THE POLAR INTERACTIONS BETWEEN PANCREATIC LIPASE, COLIPASE AND THE TRIGLYCERIDE SUBSTRATE

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1. Introduction

Pancreatic lipase is an enzyme that uniquely interacts with the interface of water-insoluble ester substrates but also with hydrophobic interfaces in general [1]. These hydrophobic interactions lead to the rapid irreversible denaturation of the enzyme and cannot be considered to be of interest under physiological conditions. In the in vivo situation – in the small intestinal contents – lipase is functioning in the presence of bile salts which by their general detergent effect desorb lipase from the substrate interface by braking or preventing hydrophobic interactions [2]. Under certain conditions of pH and salt, lipase can bind to the substrate and be catalytically active also in the presence of bile salt, indicating that polar interactions can be important [2,3]. Colipase, a polypeptide cofactor of pancreatic juice, binds to lipase substrate in bile salt solution and serves as an anchor for lipase to the substrate [4–6], thus enabling lipase to be active in bile salt solution over a wide pH range.

It is the objective of the present study to demonstrate that 'polar' rather than hydrophobic bondings are important for the attachment of lipase and colipase to the bile salt laden substrate interface and thus for the biological function of the enzyme. Evidence is also presented to show the specific binding of these two proteins most probably by hydrogen bonding to the carbonyl groups of the substrate.

2. Materials and methods

Porcine and rat pancreatic juice was prepared as described by Donnér [7] and Gidez [8], respectively. The colipase used was of type II and prepared as described [9].

Tributyrin was purified by chromatography on alumina. Triolein was freed of fatty acids by passing an Amberlite IRA-400 column in wet diethyl ether [10]. Taurodeoxycholate was synthesized in this laboratory [11]. Trioctyl glyceryl ether was synthesized as described by Bauman and Mangold [12] except that 1-octyl bromide was used in place of octyl methanesulfonate and one of the starting reactants was aceton glycerol instead of tritylated glyceryl ether.

Lipase and colipase activities were measured by potentiometric titrations using tributyrin as substrate [13]. The binding of lipase to the oil/water interfaces was determined as previously described [2] involving a separation of the dispersed oil phase by centrifugation. In the colipase experiments the emulsion was sucked through a nylon filter with 20 × 20 μm pores to separate the water from the oil phase as will be described in detail [14].

Unless stated otherwise the binding studies were performed with 2 mM Tris–maleate (pH 7.0), 1 mM in $CaCl_2$, with varying concentrations of sodium chloride and bile salt. In the case in which the pH was varied the same buffer was used but the pH adjusted and kept constant with the automatic titrator.

3. Results

3.1. *Binding of pancreatic lipase to interfaces*

In the absence of bile salts lipase adsorbs completely to a tributyrin interface and this binding is

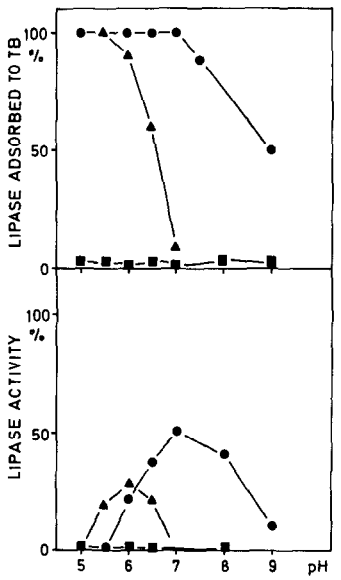

Fig.1. Upper part shows the effect of pH on the binding of 1.0 nM rat pancreatic lipase to a tributyrin/water interface in the presence of 4 mM taurodeoxycholate and three different concentrations of sodium chloride: 0.02 M (•), 0.1 M (▲), and 0.6 (■). The ordinate represents the percentage of lipase adsorbed to the interface. The lower part shows the activity of lipase against tributyrin under the same conditions as recorded by titration, calculated as a percentage of the activity obtained by a similar amount of lipase against tributyrin in the absence of bile salt pH 6.0.

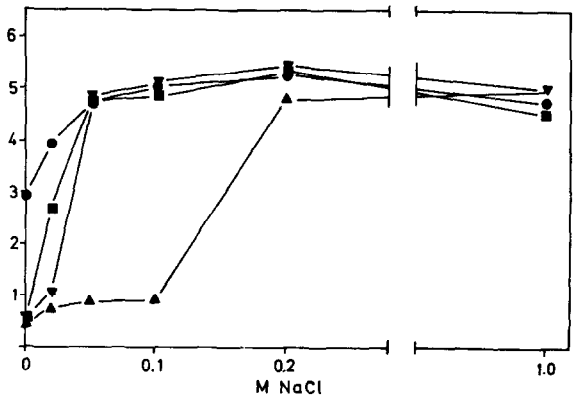

Fig.2. The effect of sodium chloride concentration on the adsorption of 2.5 nM rat pancreatic lipase in 4 mM taurodeoxycholate, at pH 6.0, to the interface of 250 μl each: tributyrin (▲), trioctylglyceryl ether (v) or hexadecane (■) in 10 ml total solution. The ordinate represents the lipase activity in the aqueous phase obtained after centrifugation, in μmol fatty acid released/min. The recovery of lipase in the blank experiments with no added oil phase is also included (•).

not affected by sodium chloride concentration (up to 1 M).

