ROLE OF COLIPASE IN THE INTERFACIAL ADSORPTION OF PANCREATIC LIPASE AT HYDROPHILIC INTERFACES

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1. Introduction

Since pancreatic lipase normally acts at triglyceride–water interfaces [1], interfacial adsorption of the enzyme can be expected to be an important step in catalysis. Indeed, lipase action on triglycerides has been shown to be inhibited by bile salts [2,3], probably because of the accumulation of the amphipath at the interface [3]. An interesting point is that this inhibition is completely reversed within certain limits by a pancreatic protein cofactor designated colipase [2,3]. A characteristic property of colipase is to bind one bile salt micelle per molecule and to form in this manner a binary complex able to fix lipase [4].

The purpose of the present work is to show that lipase, which readily adsorbs to hydrophobic interfaces, requires colipase for its adsorption to interfaces coated with an amphipath or possessing themselves an hydrophilic character. Hydrophobic interfaces were provided by siliconized glass beads. This system is likely to mimick quite well the interfacial conditions prevailing with emulsified lipase substrates, since glass beads have been reported to increase 10^3 times the activity of the enzyme on dissolved substrates [5].

2. Materials and methods

The lipase lot used throughout this work was purified in this Laboratory by Dr R. Verger [6] and it was found [7] to contain about 0.1 mol of colipase per molecule of enzyme. Colipase [2] and bovine procarboxypeptidase A were generous gifts from Dr M. Charles and A. Puigserver, respectively. Other proteins were Worthington products. Sodium taurodeoxycholate (Sigma, USA) was used without purification. Spherical siliconized glass beads were obtained from Serva (Feinbiochemica, Heidelberg, Germany). Their mean diameter determined with the aid of a Nikon comparator Model 6 C T 2 was 167 μm (164 cm^2 per g of beads). Protein adsorption on the beads was followed spectrophotometrically at 220 nm instead of 280 nm, because of the low concentration of the solutions, itself due to the low adsorbing capacity of the beads. In the case of lipase, activity was also measured [8] and the adsorption curve thus obtained was superimposable with that yielded by spectrophotometry, In the presence of bile salts which strongly absorb at 220 nm, only activity was determined.

3. Results

3.1. *Adsorption assays in the absence of taurodeoxycholate*

The assays were performed by adding small increments of the investigated protein solution to 3.5 ml of a 0.1 M Tris–HCl buffer pH 7.5 containing 1 g of beads. After gently shaking for about 1 min after each addition, the beads were allowed to settle (15–20 sec) and the protein content of the supernatant was measured. Fig.1 shows the adsorption of lipase under these conditions. Curve b shows that little enzyme can be detected in the supernatant after the first additions, due to adsorption of the enzyme to the beads. Then, after a breakpoint presumably corresponding to the saturation of the available interface,

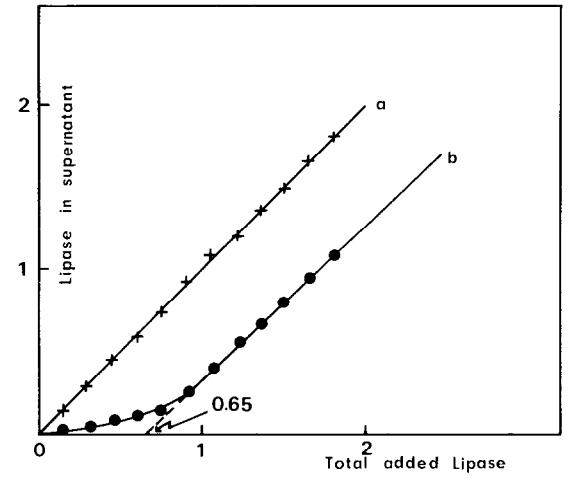

Fig.1. Adsorption of lipase on siliconized glass beads in the absence of bile salts. The enzyme concentrations are expressed in 10^{-9} mol in 3.5 ml of buffer. Curves (a) and (b), assays without and with beads, respectively.

