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EXPERT REVIEW

Effects of Surfactants on Lipase Structure, Activity, and Inhibition

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ABSTRACT Lipase inhibitors are the main anti-obesity drugs prescribed these days, but the complexity of their mechanism of action is making it difficult to develop new molecules for this purpose. The efficacy of these drugs is known to depend closely on the physico-chemistry of the lipid-water interfaces involved and on the unconventional behavior of the lipases which are their target enzymes. The lipolysis reaction which occurs at an oil-water interface involves complex equilibria between adsorption-desorption processes, conformational changes and catalytic mechanisms. In this context, surfactants can induce significant changes in the partitioning of the enzyme and the inhibitor between the water phase and lipid-water interfaces. Surfactants can be found at the oil-water interface where they compete with lipases for adsorption, but also in solution in the form of micellar aggregates and monomers that may interact with hydrophobic parts of lipases in solution. These various interactions, combined with the emulsification and dispersion of insoluble substrates and inhibitors, can either promote or decrease the activity and the inhibition of lipases. Here, we review some examples of the various effects of surfactants on lipase structure, activity and inhibition, which show how complex the various equilibria involved in the lipolysis reaction tend to be.

KEY WORDS inhibitor · interfacial enzymology · lipase · lipid-protein interaction · surfactant

ABBREVIATIONS

β-OG	β-octyl glucoside
BSA	bovine serum albumin
CMC	critical micellar concentration
DGL	dog gastric lipase
EPR	electron paramagnetic resonance
E600	diethyl *p*-nitrophenyl phosphate
GPLRP2	guinea pig pancreatic lipase-related protein 2
HPL	human pancreatic lipase
NaTDC	sodium taurodeoxycholate
PPL	porcine pancreatic lipase
SDS	sodium dodecyl sulphate
SDSL	site-directed spin labeling
TAG	triacylglycerol
TGME	tetraethylene glycol monooctyl ether
THL	tetrahydrolipstatin (Orlistat)
TLL	*Thermomyces lanuginosus* lipase
YLLip2	*Yarrowia lipolytica* Lip2

INTRODUCTION

Inhibiting digestive lipases to reduce fat absorption has become the main pharmacological approach to the treatment of obesity during the last decade (1–3), and this strategy can be associated with classical approaches such as

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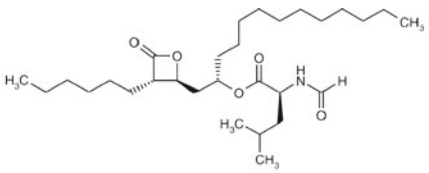
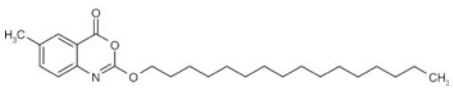
hypocaloric diets and physical exercises. With the withdrawal of appetite-suppressing drugs that act on the central nervous system, such as Fenfluramine, Rimonabant and Sibutramine, the only globally licensed anti-obesity drug remaining on the market is the lipase inhibitor Orlistat (Tetrahydrolipstatin, THL). This molecule (Table I) can be found in two different drug products: Xenical™ (Roche), which can be delivered to patients only under medical prescription, and Alli™ (GSK), which is an over-the-counter drug product containing a lower dosage of Orlistat (60 mg *versus* 120 mg in Xenical™). In addition to weight management, Orlistat produces meaningful reductions in risk factors for obesity-related conditions such as diabetes and cardiovascular disease (4). Based on these positive results of a lipase inhibitor in the treatment of obesity, Alizyme Ltd. has developed a new lipase inhibitor (Cetilistat, ATL-962) with a mode of action similar to that of Orlistat (Table I) (5). In clinical trials, although both molecules exhibit similar efficacy (6,7), it seems that Cetilistat could exhibit fewer adverse effects than Xenical™ (8), which is known to induce anal leakage and oily spotting, detrimentally affecting patient compliance. It was announced that Cetilistat was moving into Phase III clinical trials for the treatment of obesity. The success of lipase inhibitors has driven investigations into new generations of nutraceuticals, dietary supplements and pharmaceutical agents that inhibit the breakdown of fats and complex carbohydrates within the gastrointestinal tract (9,10).

Lipase inhibitors may soon be used for other pharmaceutical applications, such as the treatment of type 2 diabetes (11,12), atherosclerosis (13), tuberculosis (14–16), and probably cancer, since Orlistat was also found to have anti-tumoral effects via its inhibitory action on fatty acid synthase (17). Most of the lipase inhibitors tested so far are insoluble in water, and like the lipase substrate itself, they are often present at the interface between water and another phase or in aggregates dispersed in water. In this context, surfactants have important effects on lipase activity and inhibition, and the aim of this paper is to review some of the specific effects of surfactants, including synthetic compounds, bile salts, proteins and phospholipids.

Lipases occur widely in the microbial (14,18,19), plant (20,21) and animal kingdoms (22,23). These enzymes play an important role in fat digestion, lipoprotein metabolism, and mobilization of fat stored in lipid inclusion bodies, endosperms and adipocytes. They catalyze the hydrolysis of triacylglycerol ester bonds (24–27) and are water-soluble, whereas their substrates are insoluble in water. In this context, the catalytic reaction of lipolysis involves various interfacial processes and depends strongly on the structure of the lipid substrates present in oil-in-water emulsions, membrane bilayers, monolayers, micelles, and vesicles (28). The catalytic process can be described as a reversible lipase adsorption/desorption step occurring at the oil/water interface, followed by the formation of an interfacial enzyme substrate complex and the release of lipolysis products (29,30). In most cases, activation of the lipase is triggered by the conformational changes it undergoes in the presence of lipids and/or surfactants. It was first hypothesized that the adsorption of the lipase at the oil-water interface might be responsible for these changes (31), but it was recently established that they could also occur in solution, in the presence of micelles (32).