In the presence of bile salt above the critical micellar concentration (4 mM taurodeoxycholate) the binding of lipase to the tributyrin interface depends on the pH of the solution, the pI of the enzyme (5.2 for porcine, 6.8 for rat lipase [3]) and the salt concentration. Rat pancreatic lipase at low salt binds to tributyrin up to pH 7.5, when the salt concentration is increased the binding curve over pH is shifted to the left (shown for 0.1 M NaCl in fig.1). At high salt (0.6 M NaCl) no binding takes place at any pH. Lipolytic activity is seen in bile salt solution as long as it binds to the substrate interface in the presence of bile salt and consequently the pH optimum is displaced to the left with increase in salt. The effect of salt on the binding of rat pancreatic lipase to different oil/water interfaces at pH 6.0 is given in fig.2. In the absence of salt lipase binds to the same extent to interfaces, whether this is tributyrin, trioctyl glyceryl ether or hexadecane. With increase in salt lipase goes into the aqueous phase but the salt concentration needed is diffferent depending on the structure of the interface. For the hexadecane interface the salt concentration for desorption is the lowest, it is somewhat higher for the ether interface and for the ester interface lipase stays adsorbed up to 0.1 M salt (fig.2). The recovery of lipase in the absence of any added oil phase is also dependent on the salt concentration as shown in fig.2. This is most probably due to the adsorption of lipase to the glass surface at low salt concentrations. Similar results were obtained using porcine lipase although the binding curve over pH was displaced to the left depending on the lower pI for this enzyme [3].

3.2. *Binding of colipase to interfaces*

The adsorption of colipase to tributyrin was unaffected by variations in sodium chloride concentration between 0–1 M in the buffer (fig.3). In the presence of taurodeoxycholate above the critical micellar concentration, increase in salt concentration greatly affected adsorption, at 1.0 M sodium chloride no colipase was adsorbed.

When trioctylglyceryl ether was used as oil phase

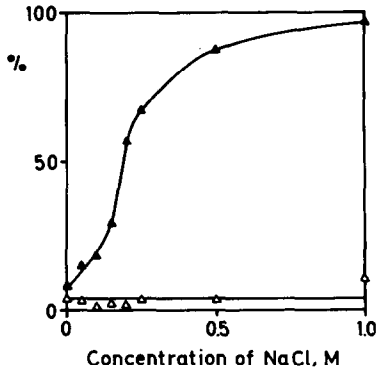

Fig.3. Effect of sodium chloride concentration on the absorption of 156 nM colipase at the tributyrin/water interface in the absence (△) of bile salt and in the presence of 2 mM taurodeoxycholate (▲). The ordinate represents the amount of colipase free in the aqueous phase as a percentage of the total amount of colipase.

at pH 7.0 and 150 mM sodium chloride there was no adsorption of colipase to the interface at concentrations of taurodeoxycholate above its critical micellar concentration.

4. Discussion

Previous investigations [1,2] have shown that pancreatic lipase and colipase adsorbs at interfaces by hydrophobic interactions in the absence of bile salts (which cannot be broken by high concentrations of salt). In the case of lipase polar interactions which are not broken by bile salts can also take place with the substrate interface [2,3]. A basic requirement for lipase to adsorb at the substrate interface in the presence of bile salt is that the pH of the bulk phase is near or below the pI of the enzyme [2,3]. The present studies have shown that the interactions of lipase in bile salt solution with a hydrocarbon or ether interface is weaker (hindered already by low salt) than with an ester interface. The carbonyls of the ester interface therefore can be implicated to be of importance binding the enzyme to the substrate by hydrogen bonding [15], the carbonyls of the substrate being hydrogen bond acceptors. Siliconized glass beads show a binding curve for lipase over salt concentration (in the presence of bile salts) that is similar to that for the hydrocarbon interface [3]. Pancreatic colipase in the presence of bile salts has the same requirements for the quality of the interface and is hydrogen bonded to the lipase substrate interface. The interactions between colipase and the lipase substrate are less sensitive to bile salt concentration [4] and to salt and decrease at high pH values in the presence of bile salts [2], possibly as an effect on the ionization of the amino groups in the protein which are engaged in the hydrogen bonding. As a consequence polar rather than hydrophobic interactions can be expected to be all important for the function of pancreatic lipase in the physiological situation, i.e., in the small intestinal contents. Under these conditions of bile salts and salt concentration, lipase can adsorb at the substrate interface by polar interactions and be catalytically active near or below the pI of the enzyme. Above the pI, lipase cannot bind directly to the substrate and colipase becomes necessary to anchor the enzyme to the substrate allowing for the proper interaction between the active site of the enzyme and the substrate ester bond.

Acknowledgement

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