PORCINE PANCREATIC PROCOLIPASE AND ITS TRYPSIN-ACTIVATED FORM

Lipid binding and lipase activation on monomolecular films

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1. Introduction

Tri- and diglycerides passing into the small intestine are hydrolyzed to fatty acids and 2-monoglycerides by pancreatic lipase assisted by its protein cofactor colipase [1,2]. Both lipase and colipase are secreted by the pancreas and colipase is synthesized as a precursor form [3]. Cleavage of the N-terminal pentapeptide by trypsin yields an activated molecule. This proteolytic cleavage is assumed to take place when colipase is secreted into the duodenum in a similar way as for the zymogens of pancreatic origin.

The importance of procolipase activation was first observed in a substrate system using the commercially available Intralipid emulsion (long-chain triglycerides emulsified with phospholipids) and was demonstrated as a decrease in a lag time before lipase hydrolysis proceeded at a high rate [3].

We have now studied the interactions of colipase and its proform with a monomolecular *rac*-1,2-dilaurin film in the presence of taurodeoxycholate and their lipase-activating properties. In this system, at high surface pressure, we have observed an all or none effect on the lipase activation by colipase and procolipase, respectively. These results support the idea that trypsin activation of procolipase yields a colipase molecule with better lipid-binding properties. This can be compared to the well-documented case of trypsin activation of prophospholipase A_2 [4,5]. In view of earlier results the relevance of procolipase activation to the in vivo situation will be discussed.

2. Materials and methods

2.1. *Lipids*

rac-1,2-Didodecanoylglycerol (1,2-dilaurin) was obtained from Serdary Research Laboratories (Ontario, Canada) and showed one major spot by thin-layer chromatography. Sodium taurodeoxycholate (TDC; Sigma) was used with no further purification. All other chemicals were analytical grade.

2.2. *Proteins*

Porcine pancreatic procolipase and colipase were prepared as described earlier [3,6]. Procine pancreatic lipase LA and LB were purified according to [7], with an additional Sephadex G-100 filtration at pH 9.2 and 0.05 M NaCl in order to remove the last traces of remaining colipase.

Iodination was performed by the triiodide method [8]. Approx. 1 atom of iodine/procolipase molecule was found to be incorporated [^{125}I]Colipase was obtained by trypsin activation of [^{125}I]procolipase [3]. The iodinated cofactors retained 50% of the original colipase activity at pH 6.5 on a tributyrin assay in the presence of TDC.

2.3. *Surface barostat technique*

Assays were performed in a special 'zero-order' trough drilled into a Teflon block [9]. The reaction compartment contained 230 ml of solution with a total surface of 123 cm^2. The subphase of the reaction compartment was thermostatically maintained at 25 ± 0.5°C by circulating water in an immersed glass coil. The subphase was continuously agitated with one magnetic stirrer (250 rev./min). 1,2-Dilaurin, in $CHCl_3$ solution (approx. 1 mg/ml), was spread over

the entire subphase with a microliter syringe. Several minutes were allowed to pass for solvent evaporation. The 1,2-dilaurin film was first equilibrated at the required surface pressure, between 32.5–40 dynes/cm. A volume of a 100 mM taurodeoxycholate stock solution was injected into the aqueous subphase of the reaction compartment to give a final concentration of 2.0 mM. Surface pressure rose immediately and then stabilized 3–10 min after the injection of bile salts. Surface pressure was then regulated with the barostat. All injections were performed behind a small Teflon bar fixed in the reaction compartment in order to avoid crossing the lipid film with the tip of the pipet. The colipase sample was always injected 1 min after lipase.

Kinetics were recorded for 15–60 min. The film was then collected and an aliquot of the bulk phase was sampled as described earlier [10].

Before each experiment, the trough was cleaned with ethanol, rinsed several times with tap water and finally with distilled water. The aqueous subphase was systematically composed of 10 mM Tris–HCl buffer, pH 8.0, 150 mM NaCl and 1 mM $CaCl_2$. Distilled water was prepared from alkaline $KMnO_4$ in an all-glass apparatus. Residual surface-active impurities were removed before each assay by sweeping and suction of the surface.