It is not easy to analyze experimental data obtained with such a soluble enzyme acting on insoluble substrates, because the partitioning of the enzyme between the aqueous phase and the substrate interface has to be taken into account. In addition, in most experimental set-ups, the enzyme activity and the partitioning process cannot be measured simultaneously, so that the Michaelis-Menten-Henri model no longer applies, and only apparent kinetic constants (k_{cat}, K_m, K_i) can be obtained (33). The K_m and K_i

Table I Structure of Digestive Lipase Inhibitors and *In Vivo* Side Effects. Adapted from (6)

Drug	Formula	Manufacturer	Mechanism	Phase of development	Side effects
Orlistat		Roche	Lipase inhibitor, inhibits intestinal fat absorption	Launched in 1998 (Xenical™), available without prescription since 2007 (Alli™ by GSK)	Fecal urgency, diarrhea, abdominal pain; case reports of liver injury under investigation
Cetilistat		Alizyme / Norgine	Lipase inhibitor	Phase III	Fecal urgency, diarrhea, abdominal pain

values often estimated for lipases and defined in terms of a volume concentration, therefore, have no relevance when working with insoluble substrates.

Furthermore, the basic steps in the process of interfacial catalysis mentioned above are highly dependent on the nature of the surfactants present during the reaction and on their quantities. Surfactants are tensioactive compounds which are preferentially located at interfaces because of their energy requirements. They are water-soluble in the micro- to millimolar range and self-assemble above a critical concentration. These substances include synthetic detergents as well as peptides, proteins, bile salts and many other compounds. Due to their specific properties, surfactants are commonly used as emulsifiers in lipase assays in order to obtain finely dispersed oil droplets in water, promoting the lipase activity by increasing the specific area of substrate accessible to the enzyme (34). However, the presence of surfactants greatly increases the complexity of the system and that of the equilibria involved. Over the last years, surfactants emerged as the most decisive factors contributing to the availability of lipase inhibitors and the lipase inhibition process.

DIRECT EFFECTS OF SURFACTANTS ON LIPASES

Surfactants and lipases can interact with each other, both in solution and at interfaces. *In vitro*, several detergents with different properties (nonionic, cationic, anionic and zwitterionic) can be used for modulating these interactions. With nonionic and zwitterionic detergents, interactions are mainly hydrophobic, while anionic or cationic detergents promoted electrostatic interactions related to the charge of the protein. Whatever the detergents used, interactions between protein and surfactant molecule can have various consequences on protein structure and activity, but also on the interaction between the protein and the lipidic interface (35,36). For example, Mogensen *et al.* described deactivation of *Thermomyces lanuginosus* lipase (TLL) in the presence of nonionic and zwitterionic surfactants at high concentrations (37). Ionic surfactants such as sodium dodecylsulphate (SDS) at low concentrations can also form complexes with most proteins in solution (35,36,38). In these complexes, the conformational stability of the protein may be altered (38), and the hydrophobicity of the protein surface may also be changed (35,36). When used at higher concentrations, ionic surfactants may lead to partial or complete unfolding of the tertiary protein structure due to additional hydrophobic interaction (39–41). By contrast, bile salts that can be regarded as natural anionic surfactants do not impair the structure of digestive lipases (42). The effects of surfactants on lipases are therefore highly variable and depend strongly on the choice of both lipase and detergent.

One particularly noteworthy feature of lipases, however, is that most of them undergo a change of conformation in the presence of surfactants or substrates, and thus adapt to the new environment without losing their catalytic properties. When several 3-D lipase structures were published in the 1990s, it was observed that the access to the active site was controlled by a so-called lid formed by a surface loop. The 3-D structures of such lipases, including *Rhizomucor miehei* lipase (43,44), TLL (45,46), *Candida rugosa* lipase (47), human pancreatic lipase (HPL) (48) and human and dog gastric lipases (49,50), have been determined. In all these cases, the lid domain was found to undergo a conformational change making the active site accessible to solvent. This conformational change is illustrated in Fig. 1, where the closed and open conformations of the lid present in various lipases are shown with blue and yellow ribbons, respectively (28).

The structural differences observed between the closed and open lipase forms range from the relatively simple rigid hinge-type motion of a single helix occurring in *R. miehei* lipase (43) to a much more complex pattern of multiple loops undergoing fundamental changes in their secondary structures (47,48,50). In some lipases, the structure of the active site (the formation of the oxyanion hole) was affected by these conformational changes. In HPL, for instance, the lid domain and the β5 surface loop were found to adopt a completely different conformation in the presence of phospholipids and bile salts (51). The β5 loop folds back on the core of the protein, and this movement creates an electrophilic region close to the active site serine, which stabilizes the negatively charged transition-state intermediate formed during the process of ester hydrolysis. These conformational changes (*i.e.* the lid opening) are therefore of great importance, since they give access to the active site as well as adjusting the catalytic machinery. However, as a large hydrophobic surface is exposed around the active site in the open conformation, only the closed form is generally observed in water (32). This hydrophobic surface has to be stabilized to achieve a sufficiently high level of activity. The stabilization can be induced at the oil-water interface by the substrate itself, as well as by surfactants such as β-octyl glucoside (β-OG) (52). It is worth noting that surfactants have often been used in crystallogenesis experiments to obtain suitable crystals for X-ray diffraction purposes, and most of the 3-D structures of lipases with the lid in the open conformation were determined in the presence of surfactants. A tetraethylene glycol monooctyl ether (TGME) molecule was found to be tightly bound to the hydrophobic cavity of the porcine pancreatic lipase (PPL) active site, which included several residues from the open lid (Fig. 2A) (53). In the case of HPL, a monomer of β-OG was observed at the entrance to the active site, stacked against the hydrophobic surface of the open lid, while the active site

