INTERACTION OF PANCREATIC COLIPASE WITH A BILE SALT MICELLE.

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Summary : Porcine pancreatic colipase was found by ultracentrifugation, dialysis and spectrophotometry to bind one NaTDC micelle per molecule. This binding is the first step of the formation of a ternary lipase-colipase-bile salt complex enabling lipase to act on long chain triglycerides in the presence of bile salts.

Colipase, a small protein (molecular weight, about 9000) synthesized by pancreas, prevents the inhibition of the *in vivo* lipase-induced hydrolysis of dietary triglycerides by physiological concentrations of bile salts (1). This effect has been assumed to imply in the first place a dimerization of colipase and the fixation of the dimer to lipase (2).

The purpose of the present work is to show by ultracentrifugation, dialysis and spectrophotometry that the first step of the colipase effect actually is the binding of one bile salt micelle to the cofactor. This binding is assumed to create in colipase a special site for the subsequent association with lipase.

MATERIAL AND METHODS

Colipase I, which contains 10 residues more than colipase II in the C-terminal region (3), was prepared as previously described (4), except for the SP-Sephadex column which was eluted by a 3.0-4.2 pH gradient in 0.25 M NaCl. Commercial NaTDC (Sigma) was found 95 % pure by thin layer chromatography. Its CMC deter-

Unusual abbreviations : NaTDC, sodium taurodeoxycholate ; CMC, critical micelle concentration.

mined spectrophotometrically was 1.0 mM at 20° C in a 0.1 M Tris-HCl buffer pH 8.0 containing 0.1 M NaCl and 1 mM $CaCl_2$ (buffer I). It attained 1.6 mM when NaCl was 0.25 mM (buffer II). Ultracentrifugation assays were performed at 20° C in a Spinco-Beckman analytical ultracentrifuge Model E equipped with temperature and speed control. The equilibrium technique of Yphantis was used, except in the case of micelles for which the low speed method of Van Holde and Baldwin (5) was preferred. Partial specific volumes were derived from density and concentration determinations. Densities were determined at 20 $\pm$ 0.01° C for varying solute concentrations with an Anton Parr digital microdensimeter Model DMA 02C. It was checked that extrapolation to 0 concentration exactly corresponded in each assay to the density of the pure solvent. Concentrations were measured interferometrically in the ultracentrifuge with a Wratten filter N° 77A (5461 Å). UV difference spectra were recorded at 20 $\pm$ 0.5° C in a Cary spectrophotometer Model 14. Dialysis assays were carried out in a multicell Teflon microdialyzer (6).

RESULTS

The sedimentation coefficient of pure colipase dissolved in buffer I is independent from the concentration of the protein and equal to 1.35 S. This value increases to 2.2 S in the presence of 4 mM NaTDC in the same buffer. Fig. 1 indicates that the transition between the 2 values takes place just after the CMC of the salt in the same buffer (1.0 mM). No detectable sedimentation coefficient change was observed with sodium taurocholate, a trihydroxy salt known (7) to form much smaller micelles than NaTDC (2400 daltons instead of 11,500 under our conditions). These findings are consistent with the view that colipase binds a micelle of bile salt.

This view was considerably strengthened by weight determinations at varying ionic strength. Considering that the size of NaTDC micelles is ionic strength dependent (7), it could be expected that the weight of the associations also varied with this parameter and was in each case equal to the sum colipase + micelle. The values obtained in the course of this work for the weight of

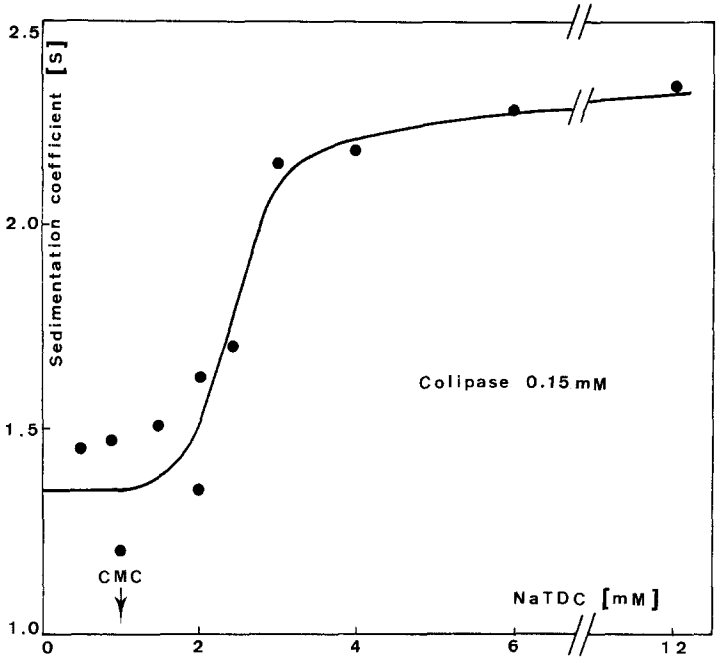

Figure 1 : NaTDC concentration dependence of the sedimentation coefficient of colipase. The concentration of colipase was 0.15 mM in buffer I.

NaTDC micelles were in substantial agreement with those previously reported by Small (7) using picnometry for density measurement. The determination of the weight of the colipase-bile salt associations was more difficult, due to poor equilibrium after the dialysis normally preceding ultracentrifugation. Therefore, colipase was simply dissolved and the concentration of bile salt in the solvent was reduced in order to compensate the amount bound to colipase in the solution. Table I shows that the weight of the associations increases as expected with ionic strength and they are, for each ionic strength value, very close to those calculated by summation of 1 colipase molecule and 1 micelle.

