BINDING OF PANCREATIC COLIPASE TO INTERFACES; EFFECTS OF DETERGENTS

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1. Introduction

Pancreatic lipase is an interfacial enzyme that adsorbs to the interface of its substrate [1] and to hydrophobic interfaces in general leading to an irreversible inactivation of the enzyme [2]. Bile salts desorb lipase from such interfaces thus affecting an inhibition of the enzyme due to physical separation from the substrate [1,3]. The inhibition is reversed by colipase [4] – a polypeptide cofactor – which makes possible the binding of lipase to the substrate interface in the presence of bile salts [1,3,5]. The complete system; lipase/colipase/bile salt/substrate, has an important physiological role and displays several interesting physico–chemical interactions of protein–protein and protein–detergent nature. Colipase has previously been found to bind to the lipase–substrate interface [1,3] even in the presence of bile salts guiding the enzyme to its active site. The present results, however, indicate that detergents will displace also colipase from interfaces and that a certain specificity is obviously related to the structure of the detergent and the quality of the interface. The combination of a triglyceride interface and bile salt seems unique for the binding of colipase and thus for the function of lipase.

2. Materials and methods

Lipase and colipase used in this work was of porcine origin and purified in this laboratory as previously described [6,7]. Colipase II containing $\simeq$ 100 amino acid residues and N-terminal valine was used [8]. Tributyrin was a product of BDH and purified by fractional destillation [9]. *n*-Hexadecane was purchased from BDH Chemicals, Poole, England and used without further purification. Sodium taurodexycholate and *N*-cholylglucosamine were synthesized in this laboratory by standard procedures [10] and were > 97% pure thin-layer chromatography. the nonionic detergent Nonipol TD 12 (tridecylpolyethoxyethanol with an average chain length of 12 oxyethylene units, molecular weight 728) was obtained from Rexoline Chemicals, Helsingborg, Sweden. Triton X-100 was a product of BDH Chemicals, Poole, England and used as purchased. The critical micellar concentrations for *N*-cholylglucosamine Nonipol TD 12, and Triton X-100 in 0.15 M NaCl were determined using iodine [11] and were found to be 3.8 mM, 0.036 mM and 0.17 mM, respectively. Siliconized glass beads were a gift from Dr Howard Brockman, the Hormel Institute, Austin, USA, with a surface area of 428 cm^2/g [5]. [^{131}J]colipase was prepared in this laboratory using a solid state peroxidase [12]. Colipase was iodinated to a level of approximately 0.25 mol iodine per mole colipase and retained its biological activity. The binding of colipase to tributyrin and hexadecane was determined as previously described in detail [1]. Briefly colipase was added to 10 ml buffer (150 mM NaCl, 2 mM Tris–maleate pH 7.0, 1 mM $CaCl_2$) with the appropriate concentrations of detergent. 500 μl tributyrin were added and the mixture emulsified for 1 min using a Super-mixer (Lab-Line Instruments Inc., Melrose Park, Ill., USA). The incubation mixture was then centrifuged at 1000 × *g* for 10 min and samples of the clear supernatant taken for determination of colipase activity titremetrically [1] or by measuring ^{131}J-activity in a γ-counter. Samples were run in duplicate plus and minus tributyrin. The binding was calculated from the differences. Adsorption of colipase to siliconized

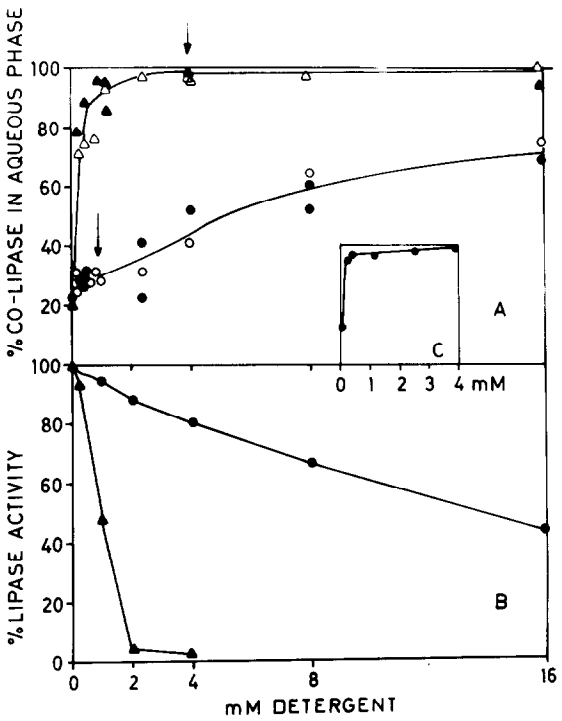

Fig.1A. Binding of pancreatic colipase to a tributyrin interface in the presence of two different detergents (●–○) taurodeoxycholate and (▲–△) *N*-cholylglucosamine. Filled symbols refer to titremetric measurements of the capacity of colipase to restore lipase activity against a tributyrin emulsion in 4 mM taurodeoxycholate [1], open symbols refer to measurements of [^{131}J]colipase in the supernatant Fig.1B. Lipase activity of the system lipase/colipase/tributyrin in the presence of varying concentrations of (●) taurodeoxycholate and (▲) *N*-cholylglucosamine [9]. The molar colipase/lipase ratio was 5:1. Fig.1C. Binding of colipase to the hexadecane interface in the presence of taurodeoxycholate of varying concentrations. The arrows refer to the critical micellar concentration for the two detergents; 1 mM for taurodeoxycholate, 4 mM for *N*-cholylglucosamine.

glassbeads was measured by adding 100 mg beads to one ml solutions of colipase in buffer or detergent solution contained in glass vials. After mixing in the Super Mixer for 1 min the beads were allowed to sediment and colipase determined from aliquots of the free solution as described above.

