

Studies on the detergent inhibition of pancreatic lipase activity

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Abstract Pancreatic lipase requires colipase, a protein cofactor, to counteract the in vitro inhibition by bile salt. Lipase activity is inhibited by nonsteroidic detergents regardless of their charge and structure. Detergent-inhibited lipase is reactivated by colipase but in all cases activation is limited to a narrow range of detergent concentration. Complementary studies on the bile salt and detergent effect on lipase activity and on interfacial tension at the substrate-water interface show that inhibition is not related to the interfacial surface tension. It is hypothesized that adsorption of amphiphilic compounds to the substrate surface modifies the distribution of the enzyme between the lipid surface and the aqueous phase. The activity of detergent-inhibited lipase is fully restored by adding bile salt to the reaction system. Bile salt might play a critical role during in vivo lipolysis by desorbing surface-active substances from the lipid-water interface thus allowing lipase and colipase to interact with substrate.—Gargouri, Y., R. Julien, A. G. Bois, R. Verger, and L. Sarda. Studies on the detergent inhibition of pancreatic lipase activity. *J. Lipid Res.* 1983. **24**: 1336–1342.

Supplementary key words colipase • detergent • bile salts • lipolysis • interfacial tension

The absorption of dietary long chain triglycerides requires their conversion to the more polar fatty acids and monoglycerides by lipolytic enzymes. Some years ago it was advanced that the lipolytic products are dispersed into the aqueous phase of the intestinal fluid in the form of mixed micelles containing bile salts from which lipid absorption occurs (1). Recently, it was proposed by Patton and Carey (2) that products dispersed in liquid crystalline lipid phases could be involved in the uptake of dietary lipids. It is well established that bile salts are in vitro inhibitors of pancreatic lipase activity against short and long chain triglycerides. Inhibition is reversed by colipase, a protein of the exocrine pancreatic secretion that specifically restores activity, even at bile salt concentrations largely exceeding their critical micellar concentration (3–5).

In many aspects, bile salt inhibition of lipase and its reversal by colipase has remained unclear. There is ev-

idence showing that, parallel to the inhibition of lipase by bile salt, the enzyme is desorbed from the interface (6, 7). Colipase, which binds to triglyceride in the presence of bile salt, would then act as an activator for lipase by anchoring the enzyme to the substrate surface (8, 9). In association with its cofactor, lipase is more resistant to surface denaturation (10, 11). According to Momsen and Brockman (12), desorption of lipase would result from the cooperative formation of a lipase-(bile salt)₄ complex in solution having low affinity for the bile salt-covered triglyceride surface. A more generally accepted view is that the enzyme is prevented from reaching its substrate by the building up of a monolayer of negatively charged bile salt molecules at the surface of the triglyceride particles (13). Other authors have related the inhibition of lipase activity to the lowering in surface tension brought about by the detergent (14). Many studies were devoted to the influence of the surface pressure on lipolysis using monolayer techniques (15, 16). In these studies, surface pressure was used only as a convenient experimental parameter to characterize the state or “quality” of lipid molecules organized at the air-water interface. All parameters such as lipid packing, lipid conformation and/or orientation, hydration state, surface potential, etc. which are susceptible to control enzyme action are related to the surface pressure. Unfortunately, up to now, it has not been possible to directly correlate the variation of enzyme activity with any of these parameters. Surface tension is solely viewed as an index of “interfacial quality”.

Intraluminal hydrolysis of fat occurs under conditions where amphiphilic compounds of various charge, structure, and surface activity might influence the lipase-catalyzed interfacial reaction. Their influence, however, remains difficult to evaluate. Previous studies have shown that detergents other than bile salt are also inhibitors of lipase (17–20).

Abbreviation: CMC, critical micellar concentration.

To get a better understanding of the effect of amphiphiles other than bile salts, we have investigated the influence of a large variety of ionic and non-ionic detergents on the activity of horse pancreatic lipase, in the absence and in the presence of colipase. Results indicate that all detergents tested are strong inhibitors of lipase and that colipase partly reverses inhibition only at low detergent concentration. Of interest is the finding that the addition of bile salt to the reaction system containing lipase, colipase, and detergent fully restores lipolysis. Then bile salt shows the ability to activate lipolysis in the presence of various inhibitory amphiphiles. This observation points to a possible essential role played by bile salt during *in vivo* lipolysis.