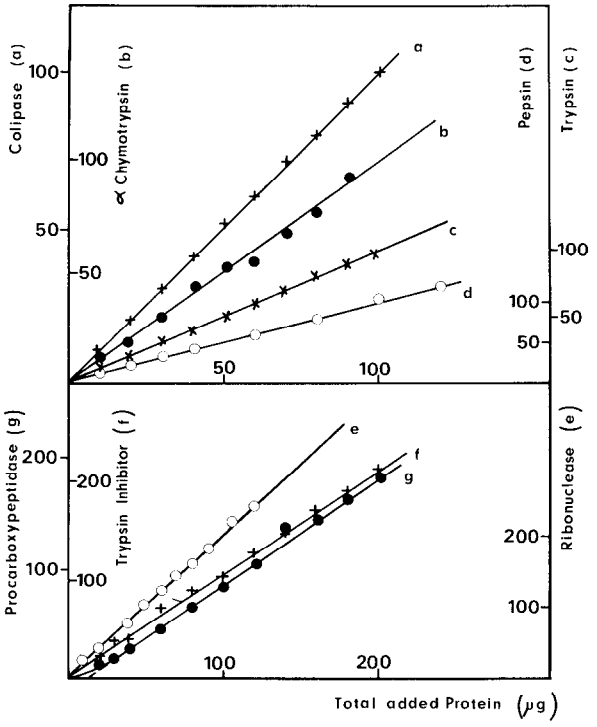

Fig.2. Adsorption assays with other proteins. Conditions were similar to those used for lipase in fig.1. Ordinates indicate the amount (μg) of protein in the supernatant. Letters near the curves refer to the proteins indicated along the ordinate axis.

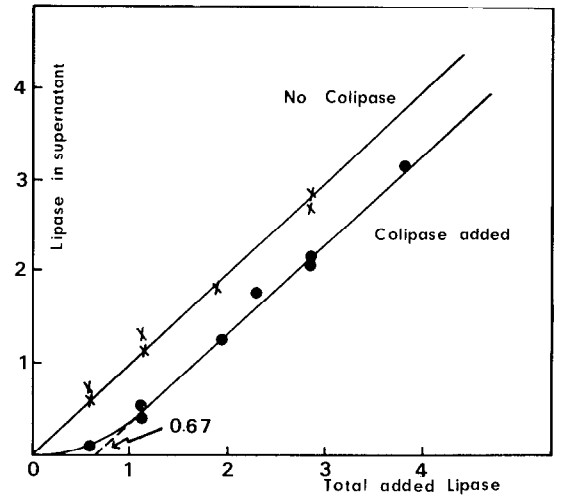

Fig.3. Adsorption of lipase on siliconized glass beads in presence of 0.85 mM bile salts without colipase (X—X) or with a 3 molar excess of colipase (•—•). Same conditions as in fig.1 except for the presence of 5 mM $CaCl_2$ in the buffer.

the plot free vs total enzyme becomes linear and an extrapolation to zero concentration in supernatant yields the amount of enzyme ($0.65 \pm 0.01 \times 10^{-9}$ mole) needed to saturate the interface (164 cm^2). This corresponds to an average density of 1 molecule of lipase per 4150 $Å^2$ of interface. A Langmuir plot of the same data leads to 3400 $Å^2$ per enzyme molecule. The dissociation constant of the enzyme–glass beads complex derived from this latter plot was found to be 2×10^{-11} mol/ml.

As shown by fig.2, several other proteins including colipase show no tendency to absorb onto siliconized glass under the same conditions. The single exception is bovine procarboxypeptidase A which is slightly bound. The fact that colipase is not adsorbed and therefore radically differs in this respect from lipase deserves special mention. It is also of interest that lipase adsorption in the absence of bile salt is not modified by a 3 molar excess of colipase.

3.2. *Adsorption assays in the presence of taurodeoxycholate*

Fig.3 illustrates the behaviour of lipase in the presence of 0.85 mM taurodeoxycholate. This concentration is smaller than the critical micelle value under the ionic strength conditions employed (1 mM). But

it is known to completely inhibit the action of colipase-free lipase on emulsified tributyrin [2]. It is therefore of great interest to note that lipase has now lost its ability to adsorb on the beads and that this ability is fully restored (0.67×10^{-9} mol of enzyme as compared to 0.65×10^{-9} moles) by addition of a 3 molar excess of colipase which also restores full enzyme activity in the tributyrin–bile salt system. Lag periods were avoided in these assays by addition of 5 mM Ca^{2+}.

3.3. *Activity assays with an hydrophilic substrate*

The diglyceride dibutyrin with a free hydroxyl group probably directed towards water at the interface is known to be a poorer substrate for lipase than its higher homolog, tributyrin. Fig.4 indicates that lipase activity on dibutyrin is very much enhanced by colipase. Although a direct correlation could not be attempted in this case because of technical difficulties, the linearity of the enzyme activity vs substrate concentration plot in the absence of colipase compared to the typical adsorption curve obtained in its presence strongly suggests that lack of adsorption is also the origin of poor activity. Another indication is that the presence of colipase considerably reduces the apparent K_m of the reaction.

4. Discussion

The observation that most proteins tested in the present work are not able to adsorb to hydrophobic siliconized glass gives more weight to the finding already reported by Brockman et al. [5] that lipase is strongly adsorbed under the same conditions. This property is most probably correlated with the ability of the enzyme to recognize the interface of hydrophobic substrates. Moreover, the value of 4150 Å^2 derived from titration assays for the area occupied by one molecule of lipase at saturation is slightly lower than that (5000 Å^2) reported by Brockman et al. [5] using the same technique and somewhat higher than that (3400 Å^2) indicated by a Langmuir plot of our own data. Considered together, these values are definitely closer to the area occupied by native lipase in a tightly packed film (3200 Å^2) than to that of the denatured enzyme (7500 Å^2 [5]). One of the most chracteristic features of lipase is perhaps to remain native at the interface or to undergo there only a slight deformation.