3. Results and discussion

3.1. *Binding of colipase to the tributyrin/aqueous interface*

The fraction of colipase ($\simeq$ 0.30) that binds to the tributyrin interface under the conditions of the experiments (0.15 M NaCl, pH 7.0) was constant over a wide concentration range (up to at least 0.2 μM). Taurodeoxycholate in low concentrations had little effect on the binding of colipase to the tributyrin interface (fig.1A). When the concentration was increased above the critical micellar concentration range ($>$ 1.0 mM) the binding decreases gradually. This contrasts to the effect of *N*-cholylglucosamine which almost completely prevents the binding of colipase to tributyrin at detergents concentration below the critical micellar concentration (fig.1A). A similar effect is also seen for the two other nonionic detergents used, Triton X-100 and Nonipol TD 12. Figure 2 includes data for Triton X-100. These results indicate a clear-cut difference between the effect of bile salts and non-ionic detergents on the binding of colipase to the tributyrin interface. Colipase binds to a hexadecane/aqueous interface to the same extent as to tributyrin but is displaced from hexadecane interface almost completely at low concentrations of taurodeoxycholate (see fig.1C). A specificity is thus displayed for the binding of colipase that is related both to the kind of detergent and to the quality of the interface.

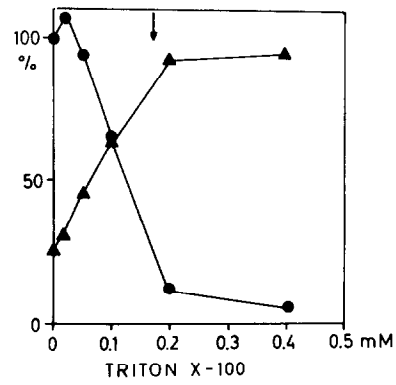

Fig.2. Effect of Triton X-100 on the % colipase in the aqueous phase of a tributyrin water emulsion (▲) and the lipase activity in the system lipase/colipase/tributyrin/Triton X-100 (●). The arrow indicates the critical micellar concentration for Triton X-100.

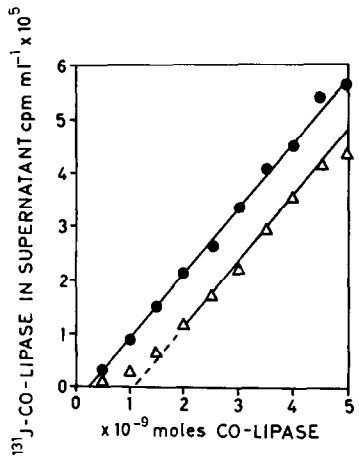

Fig.3. Binding isotherm for colipase to the interface of siliconized glass beads. Increments of 5 X 10^{-9} mol [^{131}J]-colipase were added to a total volume of 1 ml buffer in glass vials with (△) or without (●) 100 mg siliconized glass beads. The ordinate indicates the amount of radioactivity in the supernatant solution.

3.2. *Binding of colipase to siliconized glass beads*

Colipase binds to ciliconized glass beads (fig.3). The amount of colipase bound has been estimated by extrapolating to zero concentration in the supernatant solution [13]. The amount of colipase needed to saturate the interface was estimated in this way to be $25 \cdot 10^{-12}$ mol cm^2. A certain adsorption of colipase also takes place to the surface of the glass vial and subtracting this a minimum figure of $19 \cdot 10^{-12}$ mol/cm^2 is obtained. For lipase a figure of $4.0 \cdot 10^{-12}$ mol/cm^2 was reported with the same technique [13]. Chapus et al. [13] found no evidence for binding of siliconized glass beads in their investigation. A possible explanation of the difference may be that the colipase used by these authors was a partial degradation product with $\simeq$ 84 residues compared to the native colipase with $\simeq$ 100 amino acid residues used by us. Concentrations of bile salts $\geqq$ 1 mM prevent the binding of colipase to the siliconized glass beads. In this respect the siliconized glass interface is comparable to a hydrocarbon interface and does not show the specific properties of the triglyceride interface to retain colipase in bile salt solution. The property to displace albumin from an aqueous/*n*-decane interface. has previously been demonstrated for dodecyl sulfate [14] and this may be a general detergent effect. It is found in this investigation to be applicable also for the colipase/hexadecane/bile salt system as well as for colipase/tributyrin/nonionic detergents. In contrast bile salts in combination with tributyrin seems to have special properties which allows the binding of colipase to the interface.

3.3. *Relationship between the binding of colipase and lipase activity*

In the complete system lipase/colipase/detergent, lipase activity decreases with increase in detergent concentration (fig.1B). This decrease is well correlated to the fraction of colipase bound to the interface. These results indicate that the binding of colipase to the interface is an important factor determining the lipase activity in detergent solutions. A decrease in lipase activity when bile salt concentration was increased into the supra micellar range has previously been noted [15], and from kinetic data been interpreted to indicate that a binary colipase–bile salt complex is formed that acts as a competitive inhibitor for the reaction [15]. Although it has been demonstrated that bile salt binds to colipase [16,17] other interpretations of this effect are possible. The data presented in this study suggest that it is the extent of adsorption of colipase to the substrate interface that determines the lipase activity in detergent solutions and that this effect is not necessarily related to the formation of detergent micelles (the effect of the nonionic detergents is seen at concentrations below the critical micellar concentration) or to the binding of the detergent to colipase as the nonionic detergents do not bind colipase [18].

Acknowledgement

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