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Research Article

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Hydrolysis of Milk Fat Globules by Pancreatic Lipase

ROLE OF COLIPASE, PHOSPHOLIPASE A₂, AND BILE SALTS

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ABSTRACT Human milk fat globules require colipase to be hydrolyzed by pancreatic lipase in the presence of bile salts. This is contrary to a recent report in this Journal (*J. Clin. Invest.* 67: 1748-1752.) according to which inhibition of lipase by bile salt could be overcome by the addition of colipase or phospholipase A₂. This latter finding is shown to be due to contamination of commercially available pancreatic phospholipase A₂ by colipase.

INTRODUCTION

In a recent paper in this Journal (1) "a major new observation" was reported "that bile salts even at high concentration stimulated triglyceride hydrolysis of phospholipase-treated milk fat globules by pancreatic lipase also in the absence of colipase." These results are contradictory to the present concept regarding the function of the lipase/colipase/bile salt system according to which bile salts inhibit lipase activity by desorbing it from its substrate interface; a contact that is reestablished by colipase (2). The observation reported (1) can be explained by the occurrence of colipase as a contamination of phospholipase A₂ used at high concentrations in these experiments but not reported on. In the following it is demonstrated that pancreatic phospholipase A₂ purified according to standard procedures contains colipase at a level sufficient to fully explain the results obtained, and that phospholipase A₂ further purified does not stimulate the hydrolysis of milk fat triglycerides by lipase in the presence of bile salts.

METHODS

Porcine pancreatic lipase (3) and colipase₉₆ (4) with N-terminal glycine were prepared in this laboratory. Pure pancreatic phospholipase A₂ was obtained as a gift from Professor G. de Haas, Utrecht, Holland (PLA₂-U).¹ Pancreatic

phospholipase A₂ was also purified in this laboratory according to Nieuvenhuizen et al. (5) from a preparation of porcine pancreas obtained from Novo, Copenhagen (PLA₂-ML). A commercial preparation of pancreatic phospholipase A₂ was purchased from Sigma Chemical Co., (lot 41F-0427) (St. Louis, MO) called PLA₂-Σ. Tributyrin was a product of BDH (BDH Chemicals Ltd., Poole, England) passed through a column of Al₂O₃ to remove fatty acids and lower glycerides. Taurocholate (TC) and taurodeoxycholate (TDC) were synthesized in the laboratory and >97% pure.

Milk fat globules were prepared from fresh human milk by centrifugation as described by Bläckberg et al. (1). Triglyceride hydrolysis was measured by titration in a pH-stat (4). Lipase activity is expressed as micromoles of fatty acid released per minute and milliliter of incubation mixture (6). The incubation volume was 10 ml and contained 150 mM NaCl, 20 mM Ca⁺⁺, 5 mM TC, and 1 mM Tris-HCl pH 7.0 at 37°C. 400 μl human fat globules were added as substrate. The final concentration of lipase was 0.3 μg ml⁻¹ and of phospholipase 10 μg ml⁻¹. Colipase was added as indicated. The colipase content of the PLA₂ preparation was determined by pH-stat titration at pH 7.0, 20°C in a buffer containing 150 mM HCl, 1 mM Ca⁺⁺, 1 mM Tris-maleate, and 4 mM TDC with 500 μl tributyrin as substrate in a final volume of 10 ml. Lipase was added to a final concentration of 0.2 μg ml⁻¹. Under these conditions a linear relationship is obtained for colipase up to 0.02 μg ml⁻¹ incubation.

RESULTS

When human milk fat globules were incubated with bile salt and buffer as described and lipase was added (0.3 μg ml⁻¹) fatty acids were released at a rate ≈ 0.012 μmol min⁻¹ ml⁻¹. None of the PLA₂ preparations (10 μg ml⁻¹) released measurable quantities of fatty acids (except for neutralization of PLA₂-Σ which contains ammonium sulfate). The effect of adding lipase after PLA₂ varied with the PLA₂ source. Fig. 1A and B gives representative tracings when lipase was added after a preincubation of the fat globules for 3 min with PLA₂-U and PLA₂-Σ, respectively. After preincuba-

pared in our laboratory by M.L., and obtained from Sigma Chemical Co., respectively; TC, taurocholate; TDC, taurodeoxycholate.

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¹Abbreviations used in this paper: PLA₂-U, PLA₂-ML, and PLA₂-Σ, phospholipase A₂ obtained from Utrecht, pre-

