Conformational change in pancreatic lipase induced by colipase

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1. INTRODUCTION

Pancreatic lipase is a key enzyme for the intraluminar digestion of fats. The adsorption of lipase to the substrate which occurs readily when the particles are composed of pure triacylglycerol is prevented by the accumulation at the interface of various amphiphiles like bile salt and phospholipids present in the duodenum during fat digestion. In the presence of pancreatic colipase the activity of lipase is, however, fully restored. The exact mechanism for this reactivation is not known. Two different mechanisms have been suggested. The first considers colipase as an 'anchor' for lipase at the hydrophilic interface. In a series of sequential steps colipase will alone recognize and bind to the interface and from there bind to lipase thus anchoring the enzyme back to its triacylglycerol substrate [1,2]. The other claims that none of the proteins could bind to the interface but that lipase and colipase form a complex with bile-mixed micelles in solution. This complex is then adsorbed as a single unit by virtue of the bile lipids to the triacylglycerate interface [3].

Lipase is known to form a 1:1 molar complex with colipase [4]. The binding which is both electrostatic and hydrophobic has a dissociation constant of 5×10^{-7} M in buffer [5]. In bile salt solution the binding is weaker ($K_d = 3 \times 10^{-6}$ M). However, in the presence of mixed oleic acid–bile salt micelles the binding is strongly increased [6] with a K_d of 10^{-8} M [7].

In the lipase/colipase binding there has been no indication for any conformational change when the interaction has been studied in buffer or in bile salt solution as indicated by CD measurements [4]. This may, however, be due to weak binding between the two proteins under these conditions which makes necessary the use of concentrated solutions to assure a binding. Recently, a covalent complex of lipase and colipase was obtained by use of a crosslinking agent [8]. This complex had binding properties to a mixed phospholipid–triacylglycerol interface that could not be explained as an effect of colipase alone but suggested that colipase had somehow induced a conformational change in lipase enabling lipase to take part in the substrate binding.

This study shows that colipase can induce a conformational change in lipase seen as an ultraviolet spectral shift of the complex compared to the single proteins. The change was ~5-fold greater in the presence of mixed oleic acid–bile salt micelles. At pH 5, the addition of bile salt to lipase alone also induced a spectral shift. The mechanism for colipase reactivation of lipase is discussed in the light of the above experiments.

2. MATERIALS AND METHODS

Porcine pancreatic lipase was purified as in [9] with an additional Sephadex G-100 filtration at pH 9 to remove the last traces of remaining colipase. Porcine pancreatic procolipase with 101 amino acids was purified according to [10]. Activated colipase, colipase$_{85}$ was obtained by trypsin treatment [7]. Taurodeoxycholate was synthesized in the laboratory and was >98% pure as indicated by thin-layer chromatography. Ultraviolet difference spectra were recorded at room temperature in double compartment cuvettes using an Aminco spectrophotometer Model DW-2 working in split-beam mode and with a slit of 1 nm.

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3. RESULTS

3.1. *Binding of lipase and colipase$_{85}$ in the presence of mixed oleic acid–bile salt micelles*

The absorption spectrum of lipase is shown in fig.1A. Also shown is the spectrum obtained when a mixture of lipase and colipase$_{85}$ were measured againt colipase$_{85}$. If no interaction had occured between the proteins the two spectra would be expected to coincide. However, the latter spectrum showed a shift to higher wavelength. This shift could be shown more clearly by comparing the absorption spectrum of colipase alone with that obtained by measuring the mixture of lipase and colipase$_{85}$ against lipase (fig.1B). Upon subtracting the spectrum of colipase$_{85}$ from the 'mixed' spectrum in fig.1B the difference spectrum of fig.1C was obtained. This spectrum represents the absorption change induced by mixing lipase and colipase$_{85}$. The difference spectrum shows a maximum at 292 nm and a smaller maximum at 287 nm with a shifting point at 286 nm. The shift was calculated to be ~0.5 nm.