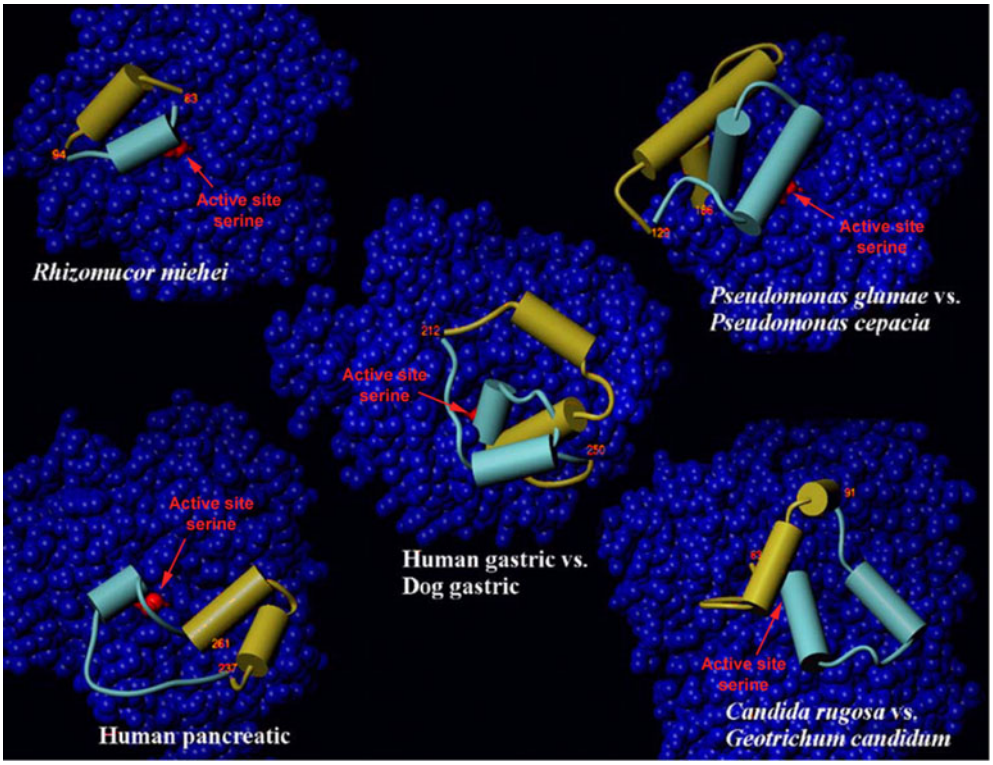


Fig. 1 Closed (*blue ribbon*) and open (*yellow ribbon*) lid conformations in various lipases. The active sites' serine residues are colored in red, and their location is indicated by an arrow. In the case of gastric lipase, the closed and open conformations of the lid were obtained with human and dog gastric lipases, respectively. In the case of the homologous bacterial lipases from *Pseudomonas sp.*, the closed and open conformations of the lid were obtained with *Pseudomonas glumae* and *Pseudomonas cepacia* lipases, respectively. In the case of the homologous fungal lipases from *Geotrichum* and *Candida sp.*, the closed and open conformations of the lid were obtained with *Geotrichum candidum* and *Candida rugosa* lipases, respectively. Adapted from (28).

itself was filled with an alkylphosphonate inhibitor. Some other surfactant molecules were also detected at the lipase-colipase junction, surrounding the colipase (Fig. 2B) (54). Likewise, studies on the dog gastric lipase (DGL) inhibited with another alkylphosphonate inhibitor showed the presence of a β-OG molecule located in the catalytic pocket (Fig. 2C) (50).

Before the closed and open conformations of lipases were observed by performing X-ray crystallography, the fact that surfactants can induce a conformational change in lipases (and give access to their active site) was previously suspected since inhibition studies with diethyl *p*-nitrophenyl phosphate (E600) performed in the presence of bile salts (55). These assumptions were only based on the kinetics of lipase inhibition, however. The opening of the HPL lid induced by bile salts in solution was recently described using combined site-directed spin labeling (SDSL) methods and electron paramagnetic resonance (EPR) (32). The results obtained in the latter study showed that the lid opening is a reversible process and that there exists an equilibrium between the closed and open conformations. When HPL was present alone in the solution, the lid was found to be closed. Adding increasing sodium taurodeoxycholate (NaTDC) concentrations induced an increase in the lid opening, which reached a maximum rate when the critical micellar concentration (CMC) of NaTDC (2 mM) was reached (Fig. 3). These experiments indicate that monomers of surfactants alone can promote and stabilize the lid opening process, contrary to the previous assumption that bile salt micelles were required to promote lid opening in the presence of colipase (56). There is, in fact, no absolute requirement of colipase to induce the lid opening, but this lipase cofactor stabilizes the open conformation of the lipase lid in the presence of bile salts (32).

Since surfactants have significant effects on the conformation of lipases, they can also decisively influence lipases' activities as well as their inhibition. It is well known that most lipases, including gastric and pancreatic lipases, require the presence of surfactants to be inhibited when the lipase and the inhibitor are mixed together before the residual activity is measured (57–60). Under these conditions, the surfactants can induce and block the active form of the protein, thus making the catalytic site accessible to the inhibitor. Bile salts have often been used to inhibit gastric and pancreatic lipases, but inhibitory effects can also be triggered by other surfactants such as TGME and β-OG (53) (Figs. 2B–C and 4). In 1978, Rouard *et al.* reported that the rate of inhibition of PPL by E600 depended on the bile salt concentration and on the state of solubilization of the organophosphate (55). Pancreatic lipase was not inhibited by E600 in the absence of bile salts, whereas other serine enzymes, such as chymotrypsin, trypsin and esterases, were inhibited by E600 without requiring the presence of bile salts. Similar effects were initially reported by Desnuelle *et al.* (61) with E600, whether or not it was emulsified with gum arabic. It was also established several years ago that