Other proofs of the binding of a micelle to the cofactor were obtained by dialysis in the presence of $[^{35}S]$-labelled NaTDC and spectrophotometry. Although equilibrium could not be reached (see above), dialysis assays clearly indicated

Table 1

Weight of the colipase-NaTDC associations at varying ionic strengths.

The colipase concentration was 0.15 mM in all assays.

Concentration (mM)		Weight of NaTDC micelles	Weight of the colipase-NaTDC associations	
NaCl	NaTDC		Calculated for 1 colipase molecule and 1 micelle	Measured
10	6	3 000	12,800	12,700
50	6	5 900	15,700	16,600
100	6	10 100	19,900	20,500
150	12	11 500	21,300	22,300

that NaTDC was fixed by colipase and that the fixation exclusively occurred above the CMC. Spectrophotometric assays were facilitated by the fact that colipase contains no tryptophan, but a cluster of 3 tyrosines (Tyr 48, 51 and 52 ref. 3)). Fig. 2a shows that micelle binding induces a strong tyrosine perturbation (about 20 % of the corresponding total absorbance). When the maximal absorbance difference at 286 nm was plotted against NaTDC concentration, typical saturation curves similar to those reproduced in fig. 2b were obtained. No response was again observable below the CMC. Moreover, as shown by Sari et al (8), any couple of points arbitrarily taken on one of the 3 curves of fig. 2b offered the possibility to calculate that the number of receptors per colipase molecule did not exceed 1. The dissociation constant of the colipase-bile salt complex was found to be 1.5×10^{-3} or 1.0×10^{-4} when expressed as usual in molarity or, more logically in this case, in micelle concentration.

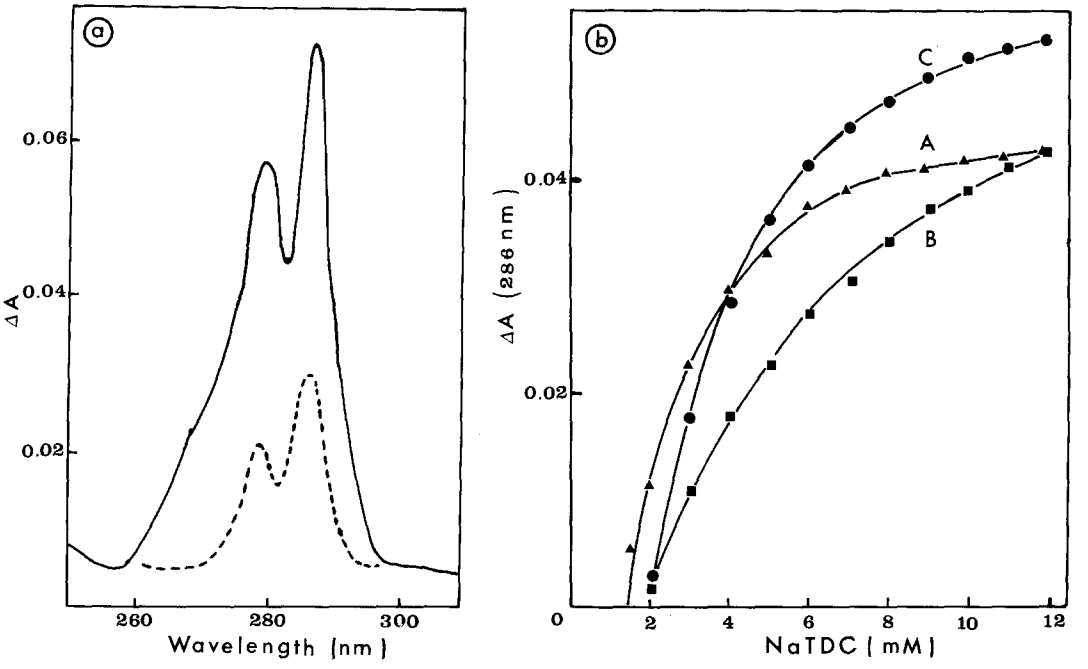

Figure 2 : Fixation of a bile salt micelle to colipase as studied by spectrophotometry. Fig. 2a : Solid line, difference spectrum of colipase (0.140 mM in buffer I) in the presence of 6.0 mM NaTDC. Interrupted line, tyrosine (0.352 mM in buffer I) with 20 % ethylene glycol. Fig. 2b : Absorbance difference of colipase at 286 nm as a function of NaTDC concentration. Curve A, 0.080 mM colipase in buffer I. Curves B and C, 0.077 and 0.120 mM colipase in buffer II. CMC, 1.0 mM in buffer I and 1.6 mM in buffer II.

DISCUSSION

A generally accepted concept is that proteins bind detergent monomers rather than micelles (9). By contrast, the data reported above are consistent with the formation of well defined associations between 1 molecule of colipase and 1 micelle of bile salt. These associations may be expected to result largely from polar interactions if preformed micelles with a polar surface are bound. However, the participation of hydrophobic forces cannot be ruled out if several monomers are attached sequentially.

The strong tyrosine perturbation (fig. 2a) indicating passage from a more hydrophilic to a more hydrophobic environment may be due to the proximity of the bound micelle or to a conformational change perhaps creating in colipase a

special site for the subsequent fixation of lipase. Lipase is already known to be included in larger complexes (4, 10, 11) which can now be assumed to contain 1 enzyme molecule, 1 colipase molecule and 1 micelle (11). These complexes represent an interesting model for protein-protein interaction mediated by an organized lipid. A part of these results have already been published in abstract form (12).

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