Activity and Stability of *Penicillium cyclopium* Lipase in Surfactant and Detergent Solutions

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ABSTRACT: Activity and stability of an alkaline lipase from *Penicillium cyclopium* var. *album* (PG 37) were studied in surfactant and detergent solutions. Three anionic surfactants [Na salts of $C_{12}SO_4^-$ (sodium dodecyl sulfate), $C_{12}ØSO_3^-$ (linear alkyl benzene sulfonate), and $C_{11}COO^-$ (laurate)] and four homologous series of nonionic surfactants of C_{12-15} polyoxethylenated fatty alcohols (AEO_3, AEO_5, AEO_7, and AEO_9) were evaluated. At a concentration range of 3.2–40 μM, sodium dodecyl sulfate and laurate stimulated the activity of PG 37 lipase. At concentrations greater than 5.6 μM, linear alkylbenzene sulfonate inhibited PG 37 lipase activity. Nonionic surfactants, AEO_5 and AEO_7, in the concentration range of 0.25–20 mM, enhanced and stabilized the activity of PG 37 lipase. The presence of PG 37 lipase in detergent formulaton improved detergency ~20%. The mechanism of inhibition of the lipolytic activity of PG 37 lipase is proposed to be partly due to the formation of inactive $(BR)_n$-E complex between the hydrophobic moiety of the surfactants and the surface of the lipase. Conversely, formation of a soluble $(RB)_n$-E complex between the hydrophilic group of the surfactant and lipase may account for the increased lipolytic activity of PG 37 lipase.
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KEY WORDS: Anionic surfactants, detergents, enzyme stability, lipase activity, microbial lipase, nonionic surfactants, *Penicillium cyclopium,* surfactants.

Alkaline lipases can be used effectively to enhance the removal of oily dirts from fabrics and hard surfaces, and to increase the detergency of compact washing powders or commercial detergents, especially at low temperatures (1–3). The role of lipase in detergency is to promote the removal of triglycerides by lipolytic hydrolysis to diglycerides, monoglycerides, and free fatty acids (3), especially during the presoaking stage. Detergents invariably contain surfactants and various auxiliary agents. These components may interact with lipase, resulting in activation or inhibition of its activity. From the viewpoint of anticipated increased use of lipase in the detergent industry, an understanding of the compatibility of lipase with surfactants and auxiliary agents is important. Although there are several studies on the effects of surfactants (mainly bile acids) on mammalian lipases, relatively little is known about the effects of surfactants on microbial lipases (3,4).

In general, the efficiency of stain removal by lipase depends largely on amount of soil, lipase concentration, washing conditions, and the substrate (5). Andree *et al.* (5) reported that lipase activity was strongest at 30°C and was strongly inhibited by the presence of anionic surfactants. The work by Kawase *et al.* (1) and more recent studies by Mozaffar *et al.* (2) showed that lipase activity was dependent on both type and concentration of the surfactant. On the basis of experimental evidence, Mozaffar *et al.* (2) proposed that the influence of surfactant was related more to interaction with lipase at the oil–water interface than to providing greater interfacial surface area. Kawase *et al.* (1) reported on the relationships between surfactants and lipases from the viewpoint of pH changes. They found that, while nonionic surfactants activated lipases, the effect of anionic surfactants on enzyme activity varied according to the lipase and, for the most part, was inhibitory. They indicated, however, that the inhibition of lipase by anionic surfactants could be avoided by mixing nonionic surfactants (1).

The source of lipase is an important consideration in compatibility studies involving lipases, surfactants, and detergent auxiliaries. This is understandable in view of the fact that that conformation and surface characteristics of lipases differ and could vary in response to environmental conditions. For example, Mucor lipase has been shown to have 16 times greater activity toward polyoxyethylene-sorbitan monooleate (Tween-80) in a nonemulsified system than Pythium lipase (2). *Chromobacterium viscosum* lipase has been shown to be competitively inhibited by sodium dodecyl sulfate (SDS) and cetyl-trimethylammonium bromide (CTAB) and noncompetitively by aerosol-OT (AOT) (2). Conversely, activity of the ex-Candida lipase was unaffected by SDS but was markedly reduced by AOT and CTAB (2).

In this paper, we report the influence of surfactants and detergent auxiliaries on activity and stability of a new alkaline lipase from a mutant *Penicillium cyclopium* var. *album.* Lipase (lipolase) from *Humicula languinosa* was used as a reference for detergency tests.