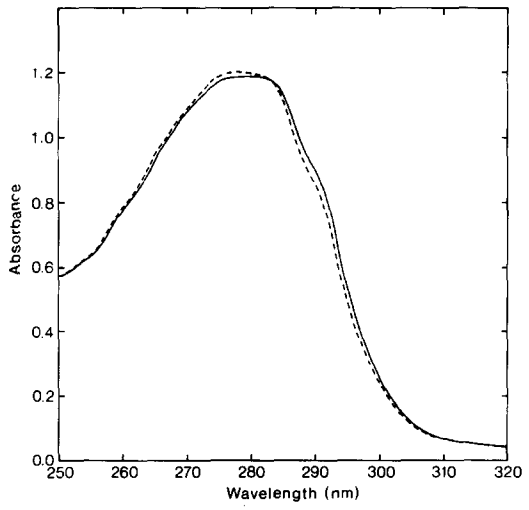

Fig.1A. Absorption spectrum of 2×10^{-5} M lipase in 0.01 M Tris–HCl buffer (pH 7) containing 1 mM oleic acid and 2 mM taurodeoxycholate; (---) the reference cell contained buffer with oleic acid and taurodeoxycholate. Spectrum of 2×10^{-5} M lipase in the presence of 2×10^{-5} M colipase$_{85}$ in the same buffer as above (——); the reference cell contained 2×10^{-5} M colipase without lipase (in the same buffer as above).

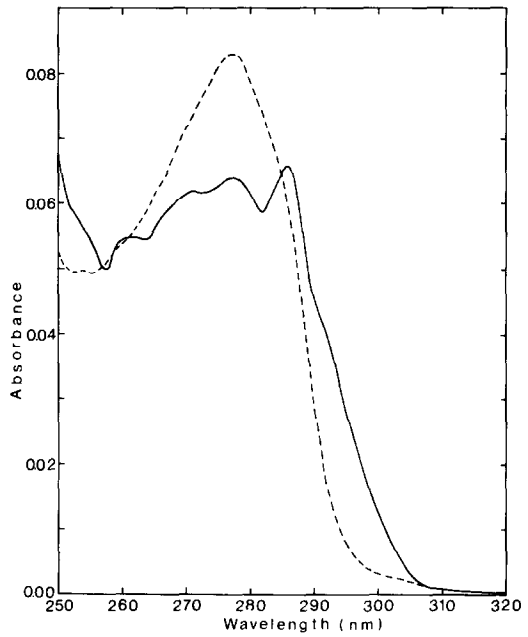

Fig.1B. Absorption spectrum of 2×10^{-5} M colipase$_{85}$ in 0.01 M Tris–HCl buffer (pH 7) containing 1 mM oleic acid and 2 mM taurodeoxycholate (curve A) (---); the reference cell contained the buffer only. Spectrum of 2×10^{-5} M colipase$_{85}$ in the presence of 2×10^{-5} M lipase in the same buffer as above (——) (curve B); the reference cell contained 2×10^{-5} M lipase without colipase.

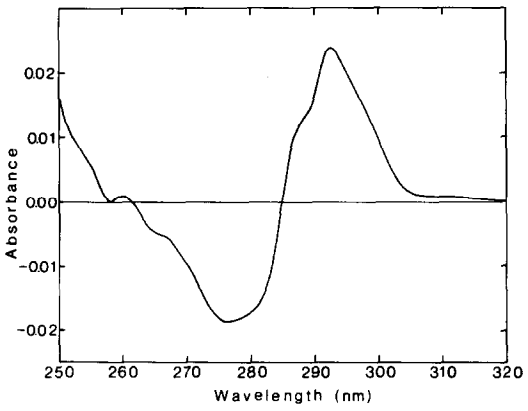

Fig.1C. Difference absorption spectrum calculated as the difference between colipase in complex (curve B, fig.2b) and colipase alone (curve A, fig.2b).

