enzymes were also identical with respect to ability to cause hydrolysis or synthesis of cholesteryl esters. These activities were, however, considerably lower than those obtained with emulsified long-chain triacylglycerols.

The most conspicuous property of these enzymes is their interaction with bile salts. Bile salts activate both enzymes. The activation is specific for primary bile salts, i.e., bile salts containing the 7α-hydroxyl group. Secondary bile salts, e.g., deoxycholate, give very low if any activity. We found here almost identical results for both enzymes using triacylglycerols and cholesteryl esters as the substrates. The concentration of bile salts needed for maximal activity was also the same and there was a requirement for relatively higher concentrations when triacylglycerols were the substrate. Thus, the carboxyl ester hydrolase is, at least functionally, identical to the bile salt-stimulated lipase in human milk.

CEH and BSSL are also similar with respect to their molecular properties. There was, however, a difference in relative molecular mass. One possible explanation of this could be qualitative and/or quantitative differences in the glycosylation of the 2 proteins [3,15]. The amino acid composition of the 2 enzymes are very similar [3,7,15]. The percentage of hydrophilic residues is 25.7 and 28.4, respectively, for CEH and BSSL while the percentage of hydrophobic residues is 36.6 for CEH and 34.3 for BSSL. These values are close, if not identical. The most distinctive feature is the unusually high content of proline; >10% of the residues. The N-terminal amino acid is alanine for both enzymes ([7]; L. B., unpublished). Apart from eserine, which inhibits both enzymes in a similar manner, organophosphates such as diisopropylfluorophosphate are very effective inhibitors [3,7]. The latter indicates that BSSL and CEH both contain a serine residue in the active site, i.e., they are serine hydrolases.

From the literature it can be deduced that there are pancreatic secretory enzymes in different species which have the property in common to be stimulated or activated by bile salts [16–23]. The interrelations between these enzymes are not clear; some of them are primarily described as esterases resembling the human carboxyl ester hydrolase whereas others are described as lipases. The findings in this study that carboxyl ester hydrolase readily hydrolyzes long-chain triacylglycerols and also has other properties in common with bile salt-stimulated lipase makes it tempting to speculate that all these different enzymes are functionally one and the same.

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