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MATERIALS AND METHODS

Materials. A homologue series of alkyl polyethoxyl ethers (AEO) [C_{12-15} triethoxyl ether (AEO_3), C_{12-15} pentaethoxyl ether (AEO_5), C_{12-15} heptaethoxyl ether (AEO_7) and C_{12-15} nonaethoxyl ether (AEO_9)] were donated by Shell Chemical Co. (Houston, TX). Anionic surfactants, SDS and sodium dodecyl sulfonate (SAS), were from Sigma Chemical Co. (St. Louis, MO); sodium salts of linear alkyl (C_{12-15}) benzene sulfonate (LAS, 36% active stock) was obtained from Shanghai Detergent Factory (Shanghai, China); alkyl (C_{12}–C_{15}) triethoxyl sulfate (AES_3, >70% active stock) was supplied by Japan Detergent Factory (Osaka, Japan). Polyethylene glycol (PEG 600, >98% purity) was from Shanghai Detergent Factory. Sodium salts of C_4–C_{18} carboxylic acids were prepared in our laboratory, and purity (>98%) was checked by thin-layer and gas chromatographies. All other chemical were reagent-grade or better.

Lipases (E C 3.1.1.3) were obtained as follows. Lipase from a mutant *Penicillium cyclopium* var. *album* (PG 37, activity 1,684 units/g, pH 7–10.5, 25°C) was donated by the Fermentation Department of Wuxi Institute of Light Industry (Wuxi, China). To obtain a hexanic acid-resistant mutant PG 37, *Penicillium cyclopium* var. *album*, PG 92 was induced by ultraviolet (UV) light and guanidine nitrite. By this process, yield of lipase from PG 37 was increased to 59.1%. Lipase (lipolase) from the fungus *Humicula languinosa* (optimum conditions: pH 10.0 and 30°C) was supplied by Novo Industri A/S (Bagsveard, Denmark). Lipolase was used as received from the supplier without further purification.

Assay of lipase activity. Lipolytic activity of lipases was tested with olive oil emulsion, essentially by the surfactant-addition method of Kawase *et al.* (1), with some modification. PG 37 lipase or lipolase was dissolved in 0.05 N glycine buffer (pH 9.4) to achieve a lipase activity of ~10 U/mL. Substrate was prepared by emulsifying 25 mL olive oil with 75 mL polyvinyl alcohol (PVA) (4% PVA in glycine buffer, pH 9.4, or in phosphate buffer, pH 7.2), henceforth referred to as olive oil emulsion. PVA served as a surfactant to stabilize the olive oil emulsion (1). In a typical assay of lipolytic activity, glycine or phosphate buffer (4 mL) and olive oil emulsion (5 mL) were combined in a clean 100-mL Erlenmeyer flask. The pH of the mixture was adjusted as necessary by the addition of 10 mM NaOH and then preincubated in a water bath (~5 min) until the desired temperature was reached. Lipolytic activity was started by addition of the diluted lipase solution (1 mL) into the flask, and incubation in the water bath was continued at the respective optimum temperatures with shaking (~250 rpm). After 10 min, 15 mL of 95% ethyl alcohol was added to the reaction mixture to stop lipase hydrolysis, and the fatty acid content in the mixture was determined by titration to the endpoint (phenolphthalein as indicator) with 0.05 N NaOH. As a blank, the assay procedure was conducted by adding the enzyme after addition of 95% ethyl alcohol. Activity of lipase (unit/g) was calculated as follows:

$$\text{unit/g} = \frac{(S-B)\,50f}{(t)(W)} = \frac{5(S-B)f}{W}$$

$$\text{unit/mL} = \frac{5(S-B)f}{V} \qquad [1]$$

where S = volume (mL) of 0.05 N NaOH to titrate lipase hydrozylates; B = volume (mL) of 0.05 N NaOH to titrate blank solution; f = dilution factor for lipase solution; 50 = μmol of NaOH contained in 1 mL 0.05 N NaOH solution; t = reaction time (10 min); W = weight (g) of the lipase powder in 10 mL assay mixture; and V = volume (mL) of the lipase solution added to the assay mixture. One unit of activity is defined as the amount of enzyme that liberates 1 μmol equivalent of fatty acid from olive oil in 1 min under the analytical conditions.