EXPERIMENTAL

Materials

Bile salts and detergents were commercially available products and were used without further purification. Sodium cholate was from Koch-Light and sodium dodecyl sulfate was from Serva. Sodium taurodeoxycholate, cetyl pyridinium chloride, benzalkonium chloride, sulfobetaines (Zwittergents TM-12 and TM-14), Brij 35, and Triton X-100 were products from Calbiochem. Tweens 20 and 80 (polyoxyethylenesorbitan monolaurate and monooleate) were obtained from Sigma. Sodium chenodeoxycholate, ursodeoxycholate, and dehydrocholate were prepared from the corresponding acids (Sigma). Sodium deoxycholate was from Fluka. Values of the critical micellar concentration (CMC) of the detergents and bile salts were taken from the literature (21–24) except for cetyl pyridinium chloride (CMC: 0.5 mM) and benzalkonium chloride (CMC: 0.04 mM) whose CMC at 20°C were determined in the laboratory by the spectrophotometric method using Rhodamine 6 G.

Methods

Lipase activity was determined with triolein emulsified in gum arabic as substrate. Hydrolysis was followed at pH 9 and 25°C with the pH-stat (25). In all assays, lipase activity was derived from the initial slope and expressed as units. One enzyme unit corresponds to the liberation of one microequivalent of fatty acid per min. Pure equine lipase and colipase (procolipase B) were used in this study (26, 27).

Interfacial tension measurements at the triolein-water interface were made at the Laboratoire de Physique des Liquides of the University of Marseille with an automatic Dognon-Abribat tensiometer connected to a chart recorder. The tensiometer was equipped with a Teflon cylinder (6 mm length, 6 mm diameter) which

had a conical shape at its lower end. In a typical experiment, 10 ml of triolein was placed on top of 50 ml of the aqueous phase (1 mM Tris-HCl buffer, pH 8, containing 1 mM NaCl and 0.5 mM CaCl₂). Concentrated solutions of bile salt or detergent were injected into the aqueous phase using a micro-syringe. Equilibrium interfacial tension was promptly obtained under magnetic stirring. The cylinder was then slowly slid down through the oil-water interface and the force was simultaneously recorded. Force reaches its maximal value when the contact angle is equal to zero. The accuracy of the equipment was tested with pure water. All measurements were made at a constant temperature of 20°C. Experimental values of the interfacial tension were not affected when gum arabic was added to the aqueous phase (10 mg per ml).

RESULTS

Effects of ionic and non-ionic detergents on lipase activity in the absence and in the presence of colipase

All detergents were checked to be nonirreversible denaturants of pancreatic lipase in the range of concentration used in the present studies. The effect of varying concentrations of sodium dodecyl sulfate, cetyl pyridinium chloride, and benzalkonium chloride on the rate of hydrolysis of triolein by lipase is presented in **Fig. 1**. Inhibition studies were also performed with zwitterionic and non-ionic detergents (**Fig. 2**). As previously observed (17–20) enzyme activity is strongly inhibited by all detergents tested. Activity decreases rapidly at a given detergent concentration threshold. Inhibition is complete below the CMC of the detergent. Inhibition is partly reversed by colipase. The cofactor failed to counteract the inhibition in a large range of detergent concentration in contrast to that observed in the case of bile salt.

Effect of enzyme concentration on the inhibition of lipase by detergent and bile salt

Inhibition of lipase by detergents and bile salts was studied with various amounts of enzyme at constant substrate concentration. In all assays, the excess of triglyceride emulsion was sufficient to obtain maximal activity. Typical results obtained with Tween 80 and sodium deoxycholate are reported in **Fig. 3**. In both cases the enzyme activity increased as the total concentration of lipase in the reaction system was increased. Curves of **Fig. 3a** and **3b** show that the concentration of detergent that causes half-inhibition is constant and not dependent upon enzyme concentration. As shown in the case of sodium deoxycholate (**Fig. 3c**), the curves obtained at

