Trypsin activation of porcine procolipase

Kinetics of activation and effects on lipid binding

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The kinetics of trypsin activation of pancreatic procolipase was investigated and the pH dependence of the binding of procolipase and colipase to a tributyrine-bile salt interface studied. The K_m was 0.06 mM and k_{cat} 8 s^{-1}, and was of the same order of magnitude as for the activation of pancreatic zymogens. At basic pH values colipase had a higher affinity for the tributyrine-bile salt interface as compared to procolipase. The trypsin activation of procolipase ensures a rapid degradation of dietary lipids in the intestine.

Colipase *Procolipase* *Trypsin activation* *Kinetics* *Lipid binding*

1. INTRODUCTION

Colipase is a protein participating in the duodenal degradation of dietary glycerides as a cofactor to pancreatic lipase [1–3]. It has recently been shown that colipase is secreted as a proform, procolipase, by the pancreas [4] and it has been suggested that it is activated in the intestine by trypsin in a similar manner to the pancreatic zymogens [5], such as trypsinogen, chymotrypsinogen [6] and pro-phospholipase A_2 [7–9]. The Arg_5–Gly_6 bond of procolipase is readily cleaved by trypsin. This limited proteolysis is particularly interesting as it decreases the lag time of lipase-catalyzed hydrolysis of phospholipid-stabilized triolein emulsion [5]. It also increases the lipid-binding and lipase-activating properties of colipase towards negatively charged and zwitterionic interfaces at high surface pressure [10,11].

The aim of this investigation was to study the kinetics of the trypsin activation of procolipase and the pH dependence of the binding of procolipase and colipase to a tributyrine-bile salt interface.

2. EXPERIMENTAL

Porcine pancreatic procolipase was purified essentially as described [12]. Two forms of procolipase could be separated after an additional ion-exchange chromatography (SP-Sephadex, pH 4.0): $procolipase_{101}$ and $procolipase_{93}$ (number denotes the amino acid content), both with a valine at position 1. Porcine pancreatic lipase was purified according to Verger et al. [13]. To obtain a lipase preparation free from colipase contaminations, an additional G-100 gel filtration step was performed at pH 9.2. Bovine trypsin and soybean trypsin inhibitor were obtained from Sigma. Preparative trypsin activation of $procolipase_{93}$ was performed using Sephadex-immobilized trypsin as in [5,14]. A complete transformation to $colipase_{88}$ was obtained. The N-terminal of this molecule was glycine in accordance with [5]. Taurodeoxycholate was synthesized in the laboratory [15] and egg phosphatidylcholine was prepared as described [16]. All other chemicals were of analytical grade.

Binding studies of procolipase and colipase to a tributyrine interface were conducted essentially as described earlier [17]. Eighteen μg protein in 10 ml 2 mM taurodeoxycholate solution and 0.2 ml

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tributyrine were stirred with a magnetic bar for 5 min at constant pH. Aliquots of 300 μl of the dispersion were transferred to a tube and centrifuged in an Ole Dick microcentrifuge (Nino Laboratory, Stockholm, Sweden) for 5 s. The tributyrine-taurodeoxycholate dispersion sedimented, but did not coalesce during this procedure. A sample was taken from the supernatant and the colipase activity measured.

The kinetics of trypsin activation of procolipase was performed according to the following procedure. Procolipase$_{93}$, 0.6–1.4 mg in 5 ml of a buffer solution composed of 50 mM Tris-HCl (pH 8.0), 10 mM $CaCl_2$ and 150 mM NaCl, was cooled on an ice bath. Five μl of a trypsin solution (1 mg/ml) was added. Aliquots were taken at different time intervals and diluted with a solution containing 10 mM diisopropyl fluorophosphate and 1 mg soybean trypsin inhibitor per ml and the colipase$_{88}$ activity measured. Colipase activity was measured by a pH-stat [18] as its lipase-activating ability against a tributyrine dispersion in a 4 mM taurodeoxycholate solution at pH 8.8.