The above standard assay protocol was modified as necessary and used to determine the stability of lipase toward anionic/nonionic surfactants and inorganic salts. The results were expressed in terms of relative activity.

Effects of anionic and nonionic surfactants on activity of lipase. Effects of surfactants on activity and stability of PG 37 were studied under the standard conditions described previously by determining the enzyme activity in olive oil emulsion with or without anionic [Na salts of $C_{12}SO_4^-$ (SDS), $C_{12}SO_3^-$ (LAS), $C_{11}COO^-$ (laurate)] or nonionic (AEO_3, AEO_5, AEO_7, AEO_9) surfactants. The ratio of the residual activity of lipase in (+) surfactant system to the activity in (–) surfactant system was termed the relative activity, hence an index of inhibition or activation.

Effects of inorganic salts. Effects of inorganic salts, generally used as builders in detergents, on lipase activity were assessed. Inorganic salts tested were sodium chloride (NaCl), sodium carbonate (Na_2CO_3), pentasodium tripolyphosphate ($Na_5P_3O_{10}$), sodium sulfate (Na_2SO_4), sodium silicate (Na_2SiO_3), and calcium chloride. Each inorganic salt was incorporated into the lipase reaction mixture (as described in lipase assay section) to give concentration of 0.01–0.2 N. The lipase assay was followed as in the standard protocol, and the relative activity was determined.

Detergency test. Detergency of lipase-containing detergent was examined in a Launder-O-Meter (Atlas Electric Devices, Chicago, IL) at 35°C by the standard method of detergency determination in China and compared with lipase-free detergent. The test detergent composition is presented in Table 1. The procedure for detergent determination consisted of three steps: fabric-specimen preparation, fabric soiling, and washing test.

Preparation of fabric specimens. White cotton fabrics (27 cm × 44 cm), purchased from Daily Chemical Research Institute, Ministry of Light Industry (Jiangsu, PR China) were put in 0.8% NaOH solution and boiled for 1 h; then the solution was decanted. Treated fabrics were rinsed with distilled water several times until the pH of the rinse was neutral. Treated fabrics were then put in 0.13% neutral soap solution, boiled

TABLE 1
Detergent Compositions (%)

Constituents	% in Formulation		
	Control	1	2
Mixed surfactants[a]	19	19	19
PEG-60[b]	3	3	3
Polyvinyl glycol[b]	6	6	6
Na_2SO_4[b]	5	5	5
Pyrophosphate[b]	5	5	5
Sodium acetate[b]	1	1	1
Ethyl alcohol (95%)[b]	4	4	4
PG 37 lipase	—	4	—
Lipolase	—	—	1
Water		Remainder	

[a]Mixed surfactant consisted of AEO9/methyl ester sulfonates/alkanolamide (15:3:1, wt%).
[b]Builders (metal-chelating agents); total = 24%.

for 30 min, and rinsed thoroughly until the fabrics were free of soap. Fabrics were air-dried overnight at room temperature prior to staining and detergency tests.

Preparation of oily-dye stain. First, an artificial carbon black colloidal suspension (CABL) was prepared as follows: arabic gum (3.2 g) was dispersed in 15 mL distilled water and heated to dissolve. Carbon black (2.3 g) was combined with 10 mL of 95% ethyl alcohol in a porcelain grinder, and 25 mL water was added; the mixture was ground for 30 min. Dissolved gum arabic was added to the mixture, followed by additional grinding for 2 min. The mixture was transferred to a large beaker, including several rinses of the grinder. Total volume of the liquor was brought to 1,500 mL with ethyl alcohol/distilled water (1:1, vol/vol). The diluted solution was the so-called CABL.

Second, oily liquor was made from castor oil, liquid paraffin, and lanolin (1:1:1, w/w), with lecithin (0.4 g/mL in 50% ethyl alcohol) as an emulsifier. The weight ratio of lecithin and mixed oil was 2:1.

To prepare the oily-dye stain, oily liquor (25 mL) was added gradually into a beaker that contained heated (55°C) CABL (500 mL). The oily-liquor flask was rinsed thoroughly with 25 mL of 50% ethyl alcohol, and all the rinses were poured into the heated beaker. The oily-CABL mixture was heated (55°C) for 30 min with continuous stirring and then cooled to ~46°C before filtration through fine cloth to remove the foam in the upper layer. The filtered oily-dye liquor (stain) was used to soil the dried fabrics.