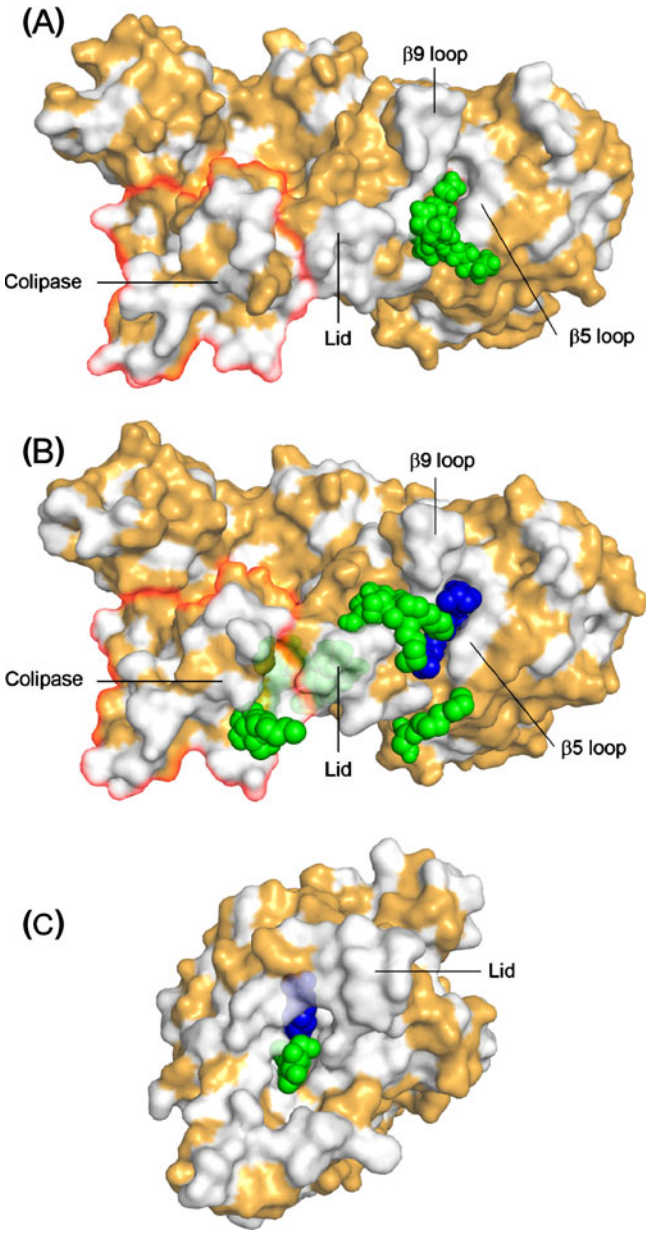


Fig. 2 Three-dimensional structures of several lipases in the open conformation showing the interactions occurring between surfactant molecules and hydrophobic parts of the lipases. Surfactants are shown in green, and inhibitors in blue. Hydrophobic residues (alanine, leucine, isoleucine, valine, tryptophan, tyrosine, phenylalanine, proline and methionine) are highlighted in white. When present, colipase is bordered in red. (**A**) Binding of a tetraethylene glycol monooctyl ether (TGME) molecule in the hydrophobic cavity of the porcine pancreatic lipase (PPL) active site. (**B**) Crystal structure of the human pancreatic lipase (HPL)-colipase complex inhibited by an alkylphosphonate inhibitor. A β-octyl glucoside (β-OG) molecule was located at the entrance of the active site, bound to the hydrophobic part of the lid. Three other β-OG molecules were also observed at the junction between the N-terminal domain of the lipase and the colipase (shown by transparency on the picture). (**C**) Three-dimensional structure of dog gastric lipase (DGL) containing an alkylphosphonate inhibitor and a detergent molecule of β-OG bound to the catalytic crevice. Structures were drawn using the PyMOL Molecular Graphics System, Version 1.2, Schrödinger, LLC, from the following Protein Data Bank files: PPL, 1ETH, HPL, 1LPB, DGL, 1K8Q.

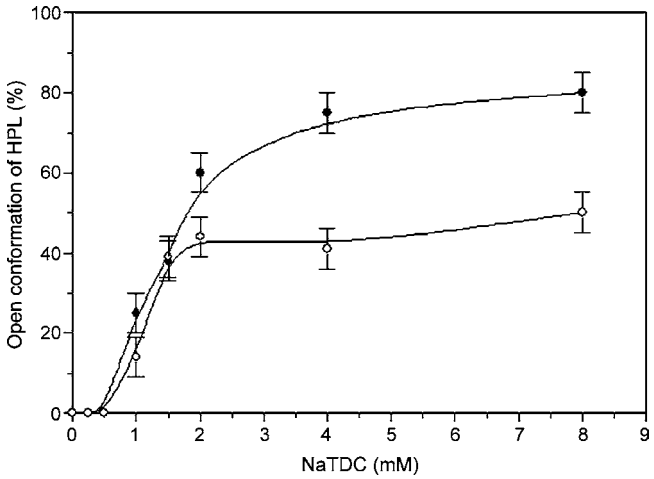


Fig. 3 Proportion of human pancreatic lipase (HPL) in the open conformation depending on the sodium taurodeoxycholate (NaTDC) concentration. (○) Experiments performed without colipase; (●) experiments performed with a 2:1 colipase to lipase molar ratio. Adapted from (32).

bile salts are required to reach the maximum inhibition of human gastric lipase by E600 (57). Similar results were subsequently obtained with the potent gastrointestinal lipase inhibitor Orlistat (62), a hydrogenated analogue of lipstatin isolated from *Streptomyces toxytricini* (63–67). Surfactants probably have similar effects to those observed with pancreatic lipases because a lid is also present in gastric lipases (Fig. 1) (49,50). In lipases without a lid, such as cutinase from *Fusarium solani pisi* (68) and guinea pig pancreatic lipase-related protein 2 (GPLRP2) (69), bile salts are not required for high inhibition rates to occur because the active site is directly accessible to solvent (58,68). However, the fact that the use of bile salts still accelerates the inhibitory process suggests that surfactants may play a direct role in the way the inhibitor is presented to the enzyme, as discussed in the second part of this review.

