Partial Purification and Characterization of Acylester Hydrolase from *Lupinus mutabilis*

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ABSTRACT: An alkaline acylester hydrolase was partially purified from germinated seeds of *Lupinus mutabilis*. Hydrolytic activity was absent in the crude extract of ungerminated lupine seed, but it increased and peaked at the fourth day in the germinating seedling. The purification scheme involved homogenization, centrifugation, acetone precipitation, anion exchange chromatography, pH precipitation, and hydrophobic interaction chromatography. The acylester hydrolase was purified 126-fold, and the overall activity yield was 10%. The molecular weight estimated by sodium dodecylsulfate-polyacrylamide gel electrophoresis was 60 kDa. The enzyme had an isoelectric point of 6.2 and showed maximal activity at pH 8.0. The enzyme showed good stability between pH 5.0 and 9.0. In the pH range 7.0–7.5, enzyme precipitation was observed. The enzyme was stable from 0 to 25°C for 5 h and at 45°C lost 50% of its activity in the same period of time. At higher temperatures, the enzyme showed low thermal stability. However, the highest initial activity was found to be at 45°C. Nonionic surfactants and cholic acid enhanced the activity of the enzyme. The activity was reduced by the addition of toluene and isooctane and increased by the addition of diethyl ether, acetonitrile, methanol, and pyridine. The activity was reduced by 37% in the presence of 1 mM Cu^{2+} ions. The enzyme-hydrolyzed triolein showing no positional specificity.

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Lipase (triacylglycerol ester hydrolase, EC 3.1.1.3) is an enzyme widely present in animals, plants, and microorganisms. This type of enzyme has become the subject of intense research during the last decade, especially due to its potential applications in industry (1), in synthetic reactions (2), and in washing processes (3). In this context, the search for new lipases, with new and better specificities, and the technologies for their use are driving these investigations. Lipases with high activity levels at alkaline conditions and with anionic surfactants present are very useful as potential detergent ingredients (4,5). For these applications, the sources of lipase have been microorganisms such as mold and yeast. Most lipases are reportedly unstable at alkaline conditions in the presence of anionic surfactants (6). Lipase from plants has been studied only to a minor extent (7). Germinating oilseeds are being explored as a possible source of lipase for the biotechnological processing of oils and fats (8). Seeds generally contain starch, proteins, and triacylglycerols as their food reserve for germination. Most of the investigations on lipase have been carried out on oleaginous seeds. In oil seeds, lipase activity is generally expressed during germination (8–10). The utilization of the storage fats is initiated by hydrolysis of triacylglycerols to free fatty acids and glycerol by the lipase. These intermediate products are then converted to sucrose by a long gluconeogenic pathway (11,12) for the support of plant growth. *Lupinus mutabilis* is a major crop in the Andean regions of Perú, Bolivia, and the northern Chile. The nutritional properties and high fat content (15–30%) of lupines are well documented (13–17). This study describes the extraction, purification, and properties of an acylester hydrolase from germinated seeds of lupine.

MATERIALS AND METHODS

Lupinus mutabilis seeds were purchased at a Sunday market in Cuzco, Perú. Phenyl Sepharose and diethylaminoethyl (DEAE) Sephadex were obtained from Pharmacia-LKB (Sollentuna, Sweden), thin-layer chromatography (TLC) plates and Silica gel 60 were obtained from Merck (Darmstadt, Germany). *p*-Nitrophenyl (pNP) esters, the dye Fast garnet GBC base, and α-naphthyl acetate were from Sigma (St. Louis, MO). All reagents and chemicals used were of analytical grade.

Germination of lupine seeds. The seeds were washed, and soaked for 20 min in distilled water, then wrapped with wet cotton and paper and placed in an incubator. The germination was carried out in darkness at 24°C with a relative humidity of 80–100%. Germinated seeds with similar root length were harvested every day for 7 d. They were then weighed, counted, and stored at –20°C.