Fabric treatment with the oily-dye stain. Five identical circles of 6-cm diameter were cut from each fabric. The white circular fabrics were dyed twice with the oily-dye stain. After each dyeing, the fabrics were air-dried. Soiled fabrics were aged three days at room temperature (~25°C) with a relative humidity of ~70%. The whiteness of the fabrics was determined by reflectometer (model 581; Shanghai Koowei Factory, Shanghai, China) with pure white magnesium carbonate as a reference standard. The reflectance reading was generally ~27%, based on a reference standard of 100% whiteness. Five readings from each side of the circular fabric were taken, and the average value of ten readings was regarded as the whiteness intensity.

Washing test. Three detergent formulations (Table 1) (one control and two test detergents) were evaluated. Washing tests were carried out in a Launder-O-Meter, consisting of 24 flasks (400-mL capacity) with 20 rubber balls (14-mm diameter). After the addition of detergent solution (300 mL) into each flask containing the soiled fabrics, the latter were washed at 35°C for 1 h at a rotational speed of 40 rpm. The fabrics were rinsed with water and dried in vacuum oven (50°C) to constant weight (~10 h). Whiteness intensity of fabrics was determined as described earlier. Detergency of the various formulations was calculated as follows (3):

$$\text{detergency } (\Delta R, \%) = \frac{Rw - Rs}{Ro - Rs} \times 100 \quad [2]$$

where Rw = reflectance of the washed fabric; Rs = reflectance of the soiled fabric; and Ro = reflectance of the fabric before soiling.

RESULTS AND DISCUSSION

Effects of surfactant on activity of lipase. Effects of anionic and nonionic surfactants on the activity of PG 37 lipase are presented in Figure 1 (A and B, respectively). Although all anionic surfactants examined showed concentration-dependent inhibitory effect on PG 37 lipase activity, the enzyme tolerated higher concentrations of SDS and laurate than LAS. At a concentration range of 3.2–40 μM, SDS and laurate stimulated the activity of PG 37 lipase (Fig. 1A). At concentrations greater than 5.6 μM, LAS inhibited PG 37 lipase, and the relative activity of lipase decreased almost linearly (Fig. 1A). The strong inhibitory effect shown by LAS in comparison to SDS may be attributed to its structure. Sulfonation of the aromatic nucleus, benzene, besides its substituted alkyl groups, greatly enhances the amphiphilic character of the LAS molecule, thus accounting for its superior surface activity over SDS. Furthermore, due to the benzene ring in the LAS molecule, LAS would be expected to involve itself in electrophilic substitution reactions. These properties make LAS a stronger protein denaturant than SDS, perhaps kexplaining the lower concentration of LAS required to inhibit PG 37 lipase activity.

Nonionic surfactants, AEO_5 and AEO_7, in the concentration range of 0.25–20 mM, enhanced and stabilized the activity of PG 37 lipase (Fig. 1B). The reason for the inhibitory effects of AEO_3 and AEO_9 on PG 37 lipase activity is not clear. While dissolution of the lipase by AEO_9 is a likely explanation for its inhibitory mechanism, it is reasonable to assume that AEO_3, due to its low EO number, was less soluble in water than the higher homologues. For all nonionics studied, however, their inhibitory effect on lipase became abrupt when their concentrations exceeded 20 mM.

Several factors could influence lipase activity, including: (i) change in ionic groups of lipase protein, (ii) change in con-