Table 1

The arrangement and result of the spectral recordings of lipase (2×10^{-5} M) in 0.01 M Tris–HCl buffer (pH 7), 0.01 M acetate buffer (pH 5) with and without 4 mM taurodeoxycholate

Sample cuvette	Reference cuvette	Difference spectrum
Buffer (pH 7)	Buffer (pH 5)	Shift to longer wavelength (0.25 nm)
Buffer (pH 7)	Bile salt (pH 7)	No shift
Buffer (pH 5)	Bile salt (pH 5)	Shift to longer wavelength (0.13 nm)
Bile salt (pH 7)	Bile salt (pH 5)	Shift to longer wavelength (0.63 nm)

3.2. *Binding of lipase to colipase$_{85}$ in buffer and bile salt*

When the same type of measurements were performed in the absence of mixed oleic acid-bile salt micelles a much smaller shift was observed, as shown by the difference spectrum in fig.2. Moreover, the shift of −0.1 nm was to shorter wavelength, with a shifting point at 293 nm and a maximum at 287 nm. In the presence of bile salt there was no sign of any spectral change that could indicate an interaction between lipase and colipase.

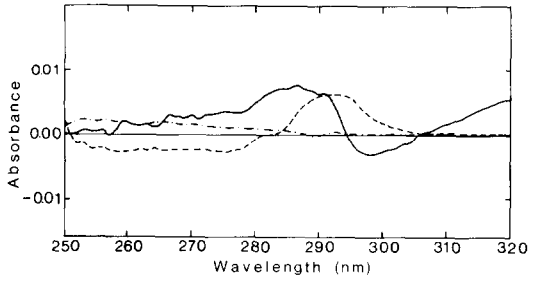

Fig.2. Difference absorption spectra calculated as demonstrated in fig.1 for (a) lipase + colipase$_{85}$ in 0.01 M Tris–HCl buffer (pH 7) (——); (b) lipase + colipase$_{85}$ in 0.01 M Tris–HCl buffer (pH 7) containing 4 mM taurodeoxycholate (–·–·–); (c) lipase + colipase$_{101}$ in 0.01 M Tris–HCl buffer (pH 7) (---).

3.3. *Binding of lipase and colipase$_{101}$ in buffer*

In fig.2 is shown the difference spectrum for lipase interacting with colipase$_{101}$ in 0.01 M Tris-HCl buffer (pH 7) at 2×10^{-5} M. In contrast to colipase$_{85}$ a shift to longer wavelength of the order of 0.2 nm was observed with a shifting point at 282 nm.

3.4. *Difference spectra of lipase at pH 7 and 5 in the presence and absence of bile salt*

As demonstrated in table 1 and fig.3, lipase when passing from pH 5 to pH 7 exhibited a difference spectrum with a shift to longer wavelength. A minimum was observed in the 250–260 nm range and a broad maximum between 280–290 nm. A similar but more marked difference spectrum was observed when the same experiment was run in the presence of bile salt. Here, two maxima at 287 nm and 295 nm were noted. The addition of bile salt at pH 7 did not give any spectral shift of lipase whereas at pH 5 addition of bile salt gave a shift to longer wavelength with a maximum at 300 nm and a shifting point at 273 nm.

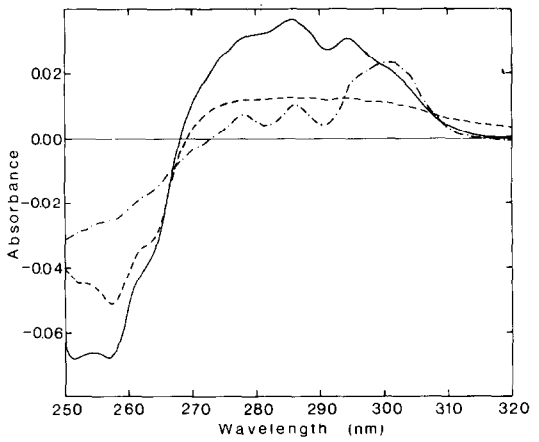

Fig.3. Difference spectra of 2×10^{-5} M lipase in 0.01 M Tris–HCl buffer (pH 7) in sample cuvette and 2×10^{-5} M lipase in 0.01 M acetate buffer (pH 5) in reference cuvette (---); lipase in 0.01 M Tris–HCl buffer (pH 7) with 4 mM taurodeoxycholate in sample cuvette and lipase in 0.01 M acetate buffer (pH 5) with 4 mM taurodeoxycholate in reference cuvette (——); lipase in 0.01 M acetate buffer (pH 5) in sample cuvette and in 0.01 M acetate buffer (pH 5) with 4 mM taurodeoxycholate in reference cuvette (_.._.).

