Hypothesis

The interaction between pancreatic lipase and colipase: a protein–protein interaction regulated by a lipid

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Pancreatic lipase readily adsorbs to a triglyceride droplet. In the intestine the triglyceride droplets are covered with bile salt and phospholipids which will prevent the adsorption of lipase. In this situation the activity of lipase is restored by colipase, another pancreatic protein. Lipase and colipase in solution form a 1:1 molar complex. I emphasize the fact that the binding and conformation of the two proteins in the complex is dependent on the type of lipids present and suggest that this lipid-determined structure of the complex is responsible for the actual function of lipase/colipase. It determines whether colipase assists lipase in binding to the bile salt-covered triglyceride droplet as is the case with tributyrin as substrate, and whether colipase in addition activates lipase as is the case with a mixed trioctanoin/lecithin monolayer substrate. In other words, lipase activity is regulated by the combined action of colipase and the lipid substrate.

Pancreatic lipase *Colipase* *Bile salt* *Phospholipid* *Triglyceride* *Activation*

1. INTRODUCTION

The key enzyme responsible for the hydrolysis of dietary fat in the intestine is pancreatic lipase. The adsorption of lipase occurs readily on a particle composed of triglycerides, but is prevented when the interface is covered with bile salt and phospholipids present in the intestine. For hydrolysis to occur in vivo the participation of another pancreatic protein, colipase, is absolutely necessary.

The mechanism for this reactivation of colipase has been reviewed [1,2]. Two different properties of colipase have been emphasized. One is the ability to bind to lipase; the other to bind to the lipid substrate. By combining these properties colipase is thought to assist lipase in its binding to the bile salt-covered substrate.

A third function, to activate lipase i.e., to increase the K_{cat} value is less clear with conflicting reports in the literature [3,4]. If these are analyzed it turns out that different types of substrates have been used to study the activation. This may be an important factor.

The binding between lipase and colipase is strongly influenced by the presence of lipids added to the solution of the proteins, increasing 100-fold with certain types of lipids [5,6]. Spectral studies have revealed that the orientation of the proteins in the complex is also dependent on the type of lipid present [7]. When these findings are coupled to the activating effect of colipase, which is also dependent on the type of lipid to be hydrolyzed, it is suggested that lipids determine the protein–protein interaction, both assuring a contact between the proteins and directing their mutual orientation. This in turn has consequences for the functional properties of the complex.

2. *Properties of lipase*

Lipase is a perfectly water-soluble globular protein with an M_r of 50000. The amino acid sequence

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is known [8]. It has a histidine in the active site, but there is no evidence of any serine involved in acylation of the enzyme [9]. A characteristic property of lipase is that it is readily adsorbed to a lipid–water interface, where the subsequent hydrolysis takes place, and at a much higher rate than on a water-soluble substrate. An 'activation' of lipase by the interface has been suggested [10]. A specific site in lipase responsible for this recognition and activation by an interface has not been found, although a serine and a Glu/Asp has been shown to play an important role [9].

In the intestine the dietary lipids, mainly triglycerides, are dispersed with bile salt and phospholipids that will cover the surface of the triglyceride droplets. In such a system lipase will no longer find its way to the triglyceride substrate but needs colipase to be active.

3. *Properties of colipase*

Colipase is another exocrine pancreatic protein. It is water-soluble, heat-stable and has an M_r of 10000. The primary structure is known [11]. It is a very compact molecule, cross-linked with 5 disulpide bridges and with 2 freely moving tails. In pancreatic juice colipase exists in a proform, procolipase, which is activated by trypsin in the intestine, whereby the two ends of colipase are shortened. The colipase molecule formed together with lipase will effect a rapid and efficient hydrolysis of a mixed triglyceride–phospholipid substrate, as is found in the intestine [5,12].

4. *Binding of lipase to colipase*

Lipase and colipase in aqueous solution form a 1:1 molar complex with a dissociation constant of the complex equal to 10^{-6} M [13,14]. The binding is both electrostatic and hydrophobic (fig.1a). The electrostatic binding is provided by Glu_{15} on colipase [15] and a lysine in lipases [16], the position of which is unknown. The hydrophobic component in the binding, probably unspecific, prevents the dissociation of the proteins at high concentrations of salt [14]. On the other hand detergents will interfere with the hydrophobic component and decrease the binding between the two proteins, the dissociation constant in the presence of bile salt micelles being 10^{-5} M (fig.1b) [13,14].

