BEHAVIOUR OF PANCREATIC LIPASE AND COLIPASE WHEN LINKED COVALENTLY

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1. Introduction

Pancreatic colipase is a protein cofactor for pancreatic lipase permitting the enzyme to work optimally in the presence of bile salt in the intestine where the triacylgycerol substrate is covered with bile salt and phospholipids [1,2]. In [3,4], evidence was presented for the existence in pancreas and pancreatic juice of a pro-form of colipase, which was activated by trypsin removing the N-terminal pentapeptide. The resulting colipase was 50–100-times more active in overcoming the lag time when a phospholipid-stabilized triacylglycerol was used as substrate for lipase [3]. A shortening in lag time was also obtained by the addition of long-chain fatty acids to the assay system containing lipase and procolipase [5].

In this work pro-colipase and lipase have been covalently linked by dimethylsuberimidate, a cross-linking agent. The resulting 1:1 protein complex exhibited no lag time with a phospholipid-covered triacylglycerol (Intralipid) as substrate. Such an immediate activity for the lipase–procolipase complex compared to the single proteins may indicate that procolipase by complexing with lipase has induced a conformational change in lipase enabling lipase to bind to the bile salt-covered mixed triacylglycerol/phospholipid substrate. Such a revelation of a substrate binding site in lipase induced by colipase was indicated in [6].

2. Materials and methods

Procolipase was prepared as in [7] and pancreatic lipase according to [8]. Chemicals used were of analytical grade. Dimethylsuberimidate was a product of Sigma (St Louis MO), Sephadex G-200 a product of Pharmacia (Uppsala). Siliconized glass beads were obtained from Serva (Heidelberg), Intralipid (Vitrum, Stockholm) is a 20% emulsion of fractionated soy bean triacylglycerol stabilized by egg lecithin.

The effect of colipase/lipase on the lag time for hydrolysis 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 Tris–maleate at pH 8.0 at 40°C. Lipase (2–10 μg) was added followed by procolipase (0.4–2 μg) after 1 min or in lipase–procolipase complex (2–10 μg) and the time extrapolated until lipase hydrolysis of the triacylglycerol was maximal using a pH stat [3]. Activity of lipase and colipase with tributyrin as substrate was measured as in [9].

3. Results

3.1. *Covalent linking of procolipase to lipase with dimethylsuberimidate*

Lipase (1 mg) in 1 ml 0.05 M NH_4HCO_3 buffer (pH 7) was added with 3 mg procolipase (final conc. 1.7×10^{-5} M lipase and 2.5×10^{-4} M procolipase). After mixing 350 mg siliconized glass beads were added, followed by 5 mg dimethylsuberimidate in 0.1 ml 0.2 M ethanolamine [10]. The pH was adjusted to 8.5 with 1 M NaOH and the solution left for 1 h at 37°C. The complex was desorbed from the beads by adding 0.2 ml 40 mM NaTDC [11]. After mixing the glass beads were spun down in a laboratory centrifuge and the clear supernatant collected. The procedure was repeated with 0.4 ml 20 mM NaTDC solution. The pooled clear supernatants were finally applied to a Sephadex G-200 column (K 15/90) equilibrated in 0.01 M NH_4HCO_3 buffer pH 8.0 and eluted. Two peaks containing both lipase and procolipase were separated, one in the void volume, the second at an elution volume corresponding to a molecule of M_r 75 000–95 000.

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The pooled fractions from these 2 peaks were ultrafiltered using an Amicon PM 10 filter. SDS–polyacrylamide gel electrophoresis showed an apparent M_r of >100 000 for the first peak indicating a macromolecular aggregate of lipase and procolipase. The second peak had an apparent M_r of 64 000 indicating a 1:1 molar complex of lipase (M_r 52 000) and procolipase (M_r 11 000).

Glass beads were present in the incubation system to increase the formation of the 1:1 molar complex of lipase and procolipase (table 1). Without glass beads most or all of the complex was recovered in the void fraction. With 1 mM oleic acid present in the incubation system it was also possible to increase the yield of the 1:1 molar complex of lipase and procolipase. The overall low yield of complex formation was probably due to the alkaline pH (8.5) of the reaction, which is not optimal for lipase–procolipase binding [8].

3.2. *Properties of the lipase–procolipase complex*

The 1:1 molar lipase–procolipase complex (in the second peak) was further investigated. With Intralipid as substrate for lipase in the presence of bile salt the lipase–procolipase complex gave no lag time. The reaction started immediately (table 2) and was maximal from the beginning (fig.1). The lipase–procolipase complex thus was similar to trypsin-activated colipase and lipase in showing a very short lag-time with Intralipid as substrate for lipase but differed by being maximal immediately (see fig.1). Hydrolytic activity of the complex against tributyrin was not affected. Assuming a 1:1 molar ratio of lipase and procolipase in the complex the specific activity of procolipase in the complex was 20 000 units/mg when measured directly and 45 000 units/mg with an excess of lipase compared to 40 000 units/mg for uncomplexed procolipase. Lipase activity of the complex was the same as for uncomplexed lipase (8000 units/mg) using tributyrin as substrate. The stability of lipase in the complex was