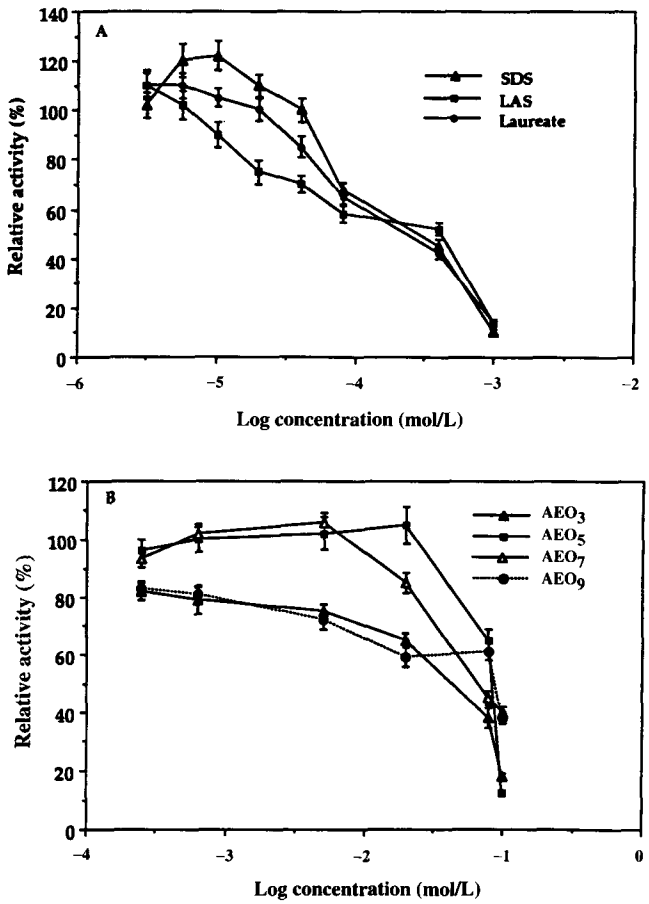

FIG. 1. Influence of anionic (A) and nonionic (B) surfactants on the activity of PG 37 lipase. Data points are means ± standard deviations (n = 6) of triplicate experiments, each analyzed twice. Concentrations of anionic surfactants in the reaction mixture ranged from 3.2–1000 μM, and nonionic surfactants ranged from 0.25–100 mM. Lipase assay was performed at 30°C (pH 9.4) for 10 min.

formation of lipase, and (iii) subsequent change in surface activity of lipase (1). The response of PG 37 lipase to anionic and nonionic surfactants in this study was similar, in principle, to the findings of Kawase *et al.* (1). They found that the presence of low concentrations of SDS increased the activities of *Rhizopus* and *Chromobacterium* lipases; at high concentration (0.005%), however, SDS was inhibitory. Their kinetic data further revealed that the mode of SDS inhibition was competitive, suggesting that the SO^3 groups of SDS perhaps adsorbed on the emulsion surface and influenced the active site of lipase (1). Conversely, the work of Mozaffar *et al.* (2) suggested that the influence of surfactant was related more to interaction with enzyme at the oil–water interface than to providing greater interfacial surface. Both proposals by Kawase *et al.* (1) and Mozaffar *et al.* (2) are plausible, although experimental conditions and lipase sources used in their investigations were different.

Another mechanistic insight of surfactant inhibition/activation of lipase may be drawn from a complex formation viewpoint. SDS is known to bind cooperatively to most proteins, the critical concentration for cooperative binding being about 25% of the critical micelle concentration (CMC) (4). Nonionic surfactants rarely induce cooperative binding and therefore do not usually denature proteins, although dissolution into inactive or less-active subunits can occur (4). Therefore anionic surfactants, such as SDS, probably interact at low concentration with lipase protein to produce a new lipase-surfactant complex that becomes more surface-active than native lipase (1). Such a complex would act on the substrate and perhaps stimulate lipase lipolytic activity, but at higher concentrations, excess surfactant would induce conformational changes or influence the active site of lipase and produce inhibition (1). Conversely, for nonionic surfactants, such conformational changes would hardly occur until high concentrations are reached because of weak electrostatic interactions of surfactant with protein and the active site of lipase (1). Indeed, our results lead to the same conclusion. In our experiments, we purposely examined the concentration ranges below the CMC of the anionics (SDS, LAS, and laurate) and above the CMC of the nonionics (AEO_3, AEO_5, AEO_7, and AEO_9). As evident from Figures 1, SDS and laurate stimulated lipase activity at a narrow low concentration ranges, whereas AEO_5 and AEO_7 stabilized and enhanced lipase activity in a broad high concentration ranges.

We propose that hydrophilic groups in surfactants interact with lipase to form a complex $(RB)_n$-E that facilitates enzyme–substrate interaction, thus increasing the rate of lipolytic activity. Here, R represents the hydrophobic moiety of surfactants; B the hydrophilic moiety of surfactants; E lipase; and n the number of surfactant molecules. At high surfactant concentration, however, hydrophobic moieties of the surfactant molecules may have access to the inner hydrophobic active center of lipase by electrostatic charge attraction and van Der Waal forces, resulting in formation of a $(BR)_n$-E–type complex. This type of complex is likely to induce micelle formation and consequently cause changes in conformation and surface properties of the lipase. In this case, the lipolytic activity of lipase would be expected to decrease due to enzyme denaturation.

