EVIDENCE FOR A PANCREATIC PRO-COLIPASE AND ITS ACTIVATION BY TRYPSIN

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1. Introduction

Pancreatic colipase is a polypeptide secreted into the pancreatic juice, necessary for the binding of lipase to its triglyceride substrate in the bile-containing content of the small intestine [1]. Colipase has been purified from the pancreatic glands of several species [1] and obtained with a varying number of amino acids. Colipases from porcine sources have been described with 68–70, 83–84 and 94–95 amino acid residues and N-terminal glycine [2]. The complete sequence of colipase$_{84}$ has been determined [3]. Colipases with 102–107 amino acids and N-terminal valine was first isolated from the porcine gland [4] and later from bovine and horse pancreas [5,6]. The N-terminal sequence of these colipases is Val$_1$–Pro$_2$–Asp$_3$–Pro$_4$–Arg$_5$–Gly$_6$–Ile$_7$–Ile$_8$–Ile$_9$– [4,5,6] (in the horse Ile$_7$ is replaced by Val [5]). It appears that these later colipases are the forms produced by the glands and that the shorter ones have been formed by limited proteolysis before or during the purification. As the –Arg$_5$–Gly$_6$– bond of the intact colipase seemed to be susceptible to cleavage by trypsin we have investigated the course of the reaction taking place when porcine colipase$_{101}$ is treated with Sephadex-linked trypsin in vitro. Such treatment leads to the rapid and complete cleavage of the N-terminal pentapeptide Val–Pro–Asp–Pro–Arg leaving a colipase$_{96}$ with N-terminal Gly–Ile–Ile–Ile–.

The cleavage of the N-terminal pentapeptide by trypsin does not result in any significant increase of the specific activity in a tributyrin-based colipase assay system.

Colipase$_{96}$, however, binds to a phospholipid stabilized triglyceride emulsion to a much higher extent than colipase$_{101}$ and is also 50–100 times more active to overcome the lag phase for triglyceride hydrolysis catalysed by lipase with such a substrate.

We propose that the colipase with N-terminal valine produced in the pancreatic gland is a *pro-colipase* that is activated by trypsin in the intestinal content. The biological importance of this activation is to produce a colipase which binds to a triglyceride interface covered by phospholipid, thereby making the triglyceride available for pancreatic lipase.

2. Material and methods

Colipase I was prepared as in [4] from a protein fraction of porcine pancreas generously supplied by Novo, Bagsvaerd. Porcine pancreatic lipase used for assaying colipase activity was prepared as in [7]. Chemicals used were of analytical grade, taurodeoxycholate was prepared in this laboratory, dextran T-500 was from Pharmacia, Uppsala and PEG-6000 was obtained from Union Carbide, New York. Intralipid (Vitrum, Stockholm) is a 20% emulsion of fractionated soy bean triglyceride stabilized by egg lecithin. Polyacrylamide gel electrophoresis was performed as in [4]. Trypsin was immobilized on Sephadex G-75 as in [8]. It had spec. act. $\simeq$ 300 mU/mg dry gel.

Amino acid analysis was performed using a Durrum D-500 Amino Acid analyser. Hydrolysis (24 h) figures were except for Val, Leu and Ile, for which 72 h hydrolysis figures were used. Serine and threonine values were given as zero-time hydrolysis values obtained by extrapolating from the 24 h and 72 h values.

N-terminal amino acids were analysed by the dansylation technique. N-terminal sequences were obtained using a Beckman 890 C Sequenator. (Kindly performed by Dr J. O. Jeppson, Department of Clinical Chemistry, Malmö). Binding of colipase to tributyrin and Intralipid was studied in a two-phase aqueous partition system as in [9].

The effect of colipase on the lag time for hydrolysis of Intralipid was determined as follows. Intralipid (0.5 ml) was diluted to 10 ml in a solution 150 mM in NaCl, 4 mM in taurodeoxycholate, 1 mM in $CaCl_2$, and 2 mM in Tris–HCl (pH 8.0), at 40°C. The colipase sample was added, followed after 1 min by 10 μl porcine pancreatic lipase (10^{-5} M). The lag time passing before lipase started to hydrolyze the triglyceride of the Intralipid emulsion was measured by pH stat titration [9].