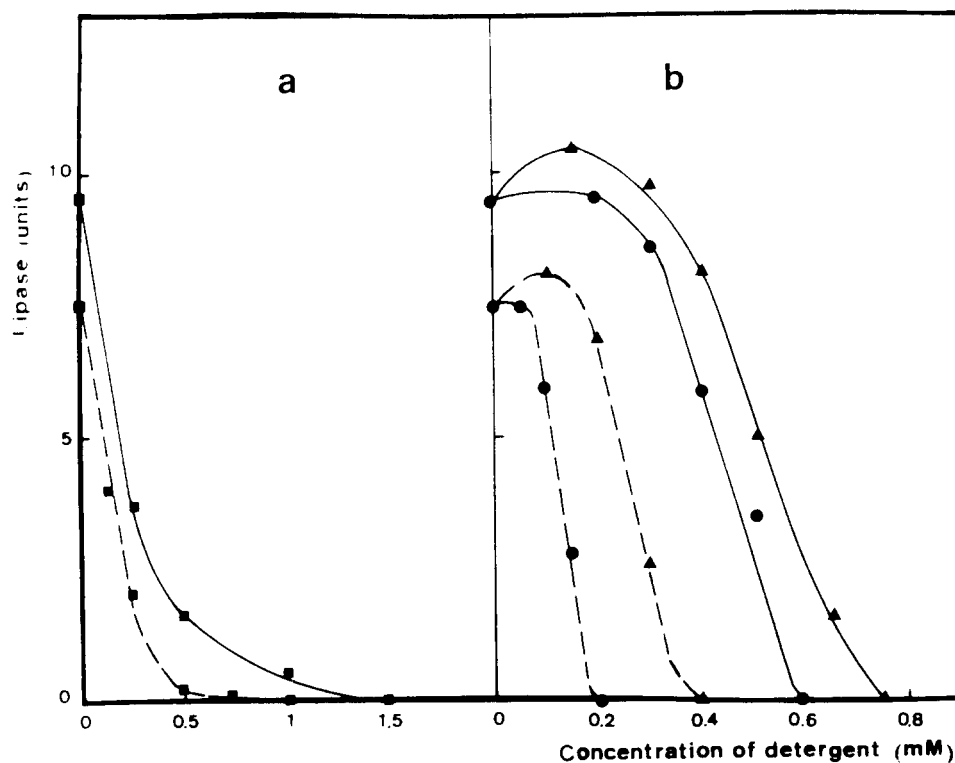


Fig. 1. Effect of increasing concentration of anionic and cationic detergents on the rate of hydrolysis of triolein by pancreatic lipase. a, Sodium dodecyl sulfate; b, cetyl pyridinium chloride (\blacktriangle) and benzalkonium chloride (\bullet). Assays were performed in absence (---) and in presence (—) of a 3-fold excess of colipase (molar ratio).

various lipase concentrations superimpose if the results are expressed as percentage of remaining enzyme activity. Comparable results were obtained with all ionic or neutral detergents used in this study (data not shown).

Effect of bile salts on detergent-inhibited lipase

Activity was measured at increasing concentrations of bile salt. As reported in **Fig. 4**, lipase activity is restored by the addition of sodium deoxycholate or var-