3. Results

To study the action of lipase on *rac*-1,2-dilaurin monomolecular films in the presence of 2 mM TDC it is necessary to work at surface pressures above 30 dynes/cm due to the high equilibrium surface pressure of the bile salts. Because of its high collapse pressure, *rac*-1,2-dilaurin is thus a suitable substrate for lipase in the presence of bile salts [11].

As is seen from the surface pressure recording (dashed curve in fig.1) there is an increase in surface

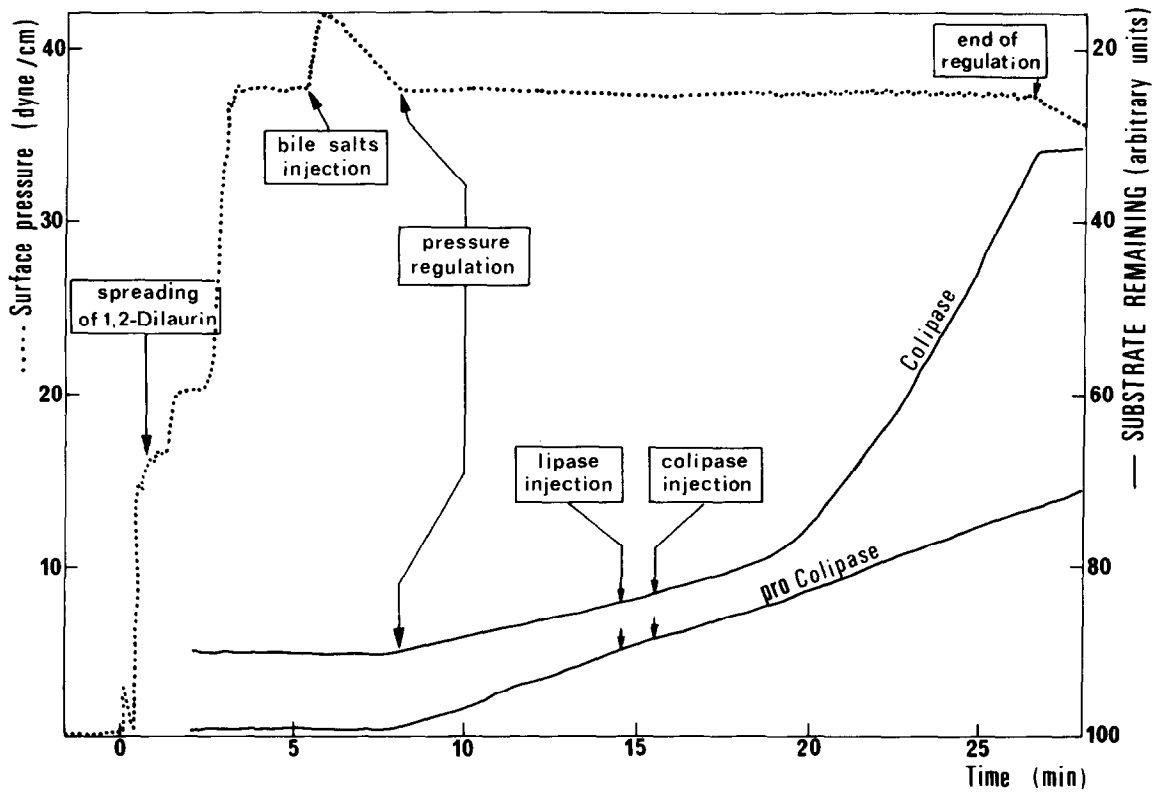

Fig.1. Kinetics of the hydrolysis by 0.9 nM pancreatic lipase of a *rac*-1,2-dilaurin film under isobaric conditions (37.5 dynes/cm) and in the presence of 2.0 mM taurodeoxycholate. Experiments performed with 8 nM colipase (middle curve) and 8 nM procolipase (lower curve); (. . . , upper curve) the surface pressure recordings. All curves are original kinetic tracings from the barostat recorder. 1 arbitrary unit of substrate remaining corresponds to 1.55 nmol of *rac*-1,2-dilaurin.