4. DISCUSSION

This study shows that the interaction of colipase with pancreatic lipase leads to an ultraviolet spectral shift in the pattern of the complex as compared to the single proteins. This shift was most obvious when lipase and colipase interaction took place in a mixed oleic acid–bile salt micellar solution (fig.1). The shift was to longer wavelength, generally interpreted as the chromophore being transferred into a more hydrophobic milieu [11]. The difference spectrum possessed two maxima at 292 and 287 nm which by comparison with model compounds suggests the involvement of a tryptophan residue [12]. If this is true, part of the conformational change observed has orginated from lipase as porcine colipase lacks tryptophan residues. The shoulder at 287 nm may well be a contribution from one or more tyrosine residues, either from lipase or colipase.

In buffer at pH 7 the spectral shift observed was small and appeared different from that of lipase/colipase interacting in the presence of mixed oleic acid–bile salt micelles. No clear identification of the chromophore(s) perturbed was possible. In bile salt solution there was no detectable spectral shift confirming earlier results [4].

Interestingly enough, the interaction of procolipase$_{101}$ with lipase in buffer led to a spectral shift of similar magnitude as for the trypsin-activated colipase$_{85}$ but was in the opposite direction. Binding studies have shown a similar dissociation constant (5×10^{-7} M) for the interaction of lipase with colipase$_{101}$ and colipase$_{85}$ [7]. The conformational changes in their interactions with lipase then obviously are not the same; the shift to shorter wavelength for colipase$_{85}$ indicates a transfer to a more hydrophilic 'exposed' milieu for the changing chromophore, while the shift to longer wavelength for colipase$_{101}$ indicates a transfer to a more hydrophobic 'hidden' milieu for the chromophore. This latter binding may represent a 'non-productive' form of the lipase/colipase complex.

As lipase by itself without colipase has a hydrolytic activity against tributyrin in the presence of bile salt at pH < 5.5. [13] it was interesting to find that lipase in the presence of bile salt exhibited a clear spectral shift by passing from pH 7–5, thus indicating a conformational change. This spectral shift was both an effect of lowering the pH and an effect of adding bile salt at pH 5. At pH 7 the addition of bile salt did not change the lipase ultraviolet spectrum. The important question, whether the conformational changes observed here are the same as occur in the interaction of lipase with colipase could not be answered definitely. The changes in lipase spectrum by lowering the pH and by adding bile salt occurred both at lower (250–260 nm) and at higher (280–290 nm) wavelengths, whereas the changes observed in lipase interacting with colipase were mainly at higher wavelengths (280–290 nm). In the latter case there could also be a contribution from colipase in the spectral shifts observed.

The above results have lead us to the following model for colipase reactivation of lipase in the intestine. Lipase and procolipase are produced as separate proteins in pancreas. Possibly they could already form a potentially harmless complex in pancretic juice where they are present at 2×10^{-6} M. In the intestine the pancreatic proteins are mixed with fatty acid-bile salt mixed micelles, the fatty acids being formed by the action of lingual lipase on dietary triacylglycerols in the stomach. Colipase$_{85}$ is formed by limited trypsin hydrolysis. This colipase induces a conformational change in lipase leading to the exposure of a hydrophobic site involving a tryptophan residue. This site perhaps together with the lipid binding sites of colipase, Tyr$_{56-57}$ [14] and Ile$_{7-9}$ [15] may form a common binding site for lipase/colipase, driving the complex to the interface. The 100-fold increased binding of lipase and colipase observed in the presence of oleic acid (and also other lipids) may be understood as a stabilization by hydrophobic interaction of the new conformation of lipase/colipase occurring in the complex formation leading to an exposed hydrophobic chromophore.

The analogy to pancreatic phospholipase A_2 with the involvement of a tryptophan$_3$ in the binding to lipid micelles is recalled [16].

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