3. Results

3.1. *Cleavage of colipase$_{101}$ with trypsin: Isolation of peptides*

Colipase I (10 mg) with the amino acid composition given in table 1 were dissolved in 8 ml 0.05 M NH_4HCO_3 (pH 8.5) and added in a test tube to 3 mg pre-swollen Sephadex-linked trypsin to 10 ml total vol. Incubation took place at room temperature with stirring. At intervals from 0.5–24 h, 1 ml samples of the solution were removed for analysis after short centrifugation to separate the gel. Polyacrylamide gel electrophoresis at pH 8.3 of the samples is seen in fig.1. A new band with higher mobility than the starting material appeared after 0.5 h and an almost complete conversion was seen after 6 h. At longer incubation another weak band started to appear with a lower mobility.

Fig.1. Polyacrylamide gel electrophoresis at pH 8.3 of colipase I (= colipase$_{101}$) at zero time and after incubation with Sephadex–trypsin for 30 min, 1,2,4,6 and 24 h as described in the text.

To isolate the main cleavage products a preparation with 100 mg colipase$_{101}$ and 30 mg Sepharose-trypsin was run in 10 ml total vol. for 24 h. After separation of the gel the solution was lyophilized, redissolved in a small volume and filtered through a Sephadex G-25 superfine column (1.5 × 90 cm). Two peaks at A_{230} were obtained. The void volume peak which had colipase activity was applied to a QAE–Sephadex column (2.6 × 40 cm) equilibrated in 0.01 M NH_4HCO_3 (pH 8.0). After elution with 200 ml of the same buffer a linear gradient was applied (0.01–0.30 M NH_4HCO_3 (pH 8.0), 500 ml of each). One major peak was obtained which at electrophoresis at pH 8.3 showed one band appearing ahead of the original colipase$_{101}$ and corresponding to the main product in the time-related reaction. The amino acid composition of this protein is given in table 1. The N-terminal sequence was Gly–Ile–Ile–Ile– and the total number of amino acid residues 96. This protein is in the following referred to as colipase$_{96}$. The second peak from the Sephadex G-25 column appeared close to the total volume and contained a pentapeptide with the amino acids given in table 1 with N-terminal valine. Colipase$_{101}$ thus was rapidly and completely cleaved by trypsin between Arg_5 and Gly_6 resulting in colipase$_{96}$ and a pentapeptide. The latter had no colipase activity.

3.2. *Binding of colipase$_{101}$ and colipase$_{96}$ to Intralipid and tributyrin*

In a two-phase aqueous partition system [9] a weak and inconsistent binding of colipase$_{101}$ to Intralipid was found; with colipase$_{96}$ binding to Intralipid occurred. The dissociation constant calculated from the Scatchard plot for colipase$_{96}$ was K_d = 1.0 ×

Table 1
Amino acid composition of colipase and the products obtained by limited proteolysis

Amino acids	Number of residues					
	Colipase I		Colipase I residue		Peptide	
	Expt[a]	Nearest integer	Expt[a]	Nearest integer	Expt	Nearest integer
Ala	4.64	5	4.73	5	–	–
Arg	5.19	5	4.12	4	1.03	1
Asx	13.06	13	12.37	12	1.00	1
Cys	n.d.[b]	10	n.d.[b]	10	0.09	–
Glx	9.90	10	10.03	10	0.05	–
Gly	8.54	8–9	8.85	9	–	–
His	2.03	2	2.01	2	–	–
Ile	5.76[c]	6	5.48[c]	5–6	–	–
Leu	9.57[c]	10	9.91[c]	10	–	–
Lys	4.15	4	4.31	4	–	–
Met	–	–	–	–	–	–
Phe	2.07	2	2.20	2	–	–
Pro	3.44	3	0.74	1	2.00	2
Ser	11.80[d]	12	10.04[d]	10	0.18	–
Thr	5.05[d]	5	5.23[d]	5	–	–
Trp	–	–	–	–	–	–
Tyr	2.64	3	3.24	3	–	–
Val	3.73[c]	4	3.01	3	0.80[c]	1
Total no. residues	101.57	101–102	96.27	95–96	4.83	5

[a] Except where noted the figures are taken from the 24 h hydrolysis value
[b] Not determined
[c] 72 h hydrolysis value
[d] Values obtained by extrapolation to zero time hydrolysis

10^{-7} M. The substrate–water interface to which colipase adsorbs may be modified by the reagents of the two phase system. This does not, however, invalidate the observation of a difference in the binding of the two colipases.

The K_d for the binding of colipase$_{101}$ and colipase$_{96}$ to a tributyrin emulsion was found to be 1.5 × 10^{-7} M with no significant difference.