The binding between lipase and colipase is influenced by the presence of lipids in different ways. Butyric acid has no influence on the binding, a medium-chain fatty acid, decanoic acid, increases the binding ($K_d = 10^{-7}$ M) and a long-chain fatty acid, oleic acid, will increase the binding 100-fold, the dissociation constant being 10^{-8} M in the presence of mixed oleic acid–bile salt micelles [5].

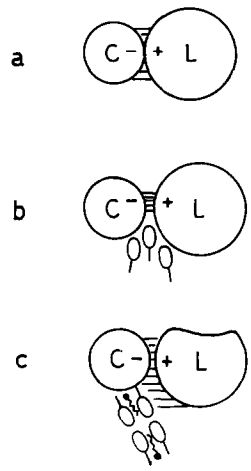

Fig.1.(a) The complex of lipase and colipase in buffer: (− +) electrostatic binding; (≡) hydrophobic binding; C, colipase, L, lipase. (b) The complex of lipase and colipase in micellar bile salt solution. Bile salt will bind to lipase and colipase and disturb the hydrophobic component in the lipase–colipase binding: (♀) bile salt. (c) The complex of lipase and colipase in a mixed oleic acid–bile salt micellar solution. Oleic acid–bile salt will bind to colipase which will then attract lipase with a greater affinity thanks to the acyl chain(s) added to the hydrophobic component in the lipase–colipase binding. At the same time a conformational change occurs with the exposure of a tryptophane residue of lipase: (ʃ) oleic acid.

With other lipids dispersed in bile salt, such as monoolein and lecithin, the binding between lipase and colipase is increased 10-fold and 3-fold, respectively ($K_d = 10^{-7}$ M and 3×10^{-6} M) (unpublished). It appears that the products of lipolysis have a greater influence on binding between lipase and colipase than the substrate.

The increased binding between lipase and colipase observed in the presence of lipids can be understood as an enlargement of the hydrophobic component in the lipase–colipase binding. In the case of oleic acid–bile salt the situation has been analyzed. Oleic acid–bile salt micelles will first

bind to colipase which will then attract lipase with a great affinity. Lipase alone does not interact with oleic acid–bile salt, but only in the presence of colipase (fig.1c), (unpublished).

5. *Conformational changes of lipase and colipase on binding*

Experiments with lipase and collipase in buffer show that on binding the proteins influence each others' conformation as seen by spectroscopic methods [7]. In the presence of bile salt micelles there was no indication of any conformational change on lipase-colipase binding. However, in the presence of mixed oleic acid–bile salt micelles the interaction between lipase and colipase clearly gave a change in conformation of the proteins involving the exposure of a tryptophan residue of lipase [7]. This has been confirmed by modifaction studies of tryptophan using *N*-bromosuccinimide (in preparation).

Two phase partition studies showed that in buffer the partition coefficient of the lipase–colipase complex was the product of the partition coefficients of lipase and colipase, respectively [14], which indicated a small contact area between the two proteins [17]. In contrast, in the presence of oleic acid–bile salt micelles the partition coefficient of the complex deviated considerably from that of the product of the partition of coefficients of the two proteins indicating a larger contact area or change in exposed surface (fig.1c) [5].

6. *Activity of lipase-colipase*

While lipase has virtually no activity on a bile salt-dispersed lipid substrate, the activity is totally restored by addition of colipase, always following a certain lag period. This lag period is very short for the short-chain triglycerides while for the long-chain triglyceride substrates, especially when mixed with phospholipids, it is considerable. In this latter case the lag period of procolipase is shortened by addition of trypsin (cutting off the ends of colipase) or by addition of long-chain fatty acids [18]. In analyzing the activating effect of colipase it is quite clear that one important function of colipase is to assist the binding of lipase to the bile salt-covered substrate. In this binding the positive charges of colipase are obligatory probably by forming electrostatic bonds to the negatively charged bile salt-covered substrate [15]. Lipase is attached to colipase either in solution or at the surface of the lipid droplet, depending on the type of substrate. In the case of a mixed triacylglycerol-phospholipid substrate the binding of the complex to the substrate needs support by lipid-binding sites, both in colipase and lipase, $Ile_{7\text{-}9}$ in the N-terminal end and the Tyr_{54}-Gly-Val-Tyr-Tyr_{58}-peptide segment in colipase and at least a tryptophan residue in lipase [7]. These are more exposed after tryptic cleavage of procolipase.