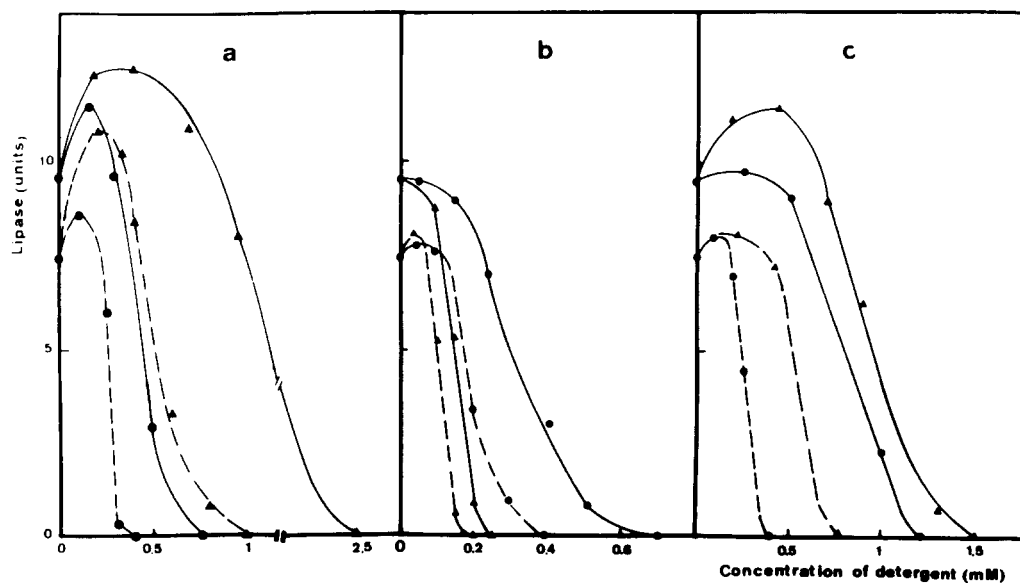


Fig. 2. Effect of increasing concentration of zwitterionic and non-ionic detergents on the rate of hydrolysis of triolein by lipase. a, Sulfobetaines TM 3-12 (\blacktriangle) and TM 3-14 (\bullet); b, Brij 35 (\blacktriangle) and Triton X-100 (\bullet); c, Tween 20 (\blacktriangle) and Tween 80 (\bullet). Assays were performed in absence (---) and in presence (—) of colipase.

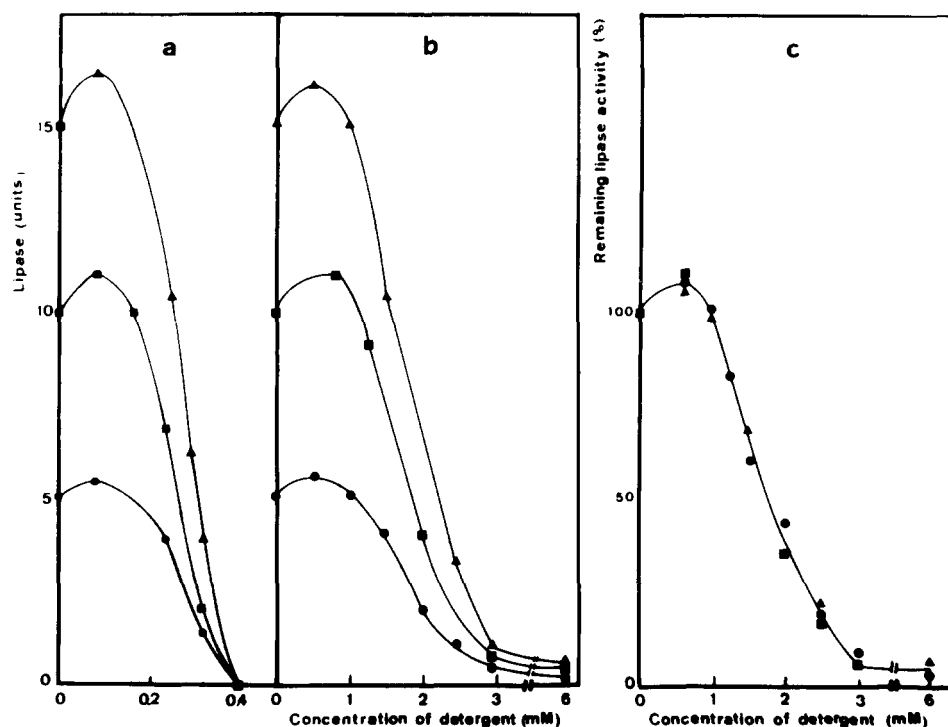


Fig. 3. Inhibition of pancreatic lipase by increasing concentrations of Tween 80 and sodium deoxycholate at various enzyme concentrations. All assays were performed in a reaction system containing 10 ml of the triolein emulsion and no colipase. Triangles, squares, and circles are experimental points obtained with amounts of lipase in ratio 3:2:1, respectively. The corresponding enzyme concentrations are 5×10^{-9} M, 3.3×10^{-9} M, and 1.65×10^{-9} M, respectively. a, Tween 80; b, sodium deoxycholate; c, same experimental values as in 3b but expressed as percentage of remaining lipase activity.