3. RESULTS AND DISCUSSION

3.1. *Binding of procolipase and colipase to the tributyrine interface in the presence of 2 mM taurodeoxycholate*

The binding profile of procolipase$_{93}$ and colipase$_{88}$ to tributyrine in the presence of 2 mM taurodeoxycholate as a function of pH is shown in fig.1. The pH values, where 50% of the proteins were bound to the dispersion, were 7.3 and 8.1 for procolipase and colipase, respectively. The data suggest that the release of the pentapeptide from procolipase induces a change in the protein, increasing its affinity for the lipase substrate at all pH values and in particular at basic pH. The pH dependence of the binding suggests that one or several amino acid residues titrating in the range pH 7–9, i.e., basic amino acid residues, might be important for the colipase interaction with the bile salt-covered tributyrine interface. The trypsin activation could thus alter the structure of the protein, inducing a change in the properties and/or environment of some basic amino acid residues like the histidines, lysines or the N-terminal amino group, in addition to the conformational change reported in the tyrosine-rich region [19].

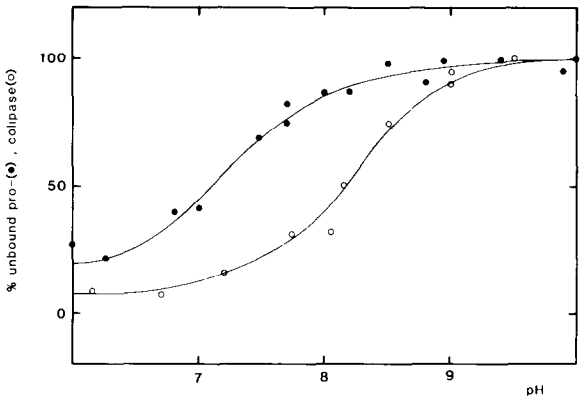

Fig.1. The pH dependence of the binding of procolipase (●) and colipase (○) to a tributyrine-taurodeoxycholate dispersion. Tributyrine (0.2 ml) was dispersed in 10 ml of 2 mM taurodeoxycholate solution containing 150 mM NaCl. The binding is expressed as % free procolipase in a supernatant following centrifugation [18].

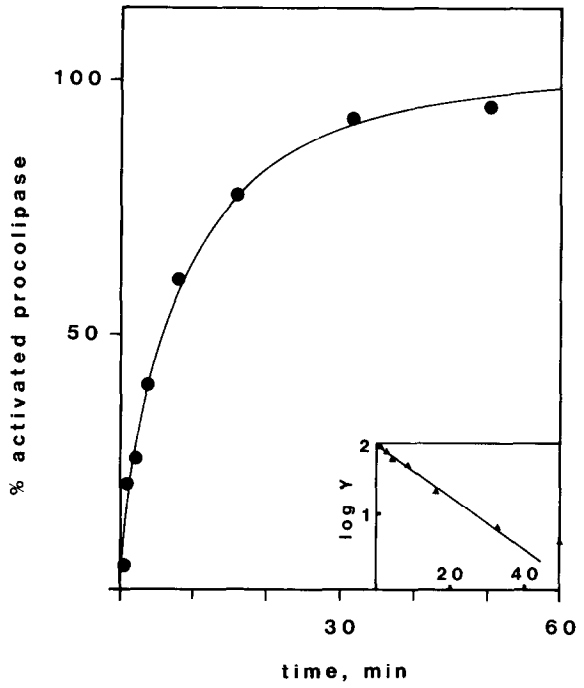

Fig.2. The kinetics of activation of procolipase$_{93}$ by trypsin. Activation was measured as the increase in colipase activity at pH 8.8. Experimental conditions: procolipase concentration, 0.3 mg/ml; trypsin concentration, 0.2 μg/ml; incubation buffer, 10 mM Tris-HCl (pH 8.0), 50 mM $CaCl_2$, 100 mM NaCl; temperature, 20°C. Inset: demonstration of the first-order kinetics of the reaction. *Y*, % procolipase.

3.2. *Kinetics of trypsin activation of procolipase*

The better binding properties of colipase, as compared to procolipase, to a tributyrine-taurodeoxycholate dispersion at basic pH values was used to discriminate between the two molecules. At pH 8.8 the specific activity (measured as lipase hydrolysis of tributyrine in the presence of bile salt) was 3000 $U \cdot min^{-1} \cdot mg^{-1}$ for procolipase and 18000 $U \cdot min^{-1} \cdot mg^{-1}$ for colipase.