Before SDSL-EPR was established as a specific approach for studying the HPL lid opening in the presence of bile salts, the fluorescence of tryptophan residues was also used to monitor the conformational changes occurring in HPL during its interactions with Orlistat in the presence of bile salts (70). Seven tryptophan residues are found in HPL, but the 3-D structures (48) showed the presence of one tryptophan residue within the lid (W252). In the open conformation of HPL lid, W252 moves away from the position it occupies in the closed conformation with an amplitude of 29Å (51). During the inactivation of HPL by Orlistat, conspicuous changes were then observed in the intrinsic tryptophan fluorescence and in the near-UV circular dichroism. The rate of HPL inactivation was found to be very comparable to that determined on the basis of the time-dependence of the spectral changes (70). It was therefore concluded that HPL undergoes a conformational

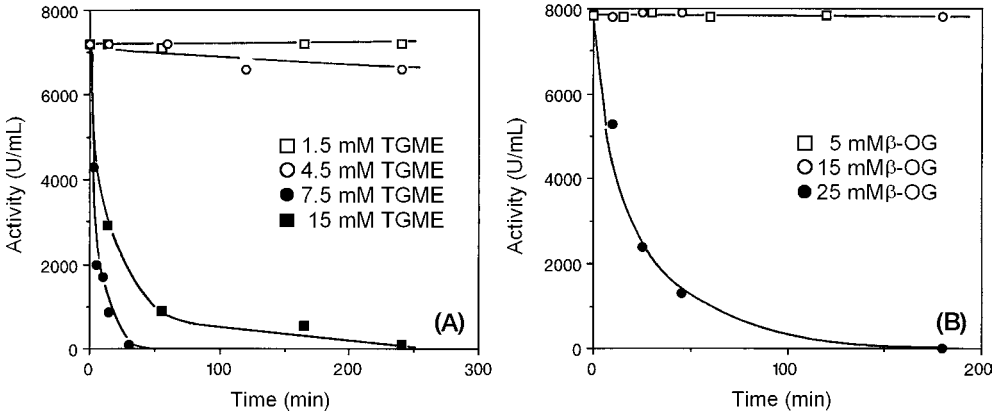


Fig. 4 Influence of nonionic detergent concentrations on pancreatic lipase inhibition by E600 in the presence of colipase. Porcine pancreatic lipase (PPL) was incubated at pH 6.0 with 4 mM E600 in the presence of colipase, at various (**A**) tetraethylene glycol monooctyl ether (TGME, CMC = 7 mM) or (**B**) β-octyl glucoside (β-OG, CMC = 20–25 mM) concentrations. At various times, aliquots were withdrawn from the incubation mixture, and the remaining lipase activity was determined at pH 7.5 using tributyrin as the substrate. Adapted from (53).

transition upon binding to Orlistat, resulting in a change in the microenvironment of the tryptophan residues. Bile salts had to be added to this system before Orlistat efficiently inactivated HPL, and the maximum inactivation rate occurred at about the CMC of bile salts, as previously observed by Rouard *et al.* with PPL (55).

Likewise, the rate of pancreatic lipase inhibition by E600 was drastically increased when other surfactants such as TGME and β-OG were used at concentrations exceeding their CMC (see Fig. 4). β-OG was also used at concentrations above the CMC to induce and stabilize active forms of several fungal lipases, including the *Candida rugosa*, *Humicola lanuginosa* and *Rhizopus delemar* lipases (52). All these results indicate that surfactants stabilize the active conformation of lipases, which is required for interactions with the substrate or the inhibitor.

EFFECTS OF SURFACTANTS ON THE LIPID-WATER INTERFACE

In water, lipids are organized spontaneously in various structures such as oil-in-water emulsions, bilayers and micelles. Surfactants as well as lipases can partition between the water phase (monomers) and these various interfaces (Fig. 5A). They have a strong impact on the physicochemistry of the lipid-water interfaces by either decreasing the interfacial tension and promoting the dispersion of the substrate in the form of oil-in-water emulsions or by forming mixed micelles. The first effect is classically observed when the triacylglycerol emulsions prepared for lipase assays are stabilized with gum arabic (acacia), which has been found to be a potent tensioactive agent (71). A larger area is then available, which improves both the lipase adsorption and the lipolysis process. It is, however, difficult