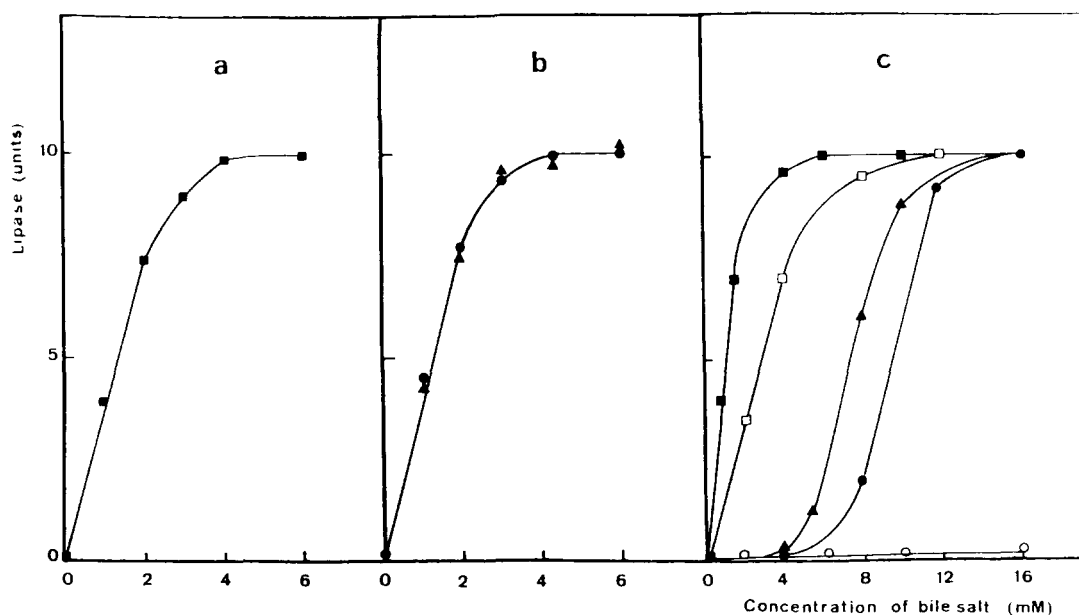


Fig. 4. Reactivation by bile salt of detergent-inhibited lipolysis. a, Lipase activity was measured after addition of increasing concentrations of sodium deoxycholate to the reaction system containing 10 ml of triolein emulsion and 1.5 mM of sodium dodecyl sulfate, lipase, and a 3-fold excess of colipase (molar ratio). b, Same conditions as in 4a except that the reaction system contained 0.75 mM cetyl pyridinium chloride (▲) or 0.6 mM benzalkonium chloride (●) in place of sodium dodecyl sulfate. c, Same conditions as in 4a except that the reaction system contained 0.65 mM Triton X-100 in place of sodium dodecyl sulfate. Lipase activity was measured after addition of increasing concentrations of deoxycholate (■), chenodeoxycholate (□), ursodeoxycholate (▲), or cholate (●). No reactivation was observed upon addition of sodium dehydrocholate (○).

ious bile salts. Reactivation begins at bile salt concentrations below their CMC. However, complete reactivation of lipolysis occurs at a bile salt concentration in the range or above the CMC. Furthermore, the activity-bile salt dependency curves are comparable regardless of charge and chemical structure of the inhibitory detergent used (Fig. 4a and 4b). Notice can be made that dehydrocholate, a bile salt analog with a high CMC, failed to restore lipase activity when added at concentrations in the same range as other bile salts (Fig. 4c).

We have also studied the influence of the order of addition of the amphiphiles (detergent and bile salt) and lipase and colipase to the reaction system on the kinetics of hydrolysis. As an example, Triton X-100 added 3 min after lipase and colipase addition causes a 90% inhibition. The lipolysis rate was fully restored and reached its maximal value by adding sodium deoxycholate to the reaction system 3 min after the Triton addition.