Extraction of acylester hydrolase. The extraction was carried out at 4°C and the entire germinating seed of lupine was used. Seedlings (50 g) were washed three times with distilled water and homogenized in 200 mL of grinding medium using a household mixer (Braun, Germany). The grinding medium

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contained: 0.5 M sucrose; 1 mM EDTA; 1 mM $MgCl_2$; 2 mM dithiothreitol; and 150 mM, pH 5.0 citrate buffer or 150 mM, pH 7.0 or 9.0 Tris-HCl buffer. The homogenate was filtered through a piece of Nitex Cloth (Petko, Elmsford, NY) with a pore size of 20 × 20 μm. The filtrate was centrifuged at 13,000 × *g* for 20 min at 10°C. Three fractions were obtained; an upper fat layer (spherisomal fraction), a pellet of particulate material, and a liquid fraction (18). The fat layer was carefully removed with a spatula and the liquid supernatant separated from the pellet. The extracted liquid was used as the crude source of acylester hydrolase.

Enzyme activity. Enzyme activity was determined with *p*-nitrophenyl acetate (pNPA) as substrate which is hydrolyzed to *p*-nitrophenol sodium (pNP). A 10-μL solution of 100 mM pNPA in methanol was mixed well with 2 mL phosphate buffer (20 mM, pH 8.0) in a 3-mL cuvette. The reaction was started by adding 50 μL of enzyme solution. The formation of pNP was measured as an increase in absorbance at 410 nm. The reaction rate was linear for the entire time interval of 2–3 min. One unit of acylester hydrolase activity was defined as the amount of enzyme that released 1.0 μmol of pNP per min. The activity observed was proportional to the amount of enzyme added.

Protein measurement. Quantification of proteins was carried out using the bicinchoninic acid method (19) with bovine serum albumin (BSA) as standard.

Purification of acylester hydrolase. To the liquid fraction obtained above (pH 7.0 unless otherwise noted) was added 60 vol% cold acetone (–20°C). The mixture was left to stand for 1 h to allow protein precipitation to occur. The precipitate was separated by centrifugation at 8,000 × *g* for 10 min. The pellet was then collected, desiccated overnight, and ground. The precipitated protein (10 g) was dissolved in 50 mL of 10 mM sodium citrate buffer (pH 6.0). DEAE-Sephadex (2 g) was then added, followed by incubation at 4°C, for 20 min on a reciprocal mixer. The mixture was filtered, and protein content and the acylester hydrolase activity of the filtrate were determined prior to lyophilization.

pH precipitation. Lyophilized enzyme (78 mg) from the previous DEAE-Sephadex treatment was dissolved in 2 mL Tris-HCl buffer 10 mM, pH 7.2, and allowed to stand at 4°C for 1 h before centrifugation (5,000 × *g* for 5 min). The precipitate containing the acylester hydrolase activity was then dissolved in 10 mM citrate buffer (pH 4.0) followed by incubation at 4°C for 1 h before centrifugation (5,000 × *g* for 5 min). The acylester hydrolase activity and protein contents were determined on the precipitates and the supernatants.

Ammonium sulfate precipitation. Protein present in the supernatant in 10 mM sodium citrate buffer (pH 4.0) was precipitated with $(NH_4)_2SO_4$ at 35% of saturation and 4°C for 1 h, then after centrifugation at 5,000 × *g* for 5 min, the supernatant was removed from the pellet. The procedure was repeated at 55, 75, and 100% saturation. At the end, four different pellets were obtained which were directly lyophilized and aliquots were dissolved in 10 mM sodium citrate buffer (pH 6.0); and the activity was assayed.

Hydrophobic interaction chromatography. A phenyl Sepharose column 50 × 2 cm was used for hydrophobic interaction chromatography. It was preequilibrated with 10 mM sodium citrate buffer (pH 6.0) with 2.0 M sodium sulfate. The sample (100 mg of protein) loaded onto the column was from the 75% $(NH_4)_2SO_4$ saturation pellet dissolved in 5 mL of 10 mM sodium citrate buffer (pH 6.0) with 2.0 M sodium sulfate. The column was washed with the same buffer to elute unbound material. The bound protein was then eluted stepwise with lowering concentrations of sodium sulfate in the same buffer (1.5, 1.0, 0.5, and 0.0 M). The absorbance of the eluate was monitored at 280 nm using a Uvicord detector from LKB (Sollentana, Sweden). The flow rate was 1.5 mL/min. Sixty fractions of 5 mL each were collected, and protein content and acylester hydrolase activity were assayed.

SDS-polyacrylamide gel electrophoresis (PAGE). SDS-PAGE of protein fractions from different stages of purification was performed using a 12.5% acrylamide gel with 5% stacking gel according to Laemmli (21), in a Bio-Rad Mini Protean apparatus. Protein was visualized by staining with Coomassie Brilliant Blue R (Sigma Chemical Co.).