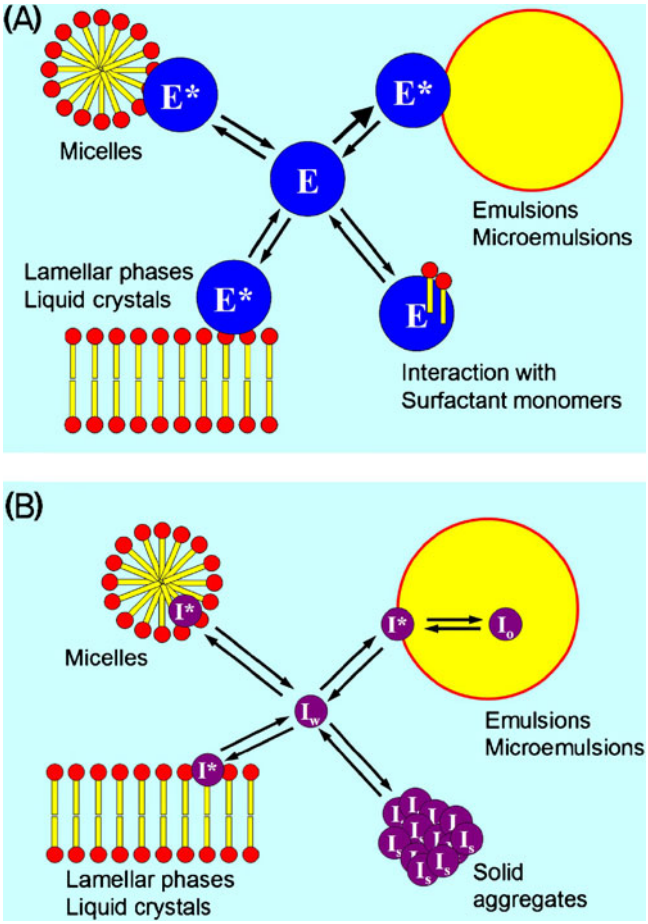


Fig. 5 Possible partitioning of either (**A**) a lipase or (**B**) a lipase inhibitor between the various phases/structures dispersed in solution in the presence of surfactants and/or lipids. Arrow's size indicates the favored interactions. E, lipase in solution; E*/I*, lipase/inhibitor present at the interface between water and emulsions/microemulsions, mixed micelles or lamellar structures (vesicles, liposomes); I_W, inhibitor in solution; I_O, inhibitor solubilized in an oil phase; I_S, inhibitor in solid aggregates.

to predict how surfactants will affect the enzyme partitioning between the various phases and interfaces. In the case of pancreatic lipase, most of the enzyme was found to be bound to oil droplets under conditions closed to the physiological ones (presence of bile salts and colipase), but the presence of an additional surfactant like gum arabic could induce the release of the enzyme in the water phase (71).

Lipase inhibitors usually have similar properties to those of the natural substrates of lipases: they are mainly insoluble and occur in the form of solid aggregates or oil-in-water emulsions (Fig. 5B). Partitioning of the inhibitors between the water phase, the interface and the lipid phase or micelles of surfactants is, therefore, likely to occur, which makes them more or less available and efficient for lipase inhibition (Fig. 5). In studies on the inhibition of PPL by E600, Rouard *et al.* reported that adding E600 to the bile salt micelles resulted in a partition coefficient of 32:1 (55). In addition, the inhibition rate was found to depend on the bile salt concentration and on the micellar concentration of the organophosphate compound. Based on these data, the authors suggested that including the E600 inhibitor in micelle of surfactants was probably a prerequisite for the organophosphate to be able to inhibit pancreatic lipase. This requirement may also be due to the effect of the surfactant on the enzyme, as previously mentioned.

The amphiphilic lipase inhibitor Orlistat, which can spread between the lipid and water phases as well as between micelles of surfactants (Fig. 5B), probably shows a similar behaviour as E600. The inhibitory efficacy of Orlistat was found to increase upon adding bile salts above their CMC in the case of HPL (70), dog and guinea pig pancreatic lipases (58) as well as human gastric lipase (62) and lipoprotein lipase (72). Although Orlistat is highly soluble in oil, Tiss *et al.* (2,73) reported that the dependence of HPL inhibition on the presence of bile salts in the incubation medium may be attributable to the formation of mixed micelles of Orlistat and bile salts (Fig. 5). This micellar solubilization may increase the availability of Orlistat in solution and thus improve its inhibitory efficiency on pancreatic lipase, the active site of which is accessible in the presence of bile salts (32). Increasing the interfacial area of the lipid-water interface by an emulsification process also promotes a stronger diffusion of Orlistat from the oil core towards the interface as depicted in Fig. 5B (74,75). This process might be essential to efficient lipase inhibition when the inhibitor is pre-dissolved in the oil phase. In a clinical study, it was observed that the effects of Orlistat on duodenal lipolysis depend strongly on the type of meal ingested (1). The TAG of a liquid test meal, finely pre-emulsified in the presence of phospholipids, was rapidly hydrolyzed by HPL before the enzyme was totally inhibited by Orlistat, resulting in a poor efficacy of the drug. By contrast, when a solid-liquid meal containing non-emulsified TAG of various sources was ingested, the rate of hydrolysis of the TAG by HPL was slower than with the liquid test meal, and the rate of HPL inhibition by Orlistat was sufficient to impair lipolysis. These results showed that lipase inhibition and lipolysis are two competitive processes and that their balance depends on the physico-chemical properties and the state of emulsification of the dietary lipids (1). The partitioning of Orlistat between the oil and aqueous phases, as well as the rate of Orlistat transfer, may contribute importantly to the inhibition of lipolysis along the gastrointestinal tract (Fig. 5B).

By decreasing the interfacial tension (and thus decreasing the interfacial energy), surfactants can also prevent the occurrence of the irreversible inactivation (unfolding) of lipases at the oil-water interface (28). For instance, both gastric and pancreatic lipases bind readily to pure trioctanoin emulsions but are rapidly inactivated in the absence of any surfactants. Adding amphiphilic compounds ranging from proteins, such as bovine serum albumin (BSA), to bile salts at low concentrations (0.5 mM) prevents the irreversible inactivation of these lipases (76–78).

COMBINED EFFECTS OF pH AND SURFACTANTS ON LIPASE ADSORPTION

Surfactants can have inhibitory effects by impairing lipase adsorption at lipid/water interfaces. Inhibitory processes of this kind can be triggered by either replacing the protein at the surface or by binding the detergent to the protein, desorbing it or preventing it from reaching the interface (79,80) (Fig. 5A). This usually occurs when surfactants are used at concentrations above their CMC. This has been found to take place with pancreatic lipase and bile salts (CMC = 2 mM). These detergents can compete with pancreatic lipase for adsorption at the interface, and when they are present at concentrations above their CMC, the interface is probably saturated by surfactant monomers, and all the lipase is recovered in the water phase, where it remains inactive (78). In this particular case, the use of the specific cofactor, colipase, makes it possible for the enzyme to anchor at oil-water interfaces even in presence of bile salts, thus restoring the lipase activity (Fig. 6A). Under similar conditions, gastric lipase was found to be insensitive to the detergent concentration and to remain present and active at the interface because it is more tensioactive (Fig. 6B) (3).

