CHEMICAL MODIFICATION OF PANCREATIC LIPASE

Effect on the colipase-reactivated and the 'true' lipase activity

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1. Introduction

The hydrolysis by pancreatic lipase of emulsified substrates is strongly inhibited by bile salt, but the hydrolytic activity is restored upon the addition of a small protein cofactor secreted from pancreas, designed colipase [1]. To explain the inhibitory effect of bile salt and the reactivating effect of colipase several studies have been undertaken. By calorimetric studies it was shown that pancreatic lipase and colipase form a 1:1 complex [2]. The inhibitory effect of bile salt was explained as a physical displacement of lipase from the substrate interface [3] and the reactivating effect of colipase was due to its adsorption to the triglyceride/water interface which occurs in spite of the presence of bile salt thereby enabling the coadsorption of lipase [3,4]. This idea of colipase acting by first penetrating the lipid film and then serving as an anchor for lipase into the film was recently confirmed using the monolayer technique for preparation of the substrate [5].

By means of chemical modification the relative importance of individual grouping within the colipase molecule was recently studied [6]. It was then found that at least two free carboxylic groups of colipase seemed important for the lipase binding, while intact lysine residue(s) were important for binding of colipase to the substrate interface in the presence of bile salt.

In this study the interest has been concentrated on the lipase moiety in the lipase–colipase complex. By gentle blocking of the lysine residues of lipase using citraconic anhydride as modifying agent lipase could no longer be reactivated by colipase in a bile salt containing emulsion of tributyrin, although it was still capable of hydrolysing an emulsion of tributyrin in the absence of bile salt. By increasing the concentration of citraconic anhydride this lipase activity on an emulsion of tributyrin was also prevented. Modification of the arginine residues of lipase by 1,2-cyclohexanedion had no effect, either on the colipase dependent activity of lipase using tributyrin in bile salt as substrate or on the hydrolytic activity of lipase alone using tributyrin in buffer as substrate.

2. Materials and methods

Porcine pancreatic lipase, low in colipase activity, was used [7]. Rat pancreatic lipase free of colipase was prepared as described [8]. Porcine pancreatic colipase was obtained as described [9]. Tributyrin was a product from BDH and was fractionally distilled free from lower glycerides and free fatty acids as detected by thin-layer chromatography (TLC). Citraconic anhydride was a product of Merck, Germany, 1-2-cyclohexanedion was bought from Fluka, Switzerland.

Lipase activity was determined by titration of tributyrin in two ways:

(1) In buffer for demonstration of the 'true' hydrolytic activity of lipase [10].
(2) In bile salt with an excess of colipase for demonstration of the ability of lipase to be reactivated by colipase [8].

Reactions were carried out a 25°C in 15 ml 2 mM Tris–maleate buffer, pH 7.0, 1 mM $CaCl_2$, 150 mM in NaCl, 0.2% NaN_3 for (1), containing in addition 4 mM NaTDC for (2). Tributyrin, 500 μl, was added and

emulsification obtained by the use of a magnetic stirrer. Between 10 and 100 μl lipase sample was added. In (2) an excess of pure colipase in 100 μl was added.

2.1. *Reaction of lipase with citraconic anhydride*

Lipase was reacted with citraconic anhydride as described [11]. 0.1–0.2 mg lipase (rat or porcine) was dissolved in 1 ml 50 mM *N*-ethyl morfoline acetic acid buffer pH 8.2. Incubation was started by addition of 10–100 μl freshly prepared solution of 100 mM citraconic anhydride in acetone (diluted 1:100) and pH maintained at 8.2 with 1 N NaOH. After different incubation periods within 1 h samples of 50 μl reaction mixture were taken and added to 1 ml 2 mM Tris–maleate buffer, 150 mM NaCl, pH 7.0. From these tubes samples of 10–100 μl were then taken for assay of lipase activity in the two different ways as described above.

2.2. *Reaction of lipase with 1,2-cyclohexanedion*

Reaction with 1,2-cyclohexanedion was performed as described [12]. Lipase, 100 μg, was incubated with 50 mM 1,2-cyclohexanedion in 1 ml 100 mM borate buffer pH 9.0. After different time intervals 50 μl aliquots were taken from the reaction mixture to 1 ml buffer solution and then assayed for lipase activity in the two different ways as described above.