Native PAGE. Nondenaturing electrophoresis for the enzyme was performed in the Mini Protean unit at 12°C using a low-pH discontinuous system (acetic acid-KOH), with a stacking gel buffer at pH 6.5 (5% acrylamide), resolving gel buffer at pH 4.5 (12.5% acrylamide), and reservoir buffer of acetic acid-β-alanine (pH 4.5). The system operated with the cathode at the bottom of the gel. The electrophoresis run lasted almost 2 h. Protein was stained as for SDS-PAGE.

Activity visualization of lipase on native PAGE-gel. After electrophoresis on acidic native gels, acylester hydrolase activity was observed as follows: A substrate coupler-staining solution was prepared by dissolving 20 mg of α-naphthylacetate (α-NA) in 4 mL of *N,N*-dimethyl formamide (DMF) and then added to a 36-mL solution of 20 mg Fast garnet GBC base in 0.05 M acetic acid-KOH buffer (pH 5.5). The gel was incubated in 0.1 M acetic acid-KOH buffer (pH 5.5) for 30 min at 37°C. It was then placed in a freshly prepared substrate coupler-staining solution for 1 h at 37°C. The reaction was stopped by decanting the coupler solution and covering the gel with 2% acetic acid.

Isoelectric focusing. The isoelectric point (pI) of purified lupine enzyme was determined using a free solution isoelectric focusing apparatus (Rotofor cell, Bio-Rad). In a first run, Biolyte 3/10 was used as the carrier ampholyte, giving an operating range of 3.8–9.3. For the second run, a carrier ampholyte with a operating range of 5.0–8.0 was used. Both ampholytes were from Bio-Rad. The isoelectric focusing was performed according to the instructions provided by the manufacturer. Lupine acylester hydrolase sample (2 mL, 10 mg protein) was used for the procedure. The enzyme was monitored by both activity and protein measurements.

Gas chromatography. For the analysis of monoolein, diolein, and oleic acid, samples were immediately extracted with diethylether, then dried and derivatized by the addition

of 10 μL of *N*-methyl-*N*-trimethylsilyl-heptafluoro-butyramine (MSHFBA). The derivatization reagent converts all hydroxyl groups to the corresponding trimethylsilyl ether. After the addition of MSHFBA, the samples were incubated for 15 min at room temperature and were then diluted with 1 mL hexane. Dried ethanol (15 μL) was added to react with any excess of MSHFBA. Analysis was carried out on a Varian 3400 gas chromatograph equipped with an 8035 auto sampler and a flame-ionization detector. An 8-meter DB-1 column (0.32 mm i.d., 0.15 μm film thickness) from J&W (Folsom, CA) was fitted to the high-performance insert of the injector. Helium was used as a carrier gas at constant pressure (55 KPa for analysis of glyceride species). The column was held at 100°C for 3 min, then the temperature was increased to 220°C at 13°C /min and held at 220°C for 3.5 min. The detector temperature was 350°C, 2-monoolein eluted before 1-monoolein and baseline separation was obtained. Standards for 2-mono, 1,3- and 1,2-diolein were used, and tricaproin was used as internal standard.

Fractionation of the lupine fats. Dried and ground spherisomal fraction (20 g) from the homogenized seedlings was dissolved in hexane, and the free fatty acids were washed away by extraction with methanol/bicarbonate buffer (1:1, vol/vol, pH 10.0). Hexane was evaporated and the oil was loaded onto a silica gel 60 column (35 × 4 cm) previously equilibrated with hexane, and the triacylglycerols were eluted with hexane/diethylether (70:30 vol/vol). The fractions collected were checked by TLC, fractions which contained lupine-triacylglycerols were pooled and the solvent evaporated.

Substrate specificity. The hydrolysis of triacylglycerol was carried out by mixing 250 μL of solution A: 100 mM monoacid triacylglycerol with one of the fatty acids; $C_{2:0}$, $C_{3:0}$, $C_{4:0}$, $C_{10:0}$, $C_{12:0}$, $C_{18:0}$, $C_{18:1}$ or $C_{20:0}$, 10% gum arabic in water (the mixtures were emulsified with a homogenizer (Disp 25, InterMed, Roskilde, Denmark) for 2 min, with 250 μL of solution B: 1% NaCl and 1% deoxycholic acid dissolved in 50 mM sodium phosphate buffer (pH 8.0). The reaction was started by adding 50 μL of enzyme solution. The mixture was incubated at 40°C, at 175 rpm on a reciprocal shaker. Samples were withdrawn, and the hydrolytic products were extracted with diethylether for TLC and GC analysis.