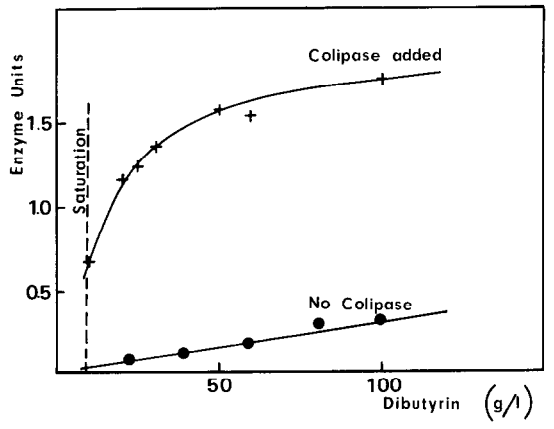

Fig.4. Michaelis plots of lipase action on dibutyrin in the presence or absence of colipase at pH 7.5 in 0.1 M NaCl.

The present work provides the first experimental indication that the inhibitory effect of bile salts on lipolysis may result from the failure of lipase to adsorb to the emulsion interface, thus preventing the first step of catalysis to occur. A plausible assumption is that bile salts preferentially occupy the interface and counteract the fixation of the enzyme by a general detergent effect. The bile salt concentration used is certainly capable of covering the whole surface of the beads by a loosely packed monolayer. Then, inhibition may result from a competition between lipase and bile salts for the possession of the interface or/and a lowering of the interfacial tension hindering lipase adsorption. This assumption is to be compared to that recently put forward by Borgström and Erlanson [3].

The fact that colipase restores both lipase activity and adsorption in the presence of bile salts lays the molecular basis of the mode of action of the cofactor under physiological conditions. The basic assumption in this respect is to relate the arrangement of detergent molecules in a monolayer covering an hydrophobic substrate interface and in a micelle. Colipase, which is already known to possess a high affinity binding site for a micelle [4] would also recognize the monolayer and adsorb to it. In the same way as lipase can associate with colipase in the presence of micelles to form a ternary complex [4], lipase would bind to

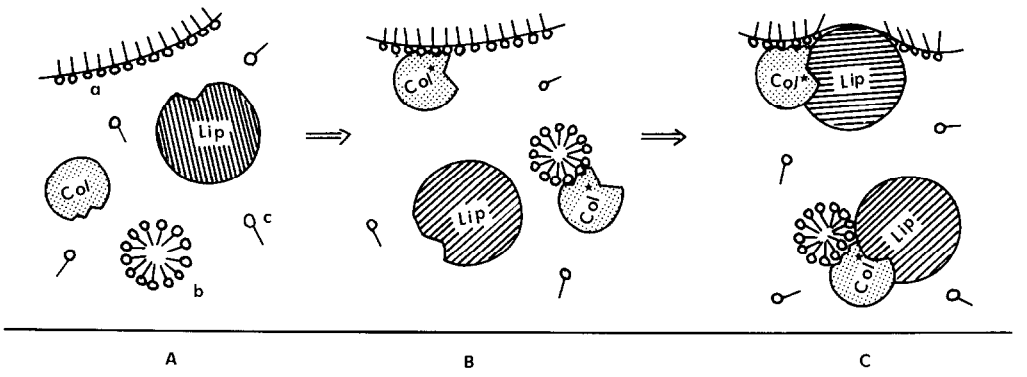

Fig.5. Tentative scheme illustrating the role of colipase for the fixation of lipase in the presence of bile salts. (a) Interface coated with detergent molecules. (b) Detergent micelle. (c) Detergent monomer. (A) When alone lipase recognizes neither the micelle nor the interface. (B) Colipase can recognize both creating a binding site for lipase. (C) Binding of lipase mediated by colipase.

the hydrophilic interface only when colipase is already adsorbed and can serve as an anchor. Since pure lipase does not recognize pure colipase [4], this means that adsorption to micelles or monolayers leads to a conformational change of colipase creating or unmasking a recognition site for lipase. This assumption illustrated by fig.5 also explains why a large excess of bile salts can, by forming more micelles competing with the substrate interface, inhibit lipolysis even in the presence of colipase.

Finally, the results obtained wtih dibutyrin offer the possibility to generalize the above considerations to any hydrophilic substrates of lipase known to be poorly attacked in the absence of colipase. A negative correlation between the hydrophilicity of the substrate (as defined by its solubility in water) and the activity of lipase has already been reported [9]. Colipase helps lipase to adsorb to hydrophobic interfaces coated with a detergent and to intrinsically hydrophilic interfaces.

Acknowledgements

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