It has also been shown that the effects of surfactants on lipase activity and interfacial binding are pH-dependent (81,82). The effects of various concentrations of NaTDC on the activity of two fungal lipases, the Lip2 lipase from

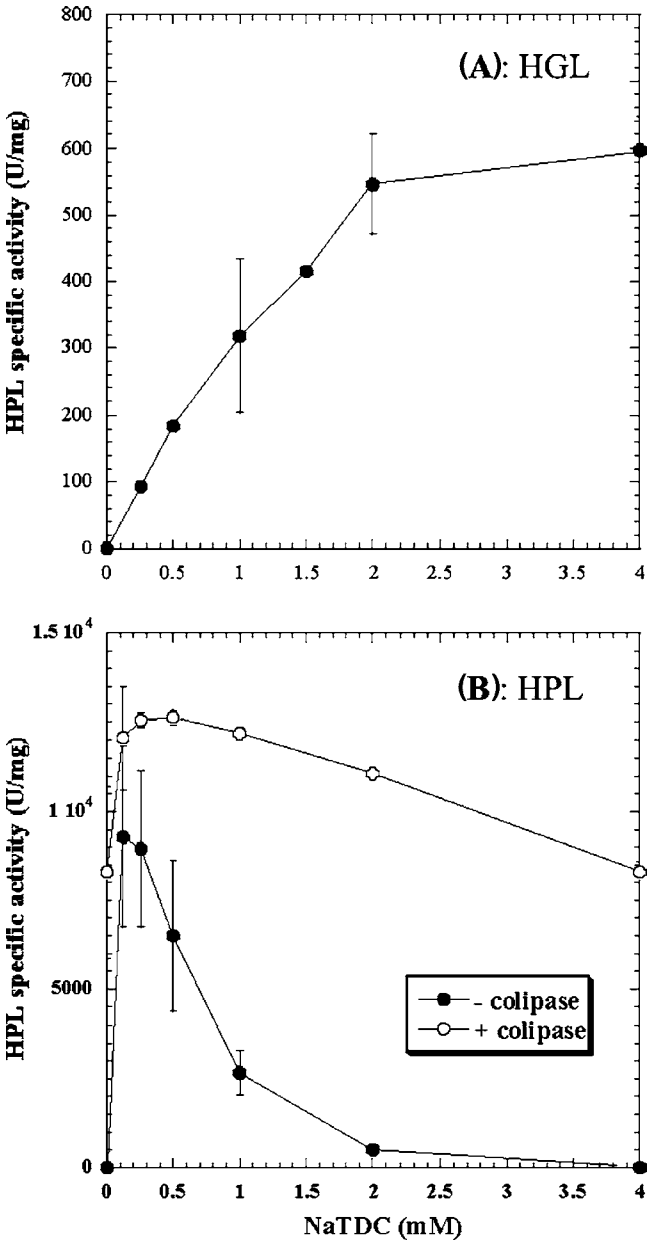


Fig. 6 Effects of bile salts on the lipase activities of human gastric lipase (HGL) and human pancreatic lipase (HPL). (**A**) Changes with the NaTDC concentration in the specific activity of HGL using tributyrin as the substrate. (**B**) Inhibition of HPL by bile salts and reactivation by colipase. Activities were determined titrimetrically using a trioctanoin substrate. Adapted from (3).

Yarrowia lipolytica (YLLip2) and the lipase from *Thermomyces lanuginosus* (TLL), were investigated with an emulsified trioctanoin substrate. Optimum activities of both lipases were obtained at very different pH values, depending on the NaTDC concentration (Fig. 7). Interfacial binding experiments performed with the same substrate confirmed that the two lipases were either found in the water phase or at the lipid-water interface, depending on the combinations of pH and NaTDC concentrations (Fig. 8). These findings suggested that the pH may affect the ability of fungal lipases to bind to oil-water interfaces, probably by inducing conformational changes in the enzyme structure, such as the opening/closing of the lid.

The effects of pH on the lid opening process were then investigated in the case of HPL, using SDSL-EPR methods (83). It turned out that decreasing the pH has similar effects to those of detergents on the opening of the lipase lid. The effects of the pH and those of detergents are therefore presumably combined in pancreatic lipase as in microbial lipases.

USE OF SURFACTANTS IN DRUG FORMULATION

The effects of surfactants on lipase inhibition suggest that these molecules could be used directly as lipase inhibitors or associated with lipase inhibitors. It has been shown, however, that most surfactants are displaced from the oil-water interface by bile salts, and their non-specific action on lipase activity observed *in vitro* is likely to be impaired *in vivo* (42,71,76,79). The association of a lipase inhibitor and surfactants is already a reality, since several surfactants are found in the formulation of Alli™ such as sodium dodecyl sulfate, sorbitan monolaurate and Polysorbate 80 (or