In the hydrolysis of pNP esters, the reaction mixture was composed of 0.95 mL of substrate [2.63 mM pNP esters in 50 mM Tris-HCl buffer (pH 8.0) containing 4% Triton X-100]. The reaction was started by adding 50 μL of acylester hydrolase solution. It was then incubated with stirring at 37°C for 15 min. The reaction was stopped by the addition of 2.0 mL of acetone and the absorbance was measured at 410 nm.

TLC. Silica gel 60 F 254 plates were used for monitoring the progress of the lupine-fat fractionation and the hydrolysis of the triacylglyceride. Mobile phases were: hexane/ether (70:30, vol/vol) and chloroform/acetone/acetic acid (88:12:1, vol/vol/vol), respectively. The detection was done by dipping in sulfuric acid/ethanol (15:85, vol/vol), and spots were visualized by heating. For studies of the positional specificity of lupine acylester hydrolase on TLC, silica gel 60 (10 × 20 cm × 0.2 mm) plates were first washed by eluting with acetone/water (60:40 vol/vol) as the mobile phase and dried. They were then dipped in boric acid, 50 g/L in ethanol, and dried at 105°C for 1 h and then kept dessicated until used for analyzing samples from fat hydrolysis.

RESULTS AND DISCUSSION

The highest acylester hydrolase activity was expressed after 4 to 5 d of germination. When grinding the seedlings, the grinding medium with Tris/HCl buffer (pH 7.0) showed the best performance for extracting and/or expressing the acylester hydrolase activity (Fig. 1). The lupine-acylester hydrolase is present in germinated seeds, but not in ungerminated ones. This finding agrees with the characteristic pattern of oilseed lipase (10,22)

Purification of lupine enzyme. The purification sequence of lupine acylester hydrolase is summarized in Table 1. The precipitation with acetone increased the specific activity fourfold. During the DEAE-Sephadex treatment, the enzyme remained in the supernatant and the activity yield increased 100%, suggesting that some inhibitors were adsorbed onto the resin. Precipitation at pH 7.2 provided an important increase of the specific activity. The enzyme was present in the precipitate. After precipitation at pH 4.0, the activity was found in the supernatant, and the specific activity was doubled by the precipitation of other proteins. Ammonium sulfate precipitation was evaluated. For the purification scheme, precipitation of the enzyme between 55 and 75% saturation was chosen. Phenyl Sepharose chromatography worked successfully only when 2 M sodium sulfate favored the hydrophobic

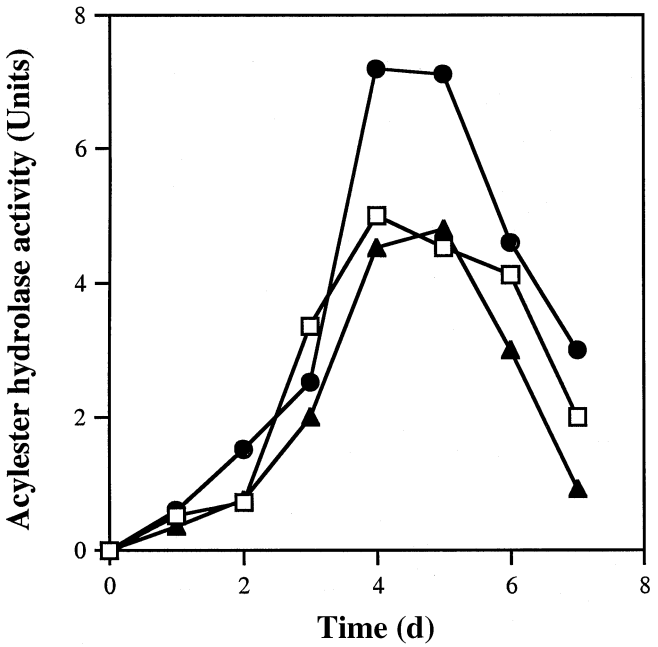

FIG. 1. Acylester hydrolase activity during germination. Extraction with 50-mM grinding medium buffers. Citrate buffer (pH 5.0) (□); Tris/HCl buffer at pH 7.0 (●); and 9.0 (▲).