3. Results

3.1. *Reaction of lipase with citraconic anhydride*

Inactivation of lipase at increasing concentrations of citraconic anhydride is shown in fig.1. As seen at low concentrations of citraconic anhydride, there is a strong inhibition of the colipase-dependent activity of lipase using tributyrin in bile salt as substrate, while lipase alone using tributyrin in buffer as substrate still retains its activity. At higher concentrations of citraconic anhydride this latter lipase activity using emulsified tributyrin as substrate is also lost. In fig.2 the same reaction is shown, using rat lipase instead of porcine lipase. As seen the two different functions of lipase can be separated by increasing the concentration of citraconic anhydride for modification of the lysine residues, although using rat lipase these two functions are more clearly separated.

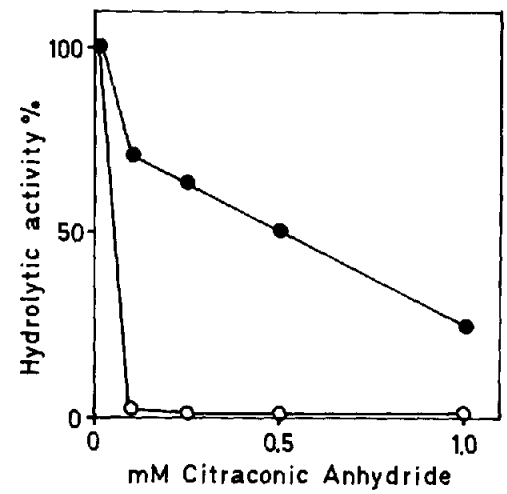

Fig.1. Reaction of porcine pancreatic lipase with increasing concentrations of citraconic anhydride. Lipase (4.0 μM) was incubated 10 min at 28°C in 50 mM *N*-ethyl morpholine (acetic acid buffer, pH 8.2, with increasing concentrations of citraconic anhydride. Samples were then taken both for assay of the 'true' lipase activity (●——●) using an emulsion of tributyrin in buffer as substrate, and for assay of the colipase-dependent activity of lipase (○——○), using a bile salt containing emulsion of tributyrin as substrate with an excess of pure colipase for reactivation of lipase.

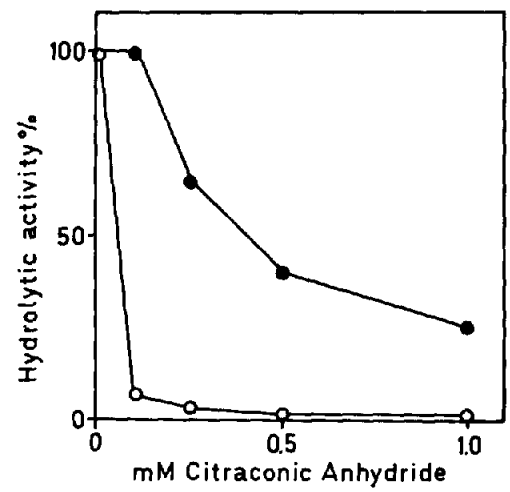

Fig.2. Reaction of rat pancreatic lipase with increasing concentrations of citraconic anhydride. Reaction conditions were similar to these described for porcine pancreatic lipase (see fig.1). 'True' lipase activity (●——●) using tributyrin in buffer as substrate. Colipase-dependent activity (○——○) using tributyrin in bile salt with an excess of colipase as substrate.

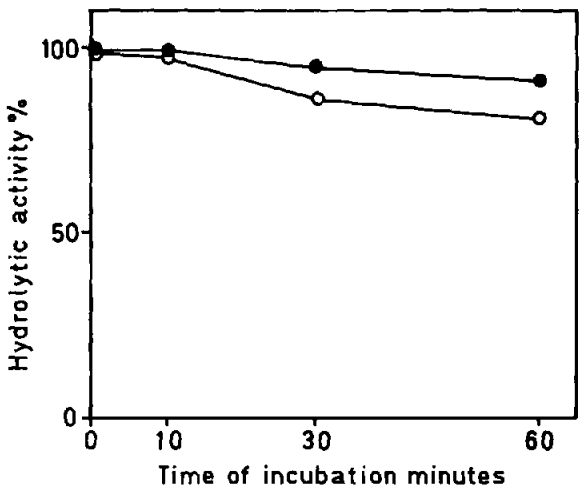

Fig.3. Reaction of porcine pancreatic lipase with 1,2-cyclohexandion. Lipase (2.0 μM) was incubated at 25°C in 100 mM borate buffer pH 9.0 with 60 mM 1,2-cyclohexanedion. After different time intervals lipase was assayed in two ways, with an emulsion of tributyrin in buffer for the 'true' lipase activity (●——●) and with an emulsion of tributyrin in bile salt with an excess of colipase for the colipase-dependent lipase activity (○——○).