Table 1

Yield of complex	Without glass beads	With glass beads	With oleic acid
Void fraction	0.03–0.12 mg	0.02–0.06 mg	0.05 mg
Second fraction	0–0.005 mg	0.12–0.24 mg	0.10 mg

Yield of lipase–procolipase complex as a macromolecular aggregate (void peak) and as a molar 1:1 complex (second peak) after filtration through Sephadex G-200 after incubation of 1 mg lipase and 3 mg procolipase with dimethylsuberimidate in the absence of glass beads, in the presence of glass beads and in the presence of 1 mM oleic acid and 2 mM NaTDC at pH 8.5

Table 2

Lag-time for rapid triacylglycerol hydrolysis with Intralipid as substrate for the lipase–procolipase complex compared to procolipase and colipase followed by lipase

Enzyme	Lag time
Lipase + procolipase	21.6 min
Lipase + colipase	2.1 min
Lipase–procolipase	0 min

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 (4×10^{-8} M) was added followed after 1 min by lipase (4×10^{-8} M) or when in complex and the time measured until maximal rate of triacylglycerol hydrolysis occurred

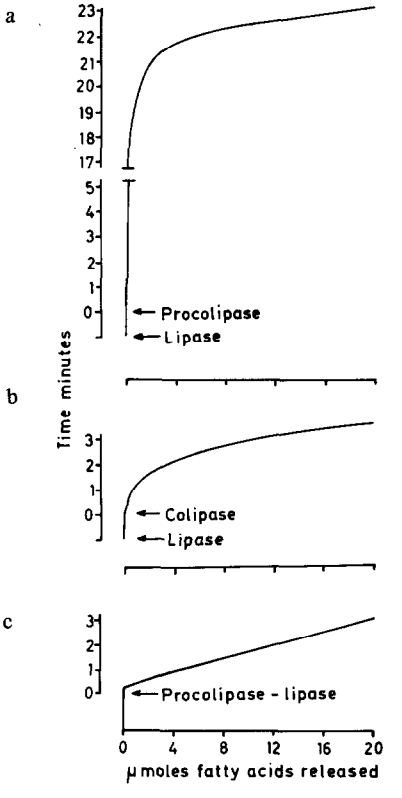

Fig.1. Kinetic of the hydrolysis of Intralipid upon injection of: (a) lipase followed after 1 min by procolipase; (b) lipase followed after 1 min by colipase; (c) covalently linked lipase–procolipase at a single time. Experimental conditions see table 2.

increased, $t_{1/2}$ being 8 min compared to 4 min when incubated at 40°C with a tributyrin emulsion at pH 7. Dimethylsuberimidate added to lipase or procolipase when separate with or without siliconized glass beads did not change the kinetic properties of the proteins.

4. Discussion

Procolipase is the precursor form of colipase. Activation with trypsin gives colipase lacking the N-terminal pentapeptide. In the presence of lipase this colipase rapidly binds to a phospholipid-covered triacylglycerol. The rapid binding has been explained by the exposure of the hydrophobic N-terminal sequence in colipase [12]. Colipase at the triacylglycerol surface has a 100-fold increased binding to lipase [1] which would thus accelerate the 'anchoring' of lipase to the substrate surface.

This work shows that a rapid binding is also obtained by covalently linking procolipase and lipase. One possible explanation for this rapid binding could be that lipase takes part in the interfacial substrate binding. As lipase by itself does not bind to the mixed triacylglycerol substrate [5], it could mean that by complexing with procolipase there is a conformational change of lipase forming a binding site for the substrate. A similar mechanism had been indicated [6] where it was shown that inhibition of lipase by diethyl-*p*-nitrophenyl phosphate, which reacts with a serine involved in the interfacial adsorption of lipase [13], was possible only in the presence of colipase.

The lag-time observed for the lipase/colipase-catalyzed hydrolysis of phospholipid covered triacylglycerol has been characterized as being dependent, i.e., dependent on the products accumulating at the substrate interface [14]. The lag-time observed here for the lipase–procolipase complex was of the independent type, i.e., independent of the concentration of the complex as described for phospholipase A [15]. One important effect of the products accumulating at the interface could therefore be to increase the binding between lipase and colipase. Fatty acids have been shown to increase the binding between lipase and procolipase [8]. The other possibility that has been discussed is that the products will increase the binding of the proteins to the surface [14]. The activity against Intralipid observed for the complex also had a different pattern compared to colipase and lipase when added separately. In the latter case there was a gradual increase in activity both for procolipase and colipase added to lipase (fig.1a,b) illustrating the activating effect of the products, while for the lipase–procolipase complex the maximal rate was reached immediately with no sign of product activation (fig.1c).

The activating effect of long-chain fatty acids can thus in light of these experiments be explained by their effect to increase the binding between lipase and procolipase.

The covalent crosslinking between lipase and procolipase was performed in the presence of siliconized glass beads. Without glass beads lipase and procolipase formed a macromolecular aggregate in the presence of dimethylsuberimidate.

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