TABLE 1
Purification Procedure of Acylester Hydrolase from *Lupinus mutabilis* Germinating Seed[a]

Step of purification	Total activity (U)	Specific activity[b] (U/mg protein)	Activity yield (%)	Protein (g)	Purification factor
Crude extract	8090	0.9	100	9	1.0
Acetone precipitation	6470	3.59	80	1.8	4.0
DEAE-Sephadex	12950	6.64	160	1.95	7.4
pH 7.2 precipitation	2020	25.9	25	0.078	29
pH 4.0 precipitation	1800	53.1	22	0.034	59
$(NH_4)_2SO_4$ (75%) precipitation	1270	83.5	16	0.015	93
Phenyl Sepharose	750	113.3	9	0.0066	126

[a]Batch of 50 g of germinating seed.
[b]Activity was determined by photometric assay with pNPA as substrate.

interaction of acylester hydrolase with phenyl Sepharose. The enzyme could be eluted with 0.5 M sodium sulfate. The entire purification procedure resulted in 126-fold increase in specific activity.

Visualization of the acylester hydrolase activity. The staining of enzyme activity in native acidic PAGE showed one band of activity, evidencing the hydrolysis of α-NA by the alkaline lupine acylester hydrolase. In a previous native electrophoresis with basic gel, the band of activity was not observed, obviously due to the fact that the enzyme could not enter that gel.

Characteristics. The apparent molecular weight of the purified enzyme was estimated to be 60 kD by SDS-PAGE. The gel showed one main band and four to five very faint bands. To confirm that the main band (60 kD) was the enzyme, a native gel electrophoresis was done and the band that gave enzyme activity was excised. The enzyme was extracted and analyzed with SDS-PAGE. The latter confirmed that the protein showing activity in the native gel migrated identically with the 60 kD protein in the SDS-PAGE. This molecular weight was within the range (60–65 kD) of other reported plants lipases (7,23,24). The pI value of this enzyme was estimated to be 6.2 using a Rotofor isoelectric focusing apparatus.

Effect of pH on activity and stability. The acylester hydrolase activity was measured in different 20-mM buffers of varying pH (from 5.0 to 8.0) using pNPA as substrate. The enzyme exhibited the highest activity at pH 8.0. At pH 9.0, the activity decreased by almost 80%. The other plant lipases, from rapeseed and mustard, also showed the same pH profile (8–10). At low pH, the nitrophenol released is not fully ionized and the molar absorbance drops. This produces errors in the activity measurements at low pH. At any rate, the enzyme showed activity at pH 5.0 and 6.0.

The effect of pH on stability was studied by dissolving the lupine acylester hydrolase in different 50-mM buffers (from pH 2.0 to 9.0) and incubating for 12 h at 30°C. Initial activity at time 0 was 100%. The activity analysis was carried out using pNPA as a substrate in 20 mM sodium phosphate buffer, at pH 8.0. Figure 2 shows that the enzyme was most stable at pH 6.0 (maximal residual activity). At pH 2.0 and 3.0 the enzyme precipitated and lost activity. At a higher pH than 6.0, the stability decreased: around 70% of the activity remained at pH 7.0 and 8.0, and 40% remained at pH 9.0.

Temperature dependence of the initial activity and stability. The effect of temperature on the initial activity was established by measuring the enzymatic activity with pNPA as substrate in 20 mM Tris-HCl buffer, pH 8.0, at different temperatures (Fig. 3). The enzyme activity was highest around 45°C.

To study the thermal stability of the lupine acylester hydrolase, pure and unpurified lupine enzyme was kept for 1 yr at −20°C. Purified lupine enzyme lost 5% of its activity, but the unpurified enzyme did not show any loss of activity. This is similar to the long-term stability found in other plant lipases (8,25). The activity was measured after incubation at different temperatures between 25 and 65°C in 50 mM Tris-