What is not clear is if colipase, in addition to the anchoring effect on lipase, also activates lipase. In studies using tributyrin as substrate there was no influence of colipase on the k_{cat}-value of lipase [4]; however, with a mixed trioctanoin–lecithin monolayer substrate there was a clear 4-fold increase in V_{max} after the addition of colipase [3].

The apparently conflicting results can be explained, in my hypothesis, by the fact that different substrates have been used, as the substrate will also influence the binding and conformation of the proteins. Thus, with tributyrin as substrate, there is no influence on binding between the proteins [6] or any conformational change to be expected from the released butyric acid which will rapidly diffuse out into the water phase, without infering with lipase–colipase at the surface. Hence, there is no activation of lipase by colipase in this case. On the other hand, with a mixed trioctanoin–lecithin substrate, there is a large increase in

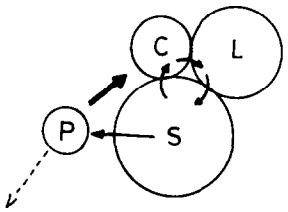

Fig.2. Circular dependence of the components in lipase–colipase–bile salt–lipid substrate system. The bile salt dispersed lipid (S) binds to colipase (C), which leads to an increased binding to lipase (L) and a change in conformation of lipase, which in turn acts to hydrolyze the lipid substrate with a certain rate according to its attained conformation. If the products (P) (fatty acids) are more effective in influencing the lipase–colipase interaction than the substrate (triglycerides) the hydrolysis is accelerating, the reason why a lag phenomenon is commonly observd in the lipase–colipase catalyzed reaction.

binding and observable conformational changes of the proteins in the complex induced by the released longer-chain fatty acids possibly also by lecithin. This can explain the observed activation of lipase by colipase in this situation.

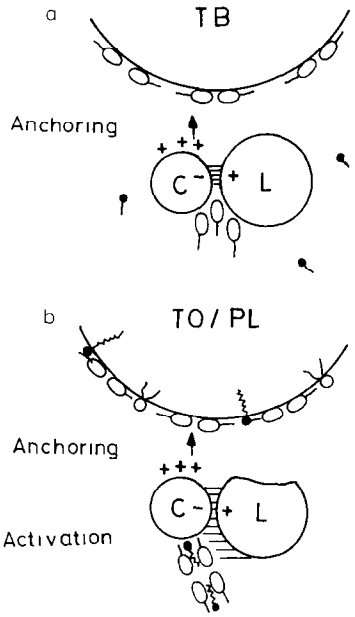

Fig.3. The lipase-colipase complex on different substrates in the presence of bile salt; C, colipase; L, lipase. (a) Tributyrin (TB): Colipase mediates binding of the complex to the substrate with its positive charges (⚲) butyric acid; (♀) bile salt. As the released butyric acids do not interfere with lipase-colipase interaction there is no observable lag time and no activation of lipase by colipase. Colipase effect: anchoring of lipase; no activation of lipase. (b) Trioctanoin-lecithin (TO/PL): Colipase iniates binding of the complex to the substrate with its positive charges but also induces a conformational change in lipase which is much increased by the long-chain fatty acids released, possibly also by the phospholipids in the substrate, leading to exposure of lipid binding sites in lipase. These together with lipid binding sites in colipase exposed by trypsin cleavage of the N-terminal and C-terminal ends of colipase are necessary for a complete and rapid binding of the complex to the mixed TG/PL substrate. As the released fatty acids interfere with and increase lipase-colipase interaction, more than the TO/PL substrate there is a lag time before hydrolysis of the substrate is maximal. In this case there is also an activation of lipase by colipase-octanoic acid (⚲); bile salt (♀). Colipase effect: anchoring of lipase; activation of lipase.

An important point is that colipase must be present in order to detect any conformational change on lipase induced by bile salt-dispersed lipids and for lipase to hydrolyze these micellar lipids at measurable rates. The activation of lipase is thus an effect both of substrate and of colipase in a circular way as depicted in fig.2.

In summary, the binding between lipase and colipase does not lead to a fixed complex always acting in a certain way. Instead the binding is modulated by the presence of lipids, different types of lipids having different effects. Following binding there are various possibilities for conformational changes and orientation of the two proteins; the structure of the complex determined by the type of lipids present, is then responsible for the function and activity of the complex (fig.3).

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