Studies of detergent and bile salt effect on interfacial tension

The effect of increasing concentrations of various bile salts on the interfacial tension of the triolein-water interface is presented in Fig. 5a. In Fig. 5b is shown the influence of varying bile salt concentrations on lipase activity against triolein. One can observe that sodium dehydrocholate, which has no effect on enzyme activity, strongly lowers the interfacial tension. Sodium cholate, strongly lowers the interfacial tension. Sodium cholate,

whose effect on the interfacial tension is comparable to that of sodium ursodeoxycholate, is much less effective in inhibition. The fact that no direct relationship could be found between the inhibition of lipase activity and the lowering of the interfacial tension is further illustrated by the results reported in Fig. 6. Curves of Fig. 6 show that the effect of increasing concentration of cetyl pyridinium chloride and Tween 80 on lipase activity are very similar although these detergents have very different effects on the interfacial tension.

DISCUSSION

Results reported in the present communication confirm that pancreatic lipase is inhibited by ionic and non-ionic detergents with large differences in charge and structure (17-20). It seems unlikely then, that lipase inhibition is due to electrostatic repulsion between the enzyme and the amphiphilic molecules adsorbed at the oil-water interface. By comparing the inhibition curves obtained with detergent and bile salt with their respective effects on interfacial tension at the triolein-water interface, no direct relationship could be established between the decrease in lipase activity and the lowering of the interfacial tension. Furthermore, the inhibition studies performed at variable lipase concentrations indicate that inhibition cannot be ascribed to a saturation of the potential lipase adsorption sites by amphiphiles on the lipid surface. At a given detergent/substrate ra-

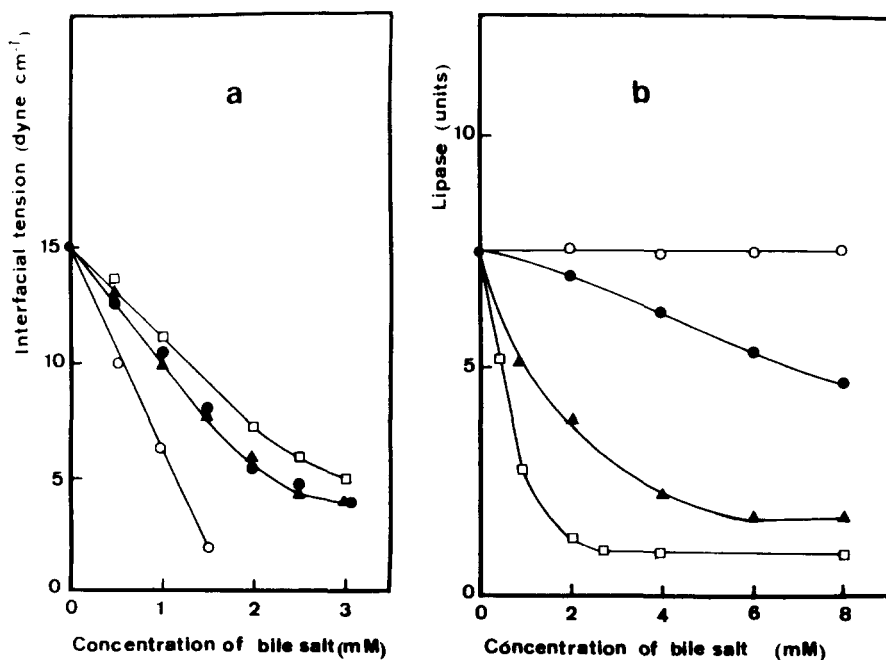


Fig. 5. Effect of increasing concentrations of various bile salts on interfacial tension (a) and on lipase activity (b) at the triolein-water interface. Experiments were performed with chenodeoxycholate (\square), ursodeoxycholate (\blacktriangle), cholate (\bullet), and dehydrocholate (\circ).

