Hydrolysis of Milk Fat Globules by Pancreatic Lipase

ROLE OF COLIPASE, PHOSPHOLIPASE A_2, 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 A_2. This latter finding is shown to be due to contamination of commercially available pancreatic phospholipase A_2 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 A_2 used at high concentrations in these experiments but not reported on. In the following it is demonstrated that pancreatic phospholipase A_2 purified according to standard procedures contains colipase at a level sufficient to fully explain the results obtained, and that phospholipase A_2 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 colipase$_{96}$ (4) with N-terminal glycine were prepared in this laboratory. Pure pancreatic phospholipase A_2 was obtained as a gift from Professor G. de Haas, Utrecht, Holland (PLA_2-U).[1] Pancreatic phospholipase A_2 was also purified in this laboratory according to Nieuvenhuizen et al. (5) from a preparation of porcine pancreas obtained from Novo, Copenhagen (PLA_2-ML). A commercial preparation of pancreatic phospholipase A_2 was purchased from Sigma Chemical Co., (lot 41F-0427) (St. Louis, MO) called PLA_2-Σ. Tributyrin was a product of BDH (BDH Chemicals Ltd., Poole, England) passed through a column of Al_2O_3 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 PLA_2 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 PLA_2 preparations (10 μg ml^{-1}) released measurable quantities of fatty acids (except for neutralization of PLA_2-Σ which contains ammonium sulfate). The effect of adding lipase after PLA_2 varied with the PLA_2 source. Fig. 1A and B gives representative tracings when lipase was added after a preincubation of the fat globules for 3 min with PLA_2-U and PLA_2-Σ, respectively. After preincuba-

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[1] *Abbreviations used in this paper*: PLA_2-U, PLA_2-ML, and PLA_2-Σ, phospholipase A_2 obtained from Utrecht, prepared in our laboratory by M.L., and obtained from Sigma Chemical Co., respectively; TC, taurocholate; TDC, taurodeoxycholate.

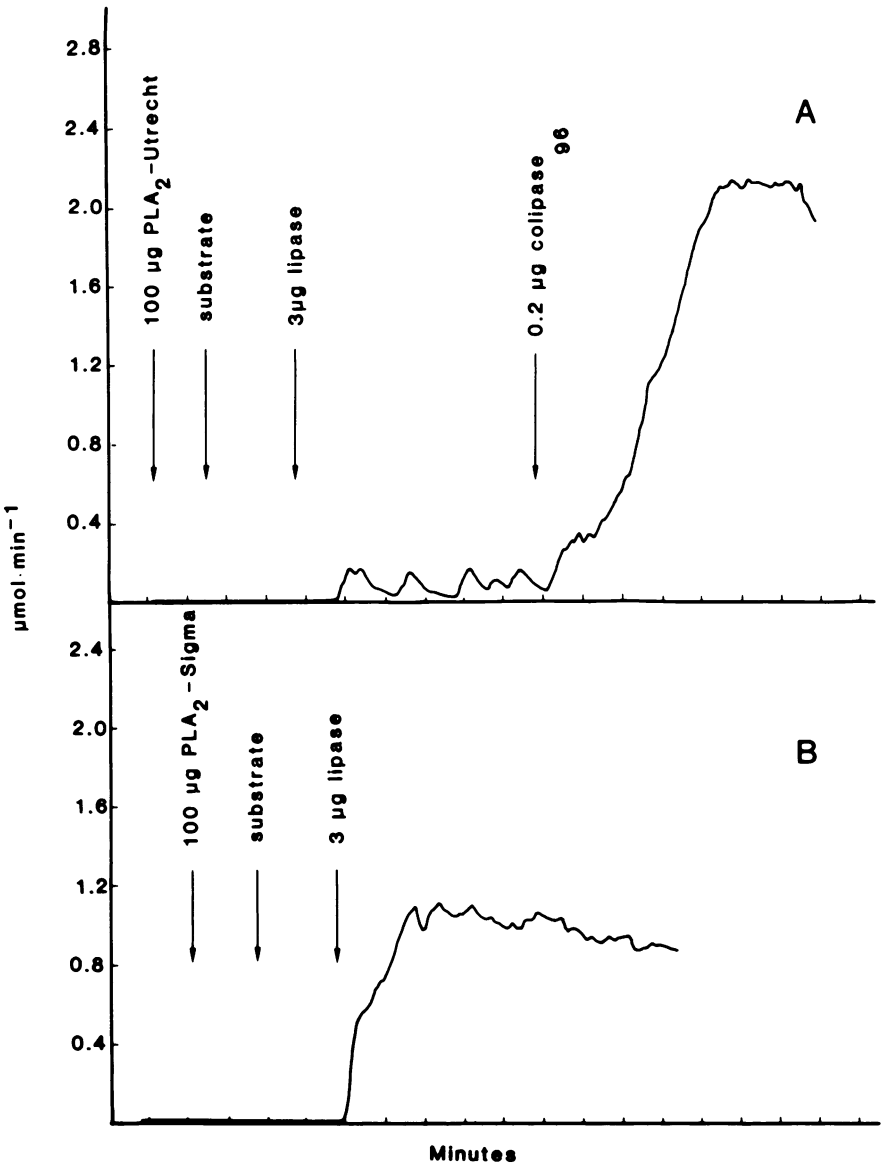

FIGURE 1 Tracings of two experiments illustrating the effect of pancreatic lipase on human milk fat globules preincubated with PLA_2-U (A) and PLA_2-Σ (B), respectively. In A, colipase was added following lipase. Total volume of incubation 10 ml, pH 7.0, 37°C, 20 mM Ca^{++} and 5 mM TC. The curves represent the rate of fatty acid release in micromoles per minute.

tion with PLA_2-U the rate of fatty acid release caused by lipase was maximally 0.016 μmol min^{-1} ml^{-1}, only slightly above the blank for lipase. With PLA_2-Σ the rate was 0.11 μmol min^{-1} ml^{-1}. When 0.02 μg $colipase_{96}$ was added per milliliter incubation medium the rate was 0.208 μmol min^{-1} ml^{-1}.

From these figures the colipase content of PLA_2 can be calculated to be $0.11/0.208 \cdot 0.02 = 0.011$ μg/10 μg PLA_2 or 0.11%. Direct determination of the colipase content of the PLA_2 preparations are given in Table I and gives for PLA_2-Σ 0.10%. These figures agree well and indicate that colipase is present in the PLA_2 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 PLA_2 produced in our laboratory according to Nieuwenhuizen 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 PLA_2 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).
‡ PLA_2-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 PLA_2 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 PLA_2 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 PLA_2 and colipase overlap on ion exchange chromatography; the purity of PLA_2 (and colipase) therefore depends on how rigorously the fractions are cut. A colipase content of PLA_2 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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