3.2. *Reaction of lipase with 1,2-cyclohexanedion*

In fig.3 is shown the reaction of porcine lipase with 1,2-cyclohexanedion with time. After 1 h incubation lipase activity in buffer and lipase activity in bile salt with an excess of colipase are both retained to about 80–90% with no clear distinction between the two activities.

4. Discussion

Studies have been performed to further characterize the lipase–colipase interaction. By calorimetric studies the decrease in entropy suggested that the interaction between the two proteins was mainly hydrophobic [2]. This binding is, however, not broken by any mild detergent like Triton as revealed by partition studies [16]. By chemical modification of colipase it was recently shown that one or two free carboxylic groups of colipase were important for the lipase binding [6]. The present experiments indicate that a lysine(s) residue of lipase is important for the reactivating effect of colipase. These experiments thus suggest that in addition to the hydrophobic interaction between lipase and colipase an electrostatic interaction is also important involving a positively charged amino group of lipase, a lysine residue, and a negatively charged carboxylic group of colipase, either an aspartic or glutamic acid residue. This ionic interaction may be important for the proper orientation between the two proteins, while the energy for the complex formation is largely hydrophobic. This pattern is in accordance with other protein–protein interactions studied, like that of trypsin and trypsin inhibitor, that of insulin monomers into dimers and the subunits of hemoglobin, where the main energy for complex formation is given by hydrophobic interaction while an ionic interaction accounts for the specificity in the reaction [13]. It is also generally accepted that an electrostatic bond which occurs in a hydrophobic microenvironment is extremely strong (cf. [18]).

That the reactivating effect of colipase on lipase is dependent on ionic interactions could explain the strong influence of salt on the lipase activity in an emulsion of tributyrin with bile salt and excess of colipase [8] while the activity of lipase on emulsified tributyrin in buffer is not affected by the concentration of salt [17].

Another point of interest is that a lysine residue is also important for the 'true' lipase activity on emulsified tributyrin in buffer. Earlier reports have shown that a serine residue of lipase is important for the hydrophobic binding [14]. Bovine serum albumin which also binds hydrophobically has been shown to contain a lysine residue in its hydrophobic binding site [15].

Modification of arginine residues of lipase had no effect, neither on the colipase-dependent nor on the 'true' lipase activity.

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References

[1] Borgström, B. and Erlanson, C. (1971) Biochim. Biophys. Acta 242, 509–513.

[2] Donnér, J., Spink, C. H., Borgström, B. and Sjöholm, I. (1976) Biochemistry 15, 5413–5417.
[3] Borgström, B. (1975) J. Lipid Res. 16, 411–417.
[4] Vandermeers, A., Vandermeers-Piret, M. C., Rathé, J. and Christophe, J. (1975) FEBS Lett. 49, 334–337.
[5] Verger, R., Rietsch, J. and Desnuelle, P. (1977) J. Biol. Chem. 252, 4319–4325.
[6] Erlanson, C., Barrowman, J. and Borgström, B. (1977) Biochim. Biophys. Acta in press.
[7] Donnér, J. (1976) Acta Chem. Scand. B30, 430–434.
[8] Borgström, B. and Erlanson, C. (1973) Eur. J. Biochem. 37, 60–68.
[9] Erlanson, C., Fernlund, P. and Borgström, B. (1973) Biochim. Biophys. Acta 310, 437–445.
[10] Erlanson, C. and Borgström, B. (1970) Scand. J. Gastroenterol. 5, 293–295.
[11] Gibbons, I. and Perham, R. N. (1970) Biochem. J. 116, 843–849.
[12] Patthy, L. and Smith, L. (1975) J. Biol. Chem. 250, 565–569.
[13] Chothia, C. and Janin, J. (1975) Nature 246, 705–708.
[14] Chapus, C. and Sémériva, M. (1976) Biochemistry 15, 4988–4991.
[15] Sweet, C. and Zull, J. E. (1970) Biochim. Biophys. Acta 219, 253–262.
[16] Patton, J., Erlanson, C., Albertsson, P.-Å. and Borgström, B. manuscript in preparation.
[17] Borgström, B. (1977) submitted.
[18] Dyson, R. (1975) in: Essentials of Cell Biology pp. 65–91 Allyn and Bacon.