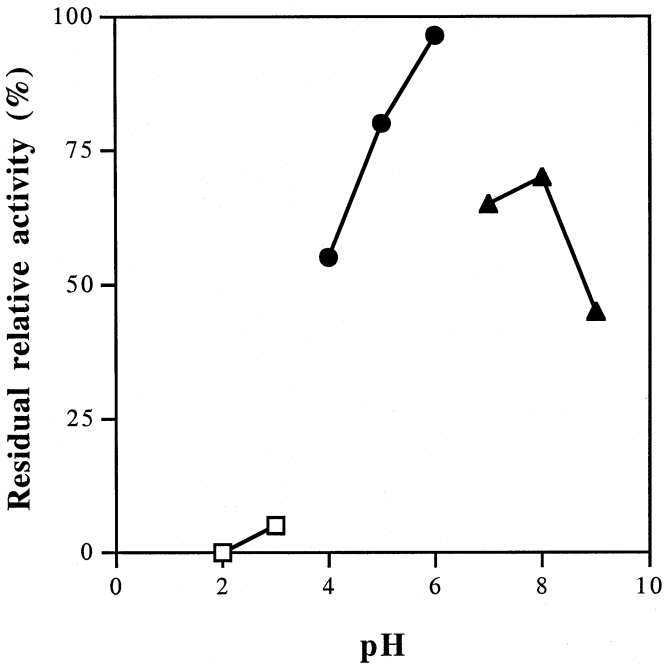

FIG. 2. Effect of pH on acylester hydrolase stability. Enzyme was incubated at 30°C for 12 h in various pH buffers (50 mM): glycine/HCl buffer for pH 2.0 and 3.0 (□), citrate buffer for pH 4.0, 5.0, and 6.0 (●), and Tris/HCl buffer for pH 7.0, 8.0, and 9.0 (▲).

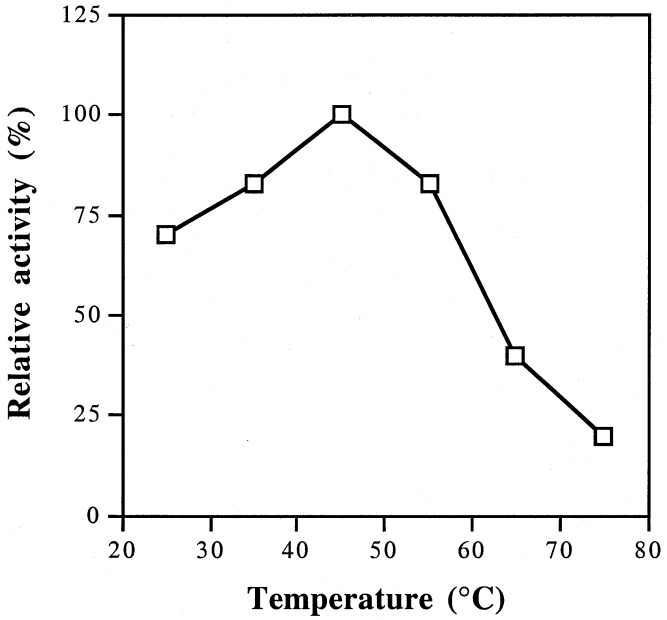

FIG. 3. The effect of temperature on the initial activity was established by measuring the enzymatic activity with pNPA as substrate in 20 mM Tris/HCl buffer (pH 8.0) at different temperatures.

HCl buffer (pH 8.0). Samples were withdrawn periodically and the residual activity was assayed using pNPA as substrate at 25°C. The enzyme was stable at 25°C for 5 h, while at 35°C, the activity was reduced by 20% after 5 h. The half-lives of the acylester hydrolase at 45 and 55°C were around 5 and 1 h, respectively. At 65 or 75°C, the half-lives were less than 0.5 h. A similar temperature stability has been reported for a peanut alkaline lipase (7).

Substrate specificity. The enzyme was active on lupine oil, olive oil, and on almost all triacylglycerols tested: tri-$C_{2:0}$, $C_{3:0}$, $C_{4:0}$, $C_{10:0}$, $C_{12:0}$, and $C_{18:1}$. Triolein was the substrate on which the enzyme showed the best hydrolytic activity, evaluated by TLC (data not shown). No hydrolysis was observed with tri-$C_{18:0}$ or with tri-$C_{20:0}$.

Hydrolysis of pNP esters. The specificity of the lupine acylester hydrolase toward pNPA, *p*-nitrophenyl propionate, *p*-nitrophenyl caprylate, *p*-nitrophenyl laureate, and *p*-nitrophenyl stearate were investigated (Table 2). The acetate ester was most rapidly hydrolyzed by lupine acylester hydrolase. Longer fatty acid chains were also hydrolyzed, but at a lower rate. Stearic acid ester was not hydrolyzed at 25°C, but at 40°C, a slow hydrolysis was observed.