Effects of inorganic salts on activity of lipase. Figure 2 shows the comparative effects of inorganic salts on PG 37 lipase activity. The effect of salts with different anionic groups on PG 37 lipase activity was examined. Figure 2 shows that neutral salts, NaCl and Na_2SO_4, at concentrations of 5–100 meq/L, effectively stimulated lipase activity; for Na_2SO_4, its stimulatory action extended to 200 meq/L. Similar stimulatory action by NaCl, in the case of pancreatic lipase, has been reported by Benzonana and Desnuelle (6). Possibly, NaCl promotes the normal poor ionization of long-chain fatty acids at alkaline pH values (6). The explanation for the stimulatory action of NaCl and Na_2SO_4 in this present study can only be speculative. First, lipase, as other hydrolases, attacks its substrate as a nucleophile, forms a substrate–enzyme complex and is thereby aculated, and transfers the acyl group to water (7). Second, as a weak nucleophilic agent, lipase requires that the substrate be sufficiently desolvated to facilitate enzyme–substrate contact (7). Probably, certain salts promote

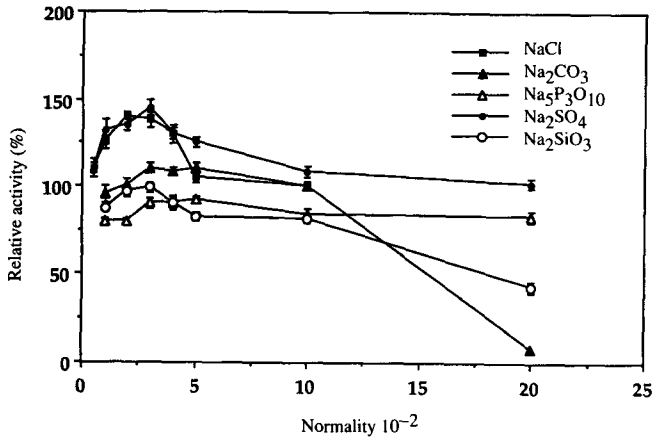

FIG. 2. Effects of inorganic salts on the activity of PG 37 lipase. Data points are means ± standard deviations (n = 4) of duplicate experiments, each analyzed twice. Each inorganic salt was incorporated into the lipase reaction mixture to give concentrations of 0.1–0.2 N. Lipase assay was performed as described in the Materials and Methods section.

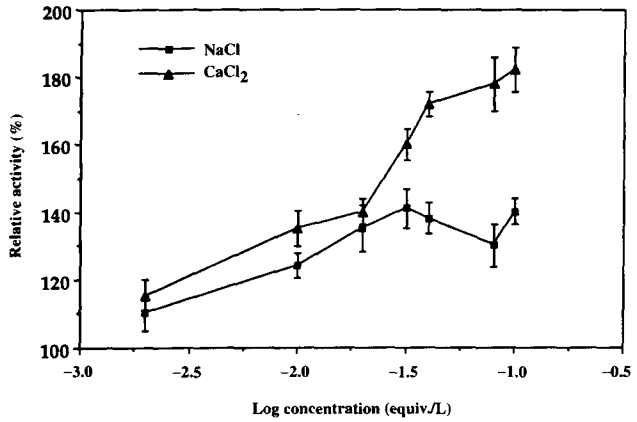

FIG. 3. Effects of NaCl and $CaCl_2$ on the activity of PG 37 lipase. Data points are means ± standard deviations (n = 4) of triplicate experiments, each analyzed twice. NaCl and $CaCl_2$ salts were incorporated into the lipase reaction mixture to give concentrations of 2.5–100 meq/L. Lipase assay was performed as described in the Materials and Methods section.

lipolysis, in part, by increasing the rate of desolvation. It is also logical to assume that the effect of these salts perhaps depended on the competitive adsorption of their anion and cation species onto the substrate and/or lipase. From this viewpoint, Na^+ ion is likely the stimulant because its adsorption to the lipase surface would be favored over Cl^- or SO_4^- due to electrostatic interaction, given the alkaline pH environment. For $Na_5P_3O_{10}$, however, for its multiple negative charges ($P_3O_{10}{}^{5-}$) may counteract the stimulatory effect of Na^+, thus causing inhibition of lipase activity (Fig. 2). This ovservation is of practical importance in lipase-containing detergents where sodium tripolyphosphate may be present as a builder.