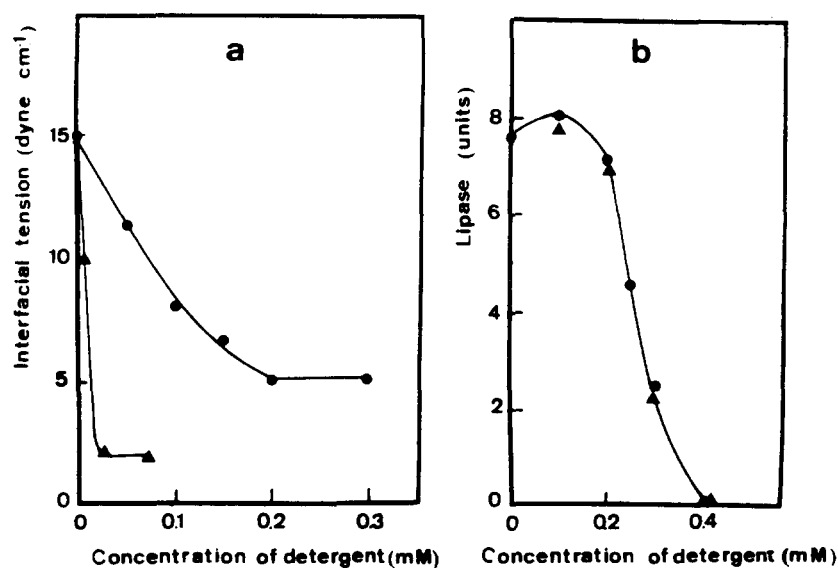


Fig. 6. Effect of increasing concentrations of detergents on interfacial tension (a) and on lipase activity (b) at the triolein-water interface. Experiments were performed with cetyl pyridinium chloride (▲) and Tween 80 (●).

to the percentage of active lipase was found to remain constant, independent of the total enzyme concentration. This latter finding suggests that the heterogeneous reaction system can be considered as a two-phase system in which the partitioning of lipase between the aqueous phase and the lipid surface would be controlled by the properties of the detergent-modified triglyceride surface to which the enzyme binds. Adsorbed amphiphiles might change the alignment and/or orientation of substrate molecules thus affecting enzyme adsorption. We have not measured the amount of enzyme adsorbed to its emulsified substrate. Indeed, this evaluation can be questioned as lipolysis occurs while the emulsion is being centrifuged and, secondly, coalescence of lipid droplets was shown to occur during phase separation. The alternative hypothesis, according to which inhibition of adsorbed lipase could only result from its direct interaction with detergent molecules bound to the lipid surface and/or equilibrium between active and inactive conformations of the enzyme (10, 28), cannot be discarded. Colipase appears to be much less effective in restoring *in vitro* lipase activity inhibited by detergent than it is in the case of inhibition by bile salts. This might reflect the difficulty for the protein cofactor to penetrate the mixed interfacial layer formed by the non-steroidic detergents adsorbed to triolein. It is worthy of notice that bile salts are different from long chain aliphatic detergents in terms of the architecture of their molecules. They possess a rigid steroid nucleus with definite hydrophobic and hydrophilic sides and they show a particular ability to form intermolecular hydrogen bonding.

There is much interest in the observation that, in all

cases, the inhibition of lipase by detergents is fully reversed by bile salts in the presence of colipase. This behavior remains difficult to interpret with certainty. The bile salt might activate the detergent-inhibited lipolytic reaction by desorbing the detergent molecules from the substrate surface and form mixed micelles, at a concentration below the CMC. This interpretation is presently supported by the observation that bile salts modify the interfacial tension of a triglyceride/water interface in the presence of detergents. Displacement of detergent from the lipid surface will be checked experimentally by using the monolayer technique.

In addition to their solubilization function during *in vivo* lipolysis, bile salts could play an essential role by counteracting the potential inhibitory effect of surface active substances adsorbed at the surface of dietary fat particles. Our conclusions are in agreement with the observation made by Borgström and Erlanson (29) that inhibition of pancreatic lipase activity by serum albumin and β -lactoglobulin is reversed by taurodeoxycholate in the presence of colipase. ■

The authors thank J. Rathelot, I. Bosc-Bierne, P. Canioni, and J. F. Baret for help in the preparation of horse lipase and colipase and for helpful discussions during this study. This work was supported in part by grants from the CNRS (L.A. 202) and from DGRST (M.R.M. 80.7.0151).

Manuscript received 18 April 1983 and in revised form 21 June 1983.

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