pressure when TDC is injected into the subphase due to the penetration of TDC molecules into the film. After the rise in pressure there follows a slow dissolution of the film which is compensated by the movement of the barrier shown in the two lower continuous tracings (fig.1). In this mixed film system at high surface pressure, lipase, with or without procolipase, did not show any hydrolytic activity (fig.2B). After injection of lipase and colipase the hydrolysis of the dilaurin-TDC mixed film proceeds with kinetics characterized by the lag time τ. Fig.2A,B show, respectively, the pressure-lag time and pressure-activity profiles of lipase hydrolysis of mixed dilaurin-TDC films. In the pressure range 32–35 dynes/cm, procolipase is able to activate lipase. However, this activating effect is progressively decreased to zero between 35–40 dynes/cm. A concomitant increase in lag time from 7 min at 32 dynes/cm to 17 min at 35 dynes/cm was observed. At 37.5 dynes/cm the lipase activity was low and steady state was not reached after 60 min. Colipase, on the other hand has different properties. Upon increasing the surface pressure, lipase activity increased, while the lag time stayed almost constant and low, in the pressure range studied.

Under no conditions could lipase activity be detected in the presence of a 2 mM TDC solution and in the absence of (pro)colipase (fig.2B).

Binding studies of [^{125}I]procolipase and [^{125}I]-colipase in the presence or absence of lipase are shown in fig.2C,D, respectively. One can note a difference in the binding properties between pro- and colipase in the absence of lipase. This difference, and the amount bound (pro)colipase, is larger in the presence of lipase and after hydrolysis has been going on, i.e. lipolytic products has been formed. The activation of lipase by radioactively labelled procolipase and colipase is less efficient than that of the corresponding unlabelled proteins (data not shown). However, its dependence of the surface pressure is similar to what was observed using unlabelled cofactors (fig.2B).

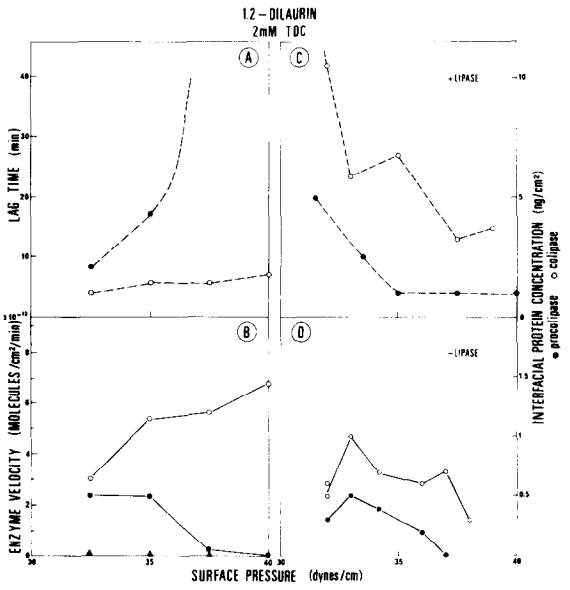

Fig.2. Lipase activity on *rac*-1,2-dilaurin films (B) and lag time, τ (A) as a function of surface pressure. Experiments were performed in the presence of 2.0 mM taurodeoxycholate in the bulk phase, and in the presence of 0.9 nM pancreatic lipase B (▲) and 8 nM procolipase (●) or 8 nM colipase (○). Binding of 21 nM [^{125}I]procolipase (●) and 21 nM [^{125}I]colipase (○) as a function of surface pressure in the absence of pancreatic lipase (D) and after 10–20 min of hydrolysis by 16 nM pancreatic lipase B (C).

4. Discussion

The activation of procolipase by trypsin should be seen in relation to the general function of the enzymes of the pancreatic juice, i.e. a fast and efficient degradation of the chyme to molecules absorbable by the enterocytes. The trypsin cleavage of the N-terminal part of the pancreatic zymogens generally yields active molecules better fitted for their metabolic role but at the same time harmful for the parenchymal tissues. Accordingly the activation of procolipase by trypsin results in the formation of a molecule with better potential lipase-activating properties. The present investigation using *rac*-1,2-dilaurin-TDC mixed monomolecular films as lipase substrate is another evidence that such a difference between procolipase and colipase exists. At 32.5 dynes/cm the lipase activity in the presence of colipase is only 25% higher than in the presence of procolipase. This situation is comparable to that prevailing in bulk system using tributyrin as substrate in the presence of TDC [3]. At higher surface pressures the procolipase activation of lipase decreases with a concomitant increase in lag time, attributable to a slow protein penetration into the film (fig.2A). This decrease in activity could be due to a decrease in binding of procolipase to the film (fig.2C). At 40 dynes/cm the lipase activity is zero and could not be restored even with a 10-fold increase in procolipase concentration. In contrast,