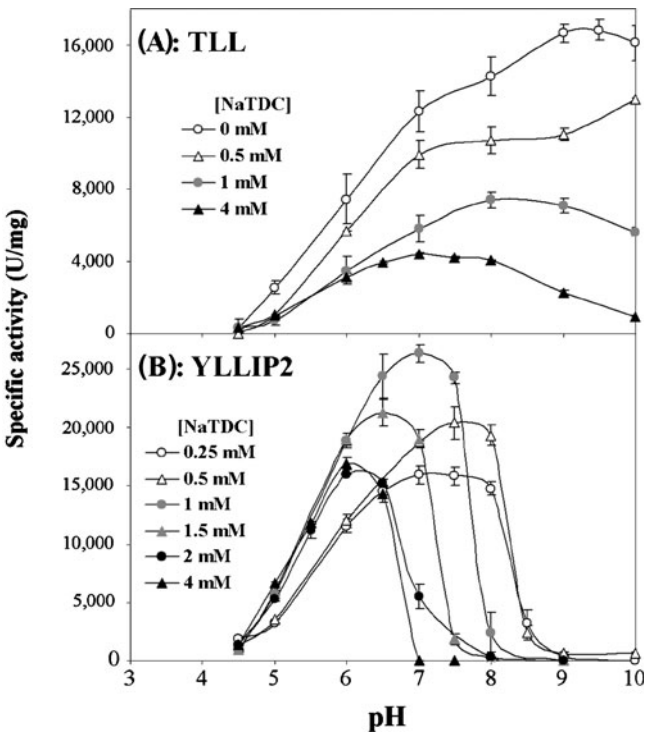


Fig. 7 Influence of pH and NaTDC on the specific activity of (**A**) *Thermomyces lanuginosus* lipase (TLL) and (**B**) *Yarrowia lipolytica* Lip2 lipase (YLLip2) on a trioctanoin substrate. All measurements were performed at 37°C using the pH-stat method. Adapted from (81).

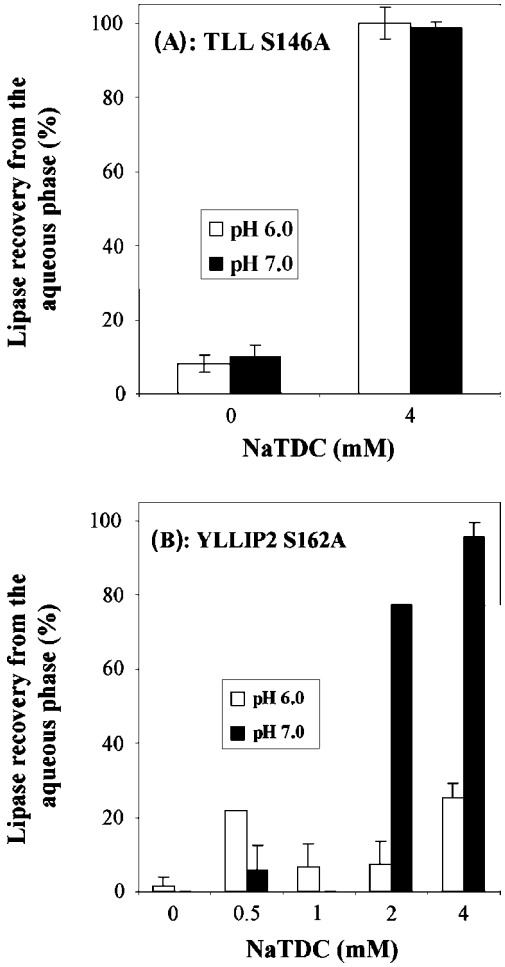


Fig. 8 Effects of pH and NaTDC on the interfacial binding of (**A**) S146A TLL and (**B**) S162A YLLip2 mutants to a trioctanoin emulsion. The interfacial binding was assayed by mixing mutants with a trioctanoin emulsion formed in a pH-stat vessel under mechanical stirring and measuring the amounts of protein (ELISA) remaining in the water phase after the separation of the oil phase. Adapted from (81).

Tween 80). It is, however, not indicated that these surfactants can affect the drug efficacy, and they are, rather, used as excipients for promoting the drug (Orlistat) dispersion and oral bioavailability. Most of the active substances developed by the pharmaceutical industry over the last 30 years for oral administration are lipophilic and therefore show poor bioavailability due to their low solubility in water (84,85). Formulation plays, then, a major role in determining the rate and extent of absorption of such drugs from the gastrointestinal tract. In recent years, and in order to improve oral biodisponibility of hydrophobic drugs, innovative formulations such as SEDDS (Self Emulsifying Drug Delivery Systems), SMEDDS (Self MicroEmulsifying Drug Delivery Systems) and SNEDDS (Self NanoEmulsifying Drug Delivery Systems) have been developed (86,87). SEDDS, SMEDDS, and SNEDDS are isotropic mixtures of oils, surfactants, co-surfactant, and drug that form, under stirring, oil-in-water emulsions, microemulsions, and nanoemulsions, respectively (88).

With regards to their toxicity, these lipid-based drug delivery systems usually include only nonionic surfactants: cationic surfactants appear to be more toxic than anionic surfactants, which seem in turn more toxic than nonionic surfactants (89). Moreover, nonionic surfactants have generally been considered as acceptable for oral ingestion, and the emergence of several successfully marketed products (90) has given the industry confidence in the use of surfactants.

One has to be aware, however, that nonionic surfactants such as polyethyleneglycol esters are also substrates for digestive lipases (91–94). Their association with lipase inhibitors is, therefore, a difficult challenge.

CONCLUSION

Due to their ability to interact directly with lipases and their inhibitors as well as with lipids at oil-water interfaces, surfactants greatly affect these enzymes' activity and inhibition. On the one hand, they can improve the availability of the substrate and the inhibitor by promoting emulsification, by forming mixed micelles and by triggering conformational changes in the enzyme, which improve the accessibility of the active site. On the other hand, surfactants can also significantly reduce the lipolytic efficiency by forming inactive aqueous enzyme-surfactant complexes or by impairing the enzyme's binding at the interface. Depending on the nature of the surfactants and their concentration, they can result in either activation or deactivation. It is therefore of great importance to study the effects of surfactants with great care before carrying out any activity or inhibition assays on lipases.

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