Positional specificity. The positional specificity of lupine acylester hydrolase on triolein was examined by TLC and gas chromatography (GC) of the hydrolytic products (26). The TLC analysis on boric acid-impregnated plates showed that substantial amounts of 1,2-dioleyl glycerol and 2-monooleyl glycerol were formed as reaction products; also 1,3-dioleyl glycerol was formed, but at a lower rate. This suggested that the lupine enzyme could cleave in position 1 (3) faster than in position 2. The enzyme seems to hydrolyze all three positions in triacylglycerides and can be regarded as unspecific

TABLE 2
Substrate Specificity of Lupine Acylester Hydrolase Toward Several p-Nitrophenyl Esters[a]

	Relative activity (%)	
Substrate	25°C	40°C
p-Nitrophenyl acetate	75	100
p-Nitrophenyl propionate	65	85
p-Nitrophenyl caprylate	63	75
p-Nitrophenyl laurate	5	10
p-Nitrophenyl stearate	0	5

[a]The enzyme activity was measured by the spectrophotometric method described in the Materials and Methods section.

for this substrate. The results were also confirmed by GC analysis of the silylated reaction products. Spontaneous isomerization of the reaction products was considered negligible owing to the short reaction times (30 min).

Effect of solvents on acylester hydrolase stability. The effect of organic solvents on stability was studied with lupine acylester hydrolase. The remaining activity was measured after incubation with various solvents. The enzyme was incubated in 50 mM sodium phosphate buffer (pH 8.0), containing 20% vol/vol of various solvents for 60 min at 30°C. The residual activities, on samples taken from water phase, were assayed by the spectrophotometric method described in the Materials and Methods section. The lupine acylester hydrolase was stable over the incubation period with most of the water-miscible solvents added. This behavior was also observed for a fungal lipase by Lin *et al.* (26). The activity increased 50% with diethyl ether, 30% with acetonitrile, 18% with methanol, and 13% with pyridine, whereas toluene and isooctane reduced the activity by 50 and 40%, respectively. Other solvents that caused less than 10% increase of activity were: 3-methyl-3-pentanol, ethyl acetate, 2-propanol, and acetone. Solvents that caused less than 20% decrease were: dioxane, tetrahydrofuran, hexane, dichloromethane, dimethylsulfoxide, and ethanol.

Effect of metal ions on acylester hydrolase activity. The effect of various metal ions on activity was studied. The enzyme was incubated in 50 mM sodium phosphate buffer (pH 8.0), containing various metal chlorides at 1 mM concentration, for 30 min at 30°C. The residual activities were assayed by the spectrophotometric method described in the Materials and Methods section. The lupine enzyme was significantly inhibited (40%) by 1 mM of Cu^{2+} and less inhibited by Cs^{+}, Fe^{2+}, Sn^{2+}, Co^{2+}, Mg^{2+}, and Ca^{2+}. Metal ions generally form complexes with ionized fatty acids, changing their solubility and behavior at the interface. However, the inhibition might involve the catalytic site directly, although alteration of the properties of the interface must also be considered. Almost no inhibition was shown with Cd^{2+}, Mn^{2+}, Ni^{2+}, and Zn^{2+} ions.

Effect of detergents. The effect of detergents was tested on lupine acylester hydrolase activity. The enzyme was incubated in 50 mM sodium phosphate buffer (pH 8.0), containing 0.1% (wt/vol) detergents for 60 min at 30°C. Inhibition (20%) was observed with the addition of dioctyl sulfosucci-

nate, sodium salt. With sodium dodecyl sulfate and sodium deoxycholate, no effect was observed. All the nonionic surfactants tested produced an activating effect: sorbitan tristearate (27%), Tween 80 (19%), and Triton X-100 (10%). Furthermore, cholic acid, which is known to stimulate pancreatic lipase, stimulated lupine acylester hydrolase activity by 30%. Whether the stimulation of acylester hydrolase activity was due to a direct interaction of surfactants with the enzyme or to an alteration of emulsion properties which in turn affected activity is not known. Similar results have been reported by others for lipases from different sources (26–28).

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