the colipase activation of lipase increases with surface pressure (fig.2B) although there is a decrease in the amount of colipase bound to the film (fig.2C). This might indicate that the activation of colipase also affects the binding of lipase to colipase at the interface and/or the turnover number of enzyme [12,13]. This phenomenon will be further investigated using simultaneously radioactively labelled lipase and colipase. Nevertheless the better binding properties of colipase compared to procolipase are illustrated by the large difference in lag time between the two proteins (fig.2A). The increase in binding of the both colipases after hydrolysis started clearly indicates that reaction products at the interface enhance the binding of colipase.

Apparently at a surface pressure of 40 dynes/cm, procolipase does not activate lipase against a *rac*-1,2-dilaurin substrate, while colipase does. Is this relevant to the in vivo situation? The diglycerides certainly are one of the principal physiological substrates of pancreatic lipase. It is well documented that the dietary triglycerides are partially degraded to diglycerides and fatty acids already when they enter the duodenum due to the action of the lingual lipase [14–18]. These glycerides are emulsified by the fatty acids, alimentary phospholipids and mixed with bile. All these compounds are likely to form a highly packed structures. The colipase molecule thus has to overcome these barriers by having efficient binding properties.

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References

[1] Borgström, B., Erlanson-Albertsson, C. and Wieloch, T. (1979) J. Lipid Res. 20, 805–816.
[2] Sémériva, M. and Desnuelle, P. (1979) Adv. Enzymol. 48, 319–370.
[3] Borgström, B., Wieloch, T. and Erlanson-Albertsson, C. (1979) FEBS Lett. 108, 407–410.
[4] Pieterson, W. A., Vidal, J. C., Volwerk, J. J. and de Haas, G. H. (1974) Biochemistry 13, 1455–1460.
[5] Pattus, F., Slotboom, A. J. and de Haas, G. H. (1979) Biochemistry 13, 2691–2697.
[6] Erlanson, C., Fernlund, P. and Borgström, B. (1973) Biochim. Biophys. Acta 310, 437–445.
[7] Verger, R., de Haas, G., Sarda, L. and Desnuelle, P. (1969) Biochim. Biophys. Acta 188, 272–282.
[8] Slotboom, A. J., Verheij, H. M., Pujik, W. C., Dedieu, A. G. R. and de Haas, G. H. (1978) FEBS Lett. 92, 361–364.
[9] Verger, R. and de Haas, G. H. (1973) Chem. Phys. Lipids 10, 127–136.
[10] Rietsch, J., Pattus, F., Densuelle, R. and Verger, R. (1977) J. Biol. Chem. 252, 4313–4318.
[11] Lairon, D., Charbonnier-Augeire, M., Nalbone, G., Leonardi, J., Hauton, J. C., Pieroni, G., Ferrato, F. and Verger, R. (1980) Biochim. Biophys. Acta 618, 106–118.
[12] Verger, R. (1980) Methods Enzymol. 64, part B, 340–392.
[13] Pieroni, G. and Verger, R. (1979) J. Biol. Chem. 254, 10090–10094.
[14] Ramsey, H. A., Wise, G. H. and Tove, S. B. (1956) J. Dairy Sci. 39, 1319–1322.
[15] Borgström, B., Dahlquist, A., Lundh, G. and Sjövall, J. (1957) J. Clin. Invest. 36, 1521–1536.
[16] Olivecrona, T., Billström, Å., Fredrikzon, O., Johnson, O. and Samuelson, G. (1973) Acta Paediatr. Scand. 62, 520–522.
[17] Hamosh, M. and Scow, R. O. (1973) J. Clin. Invest. 52, 88–95.
[18] Plucinski, T. M. Hamosh, M. and Hamosh, P. (1979) Am. J. Physiol. 237, 541–547.