3.3. *Biological activity of colipase$_{101}$ and colipase$_{96}$*

The activity of colipase$_{96}$ to restore lipase activity of an emulsion of tributyrin dispersed with bile salt was only 1.2–1.5-times greater than that for colipase$_{101}$, as measured as V_{max} from a Lineweaver-Burke plot. In these experiments lipase and colipse in equimolar concentrations were incubated with increasing amounts of tributyrin emulsified in a constant volume of buffer containing bile salt at pH 7.0.

When lipase was incubated with Intralipid in bile salt suspension no significant hydrolysis took place. With colipase added, triglyceride hydrolysis occurred at a high rate (dependent on lipase concentration) after a lag time. The length of the lag period was dependent inter alia on the concentration and species of colipase. Figure 2 shows a plot of the length of the lag phase for triglyceride hydrolysis versus log colipase concentration for the two colipases; parallel curves are obtained and 50–100-times higher concentration is needed of colipase$_{101}$ compared to colipase$_{96}$ in order to reach the same lag time. A comparison at the concentration of colipase found in pig small intestinal content, 2 × 10^{-7} M [9], showed a lag time for colipase$_{101}$ and colipase$_{96}$ of $\simeq$ 15 min and $\simeq$ 0.8 min, respectively.

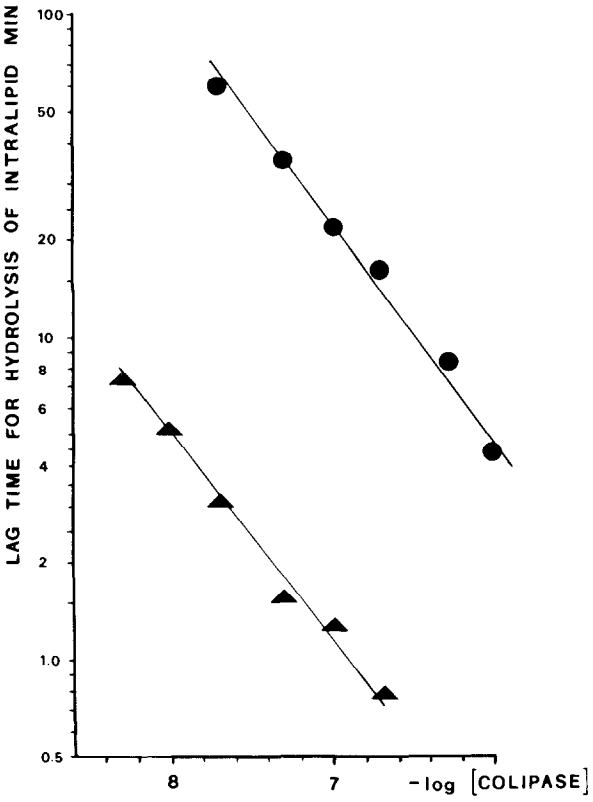

Fig.2. Effect of colipase$_{101}$ (•) and colipase$_{96}$ (▲) on the lag time for rapid triglyceride hydrolysis with Intralipid as substrate. Intralipid emulsion (0.5 ml) in a 10 ml total vol. buffer, 4 mM in TDC was titrated at pH 8.0 and 40°C. Colipase was added followed after 1 min by lipase (final conc. 2×10^{-8} M) and the time measured until a high rate of triglyceride hydrolysis occurred.

4. Discussion

Porcine colipase I with N-terminal valine analysed in this investigation was designed colipase$_{101}$. The present experiments show that a limited proteolysis of colipase$_{101}$ with trypsin gives colipase$_{96}$. Extensive treatment with trypsin causes degradation also at the C-terminal end but this has probably no biological importance.

Colipase$_{96}$ has a hydrophobic N-terminal sequence that has been found in the structure of colipases from all species analysed [1]. So far the only unique property recognized for colipase$_{96}$ is its activation of a phospholipid covered triglyceride emulsion. This, however, may be an important biological function as dietary fat in general contains phospholipid and phospholipid is contained in appreciable quantities in the bile admixed to the small intestinal contents. The hydrophobic N-terminal tail of colipase$_{96}$ may interact specifically with phospholipid monolayers, as indicated by its binding to the interface of Intralipid. Such interactions are still under investigation. Preliminary experiments have revealed that no significant spectroscopic changes (NMR, ultraviolet) occur as a consequence of the conversion of colipase$_{101}$ to colipase$_{96}$. If colipase is synthesized and secreted as a pro-colipase, the reason might be that the activated form is incompatible with the pancreatic tissue. Perhaps the hydrophobic tail of the activated colipase would interact with the phospholipids of the intracellular membranes and disrupt the sequence of secretion.

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