It is interesting that salts with the same valence of the anions showed different effects: Na_2SO_4 was stimulatory, while Na_2SiO_3 and Na_2CO_3 were inhibitory. At concentrations above 100 meq/L, inhibition of lipase activity by Na_2CO_3 was more drastic than $Na_5P_3O_{10}$ or Na_2SiO_3 (Fig. 2). This observation seems logical because Na_2CO_3 is a strong alkali, whereas $Na_5P_3O_{10}$ and Na_2SiO_3 are known (5) to possess buffering and chelating properties. The adverse effect of sodium carbonate on lipase activity may be attributed to its strong tendency to complex or precipitate calcium, and necessary co-factor for lipase action. The order of effects may be expressed as follows: Na_2SO_4 = NaCl > Na_2CO_3 > $Na_5P_3O_{10}$ = Na_2SiO_3. This implies that the activity of PG 37 lipase is inversely proportional to the alkalinity of salt solutions.

To investigate the influence of divalent vs. monovalent cations on lipase activity, a set of experiments was conducted with $CaCl_2$ in parallel with NaCl. The result is presented in Figure 3. The presence of $CaCl_2$ greatly stimulated PG 37 lipase activity over NaCl at 100 meq/L. Calcium appears to stimulate the activity of various lipases (8). Several theories have been advanced to explain the basis of the stimulatory effect of calcium ions on lipase activity. An earlier theory suggested that calcium ions may remove free fatty acids, produced during lipolysis, as insoluble calcium soaps, thereby permitting lipolysis to continue (8). Also, the valence disposition of the calcium atom has been viewed as a factor. It is well known that the nature of divalent calcium is quite different from that of monovalent sodium atoms. The polarization of Ca^{2+} is stronger than Na^+, and therefore, Ca^{2+} is more prone to interact with lipase than Na^+. Furthermore, coordination bonding of the vacant orbit of the calcium atom with lone-pair electron-containing oxygen, sulfur, or nitrogen atoms of the lipase molecule is likely (9).

In general, low concentrations of calcium, sodium, potassium, and magnesium salts are reported to stimulate lipolysis, and salts of heavy metals are strongly inhibitory toward most of the microbial lipases (10,11).

Detergency test. Table 2 compares the detergency of lipase-free with lipase-containing detergents. The presence of PG 37 lipase improved the detergency by ~20%, and its performance was comparable to that of lipolase, a lipase used in household detergents. This suggests that PG 37 lipase may potentially enhance the detergency of synthetic detergents. Much more research is needed to fully explore this enzyme in terms of optimum conditions and synergistic properties; nevertheless, this preliminary finding is encouraging.

The potential use of lipase in detergents is gaining interest in the detergent industry, as reflected in several review articles (12,13). Many studies with lipases have demonstrated that the best effect on fat removal is seen after more than one wash (13). Andree *et al.* (5) indicated that commercially available microbial and pancreatic lipases may contribute to the washing efficiency of presoak, 60°C, and woolens detergents, if they are used as moderate temperatures in the range of 20–30°C. They (5) noted that lipase activity was strongest at 30°C and that the presence of anionic surfactants in the detergent formulations strongly inhibited lipase activity. Fur-

TABLE 2
Comparison of Detergency of Lipase-Containing and Lipase-Free Detergent Formulations[a]

Formulation	Detergency (%)	Improvement in detergency (%)
Lipase-free detergent	56.3 ± 3.2	—
Detergent + PG 37 lipase	67.5 ± 2.9	19.9
Detergent + lipolase	68.4 ± 4.4	21.5

[a]Mean ± standard deviation of five washings.

thermore, Andree *et al.* (5) demonstrated that the improvement in detergency, obtained by the addition of lipase to the detergent formulation, was similar to that achieved by increasing the concentration of nonionic surfactants in the detergents (5).

In conclusion, inhibition of the activity of PG 37 lipase by anionic surfactant is concentration-dependent. The enzyme tolerates higher concentrations of SDS and laurate than LAS. However, nonionic surfactants, AEO_5 and AEO_7, at low but above CMC values, enhances and stabilizes the activity of PG 37 lipase. Calcium ions and dilute solutions of neutral salts, NaCl and Na_2SO_4, effectively stimulate lipase activity. The presence of PG 37 lipase in detergent formulations improves their detergency by ~ 20%, suggesting that PG 37 lipase may potentially enhance the detergency of synthetic detergents.

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