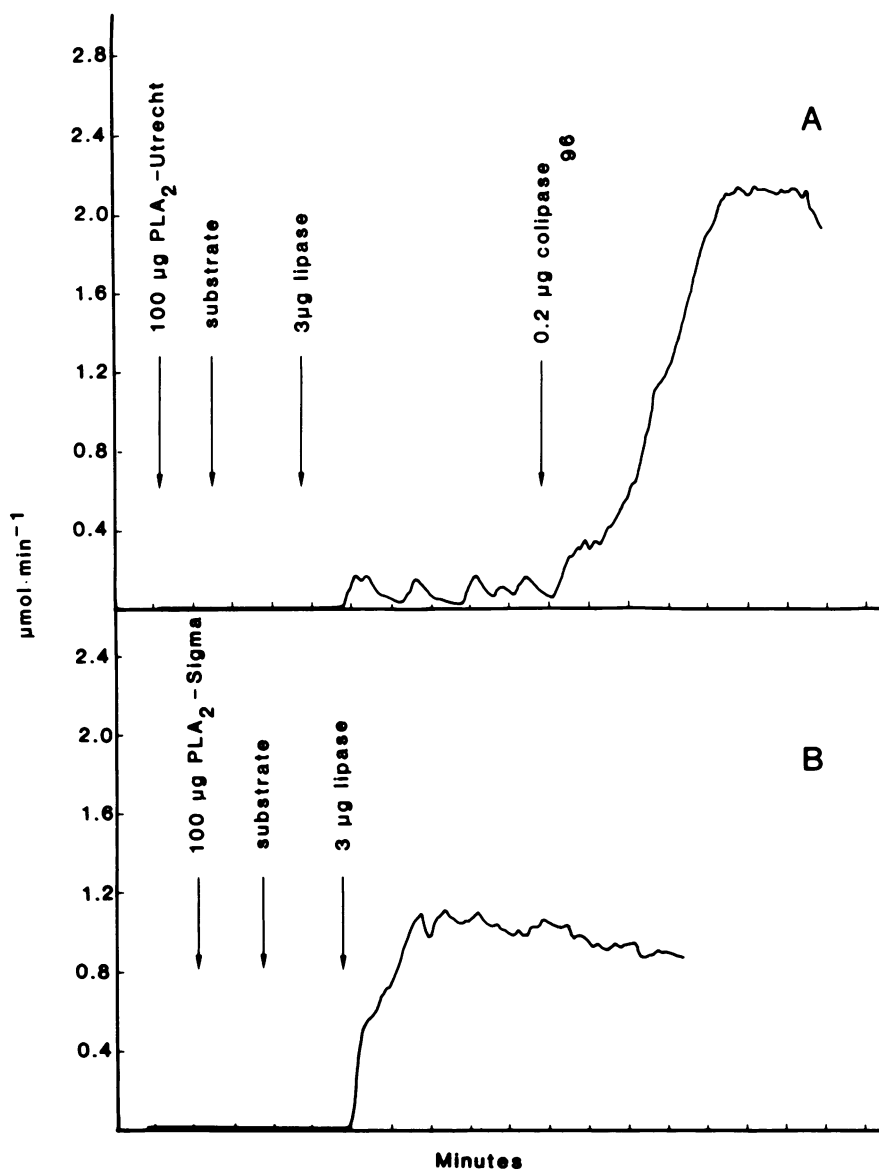


FIGURE 1 Tracings of two experiments illustrating the effect of pancreatic lipase on human milk fat globules preincubated with PLA₂-U (A) and PLA₂-Σ (B), respectively. In A, colipase was added following lipase. Total volume of incubation 10 ml, pH 7.0, 37°C, 20 mM Ca⁺⁺ and 5 mM TC. The curves represent the rate of fatty acid release in micromoles per minute.

tion with PLA₂-U the rate of fatty acid release caused by lipase was maximally 0.016 $\mu\text{mol min}^{-1} \text{ml}^{-1}$, only slightly above the blank for lipase. With PLA₂-Σ the rate was 0.11 $\mu\text{mol min}^{-1} \text{ml}^{-1}$. When 0.02 $\mu\text{g colipase}_{96}$ was added per milliliter incubation medium the rate was 0.208 $\mu\text{mol min}^{-1} \text{ml}^{-1}$.

From these figures the colipase content of PLA₂ can be calculated to be $0.11/0.208 \cdot 0.02 = 0.011 \mu\text{g}/10 \mu\text{g PLA}_2$ or 0.11%. Direct determination of the colipase content of the PLA₂ preparations are given in Table

I and gives for PLA₂-Σ 0.10%. These figures agree well and indicate that colipase is present in the PLA₂ preparation and can be responsible for the activity of lipase against the milk fat triglycerides in the presence of 5 mM TC. Table I also indicates that a preparation of PLA₂ produced in our laboratory according to Nieuvenhuizen et al. (5) contained 0.22% colipase that could be almost completely removed when the preparation was subjected to another CM-cellulose chromatography (after trypsin activation).

TABLE I
Colipase Content of Different PLA₂ Preparations*

Preparation	Colipase content
	%
PLA ₂ -U	<0.01
PLA ₂ -Σ	0.10
PLA ₂ -ML	0.22
PLA ₂ -ML†	0.01
Lipase blank	0.004

* This was determined using tributyrin as substrate in the presence of 4 mM TDC and 0.2 μg lipase ml⁻¹. Colipase content was calculated assuming a specific activity of 40,000 U/mg (7).

† PLA₂-ML rechromatographed on a CM-cellulose column (5).

The rate of fatty acid release obtained by Bläckberg et al. (1) under similar conditions was 0.04–0.05 μmol min⁻¹ ml⁻¹, indicating that the colipase content of their PLA₂ batch was about half ours. Their figures are based on the rate of titration during 20-min incubation time and may well be too low due to the well-known nonlinearity of the reaction caused by a temperature-dependent denaturation of lipase (8).

DISCUSSION

The results presented indicate that PLA₂ prepared according to standard procedures (and commercially obtained) contains colipase as a contamination in minor quantities (0.1–0.2% by weight). This contamination is due to the fact that PLA₂ and colipase overlap on ion exchange chromatography; the purity of PLA₂ (and colipase) therefore depends on how rigorously the

fractions are cut. A colipase content of PLA₂ of 0.1–0.2% is, however, sufficient to stimulate hydrolysis of milk fat triglyceride by lipase in the presence of bile salts and explains the results reported by Bläckberg et al. (1). Phospholipase-treated milk fat globules therefore require colipase to be hydrolyzed by lipase in the presence of bile salts.

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