The time course of the activation of procolipase$_{93}$ by trypsin at 20°C is shown in fig.2. The inset shows the first-order kinetics of the activation process. The initial velocity was determined by calculating the amount of procolipase transformed during the first minute of the reaction. To compare the activation process to other trypsin activation reactions reported earlier, the reaction was conducted at 0°C. The Lineweaver-Burk plot of the activation reaction is shown in fig.3. The K_m was calculated using a least-square procedure and found to be 0.06 mM. The k_{cat} was obtained using the relation $V = k_{cat}/(E_o)$ and was calculated to be 8 s^{-1}. The low K_m value reported here for the trypsin activation of procolipase is in the same order of magnitude as for several of the zymogens of pancreatic origin (table 1). The high k_{cat} value stresses the efficiency of the trypsin activation process and ensures a rapid activation of procolipase in the intestinal contents.

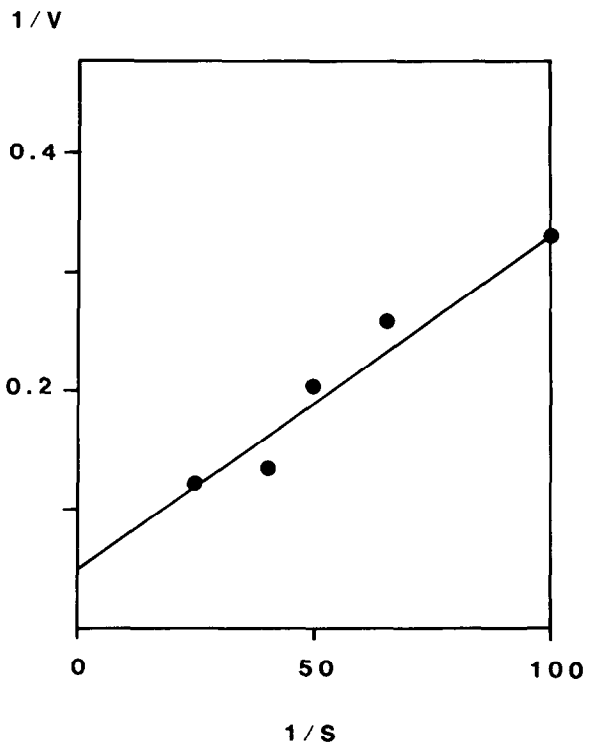

Fig.3. Lineweaver-Burk plot of the activation of procolipase by trypsin. Experimental conditions: 1 μg trypsin/ml; incubation buffer, 10 mM Tris-HCl (pH 8.0), 50 mM $CaCl_2$, 100 mM NaCl; temperature, 0°C. V is given as μmol procolipase$\cdot min^{-1} \cdot$mg trypsin^{-1}. S, mM.

3.3. *Effects of micelles on the activation process*

Ten μg of procolipase$_{93}$ was incubated with 10 μg trypsin in 1 ml of solutions containing respectively, 10 mM taurodeoxycholate, 10 mM taurodeoxycholate + 2 mM oleic acid, 10 mM taurodeoxycholate + 1 mM egg phosphatidylcholine and 0.1 ml native bile in the presence and absence of 50 μg pancreatic lipase at 37°C. In all cases full transformation of procolipase to colipase was obtained after 2 min. Thus different compositions of micelles, which are likely to be present in the small intestinal content, do not affect the activation of procolipase. In conclusion, the present investigation has shown that the trypsin activation of procolipase is a process comparable to the activation of other pancreatic zymogens, and that the process is not affected by the presence of bile constituents. The colipase molecule formed by trypsin activation has better lipid binding properties, in particular at basic pH values.

Table 1

Kinetic parameters of the trypsin activation of some pancreatic zymogens and procolipase

Zymogen bond cleaved	K_m (mM)	k_{cat} (s^{-1})
Porcine trypsinogen (7) Lys_6-Ile_7	0.48	0.0055
Chymotrypsinogen A (7) Arg_{15}-Ile_{16}	1.09	0.18
Prophospholipase A_2 (8)	2.2	7
Arg_7-Ala_8 (9)	0.04	0.36
Procolipase Arg_5-Gly_6	0.06	8

Conditions: 10 mM Tris-HCl, 100 mM NaCl, 50 mM $CaCl_2$, temperature, 1°C

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