Partial Purification and Some Properties of *Brassica napus* Lipase

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Lipase, Rape, Purification, Properties

Lipase (triacylglycerol acylhydrolase EC 3.1.1.3) from rape (*Brassica napus cv.* Ceres) was isolated from cotyledons of dark-grown seedlings. The enzyme was partially purified by polyethylene glycol precipitation. Delipidation of the lipase with *n*-hexane was required prior to further purification by anion exchange chromatography and size exclusion chromatography. A purification factor of 337 was ultimately achieved and the purification process was monitored by SDS-PAGE. Here, at least two protein bands with molecular masses of 62 and 64 kDa respectively were found in the active fraction obtained by size exclusion chromatography. Sodium deoxycholate was found to stimulate the lipase activity, but appeared to cause aggregation of the enzyme. It was not possible to estimate the isoelectric point of the dialyzed rape lipase due to the high molecular mass of the aggregates. Two simple methods to detect lipase activity directly on polyacrylamide gel were applied. No esterase activity was found by using *p*-nitrophenyl acetate as substrate.

Introduction

Most investigations on plant lipases have been carried out on oil seeds in which lipase activity is relatively high during germination. Lipase activity is absent in ungerminated seeds, except castor bean, but increases rapidly after seed germination. During germination the lipase hydrolyses stored triacylglycerols stepwise to release free fatty acids and glycerol. The free fatty acids are converted into sugars which provide building material for seedling growth.

Brassica napus lipase is associated with both lipid bodies and microsomes. It should be noted that about 75% of lipase activity has been found in the microsomal fraction (Hills and Murphy, 1988; Theimer and Rosnitschek, 1978). However, it is still unclear, whether lipase activity found in lipid bodies and microsomes resides in the same enzyme protein. Hills and Murphy (1988) reported that microsomal and lipid body lipases differ in their pH activity curves, kinetics and substrate specificities.

Theimer and Rosnitschek (1978) found that the activity of *Brassica napus* lipase in cotyledons initially rises until day 4 and then decreases until the lipids are exhausted. Furthermore, these authors assessed the lipase pH optimum at 9.0. According to Lin and Huang (1983) the maximum activity of rape lipase using N-methylindoxyl myristate as substrate is at pH 8.0.

To date maize scutellar and castor bean endosperm glyoxysomal lipases are the only oilseed lipases which have been purified in soluble form. Lin and Huang (1984) also found that the molecular mass of maize lipase is about 270 kDa, as determined by sucrose density gradient centrifugation in 1% sodium deoxycholate. Weselake *et al.* (1989) reported a figure of 250 kDa for rape lipase. The molecular mass of corn lipase was estimated to be 260 kDa (Lin *et al.,* 1986). Using SDS-PAGE, the molecular masses of the subunits of the castor bean and maize lipases were found to be 62 and

Abbreviations: DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; PAGE, polyacrylamide gel electrophoresis; PEG, polyethylene glycol; PMSF, phenylmethane sulfonyl fluoride; *p*NPA, *p*-nitrophenyl acetate; SDS, sodium dodecyl sulphate; Tricine, N-[tris-(hydroxymethyl)-methyl]glycine; Tris, tris(hydroxymethyl) aminomethane; Tween 60, polyoxyethylene-sorbitan monostearate.

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65 kDa respectively (Lin and Huang, 1984; Maeshima and Beevers, 1985).

The 62 kDa polypeptide was found in seedlings from many oilseed species and copurified with lipase activity (Hills and Beevers, 1987). However, it was later implicated to be malate synthase (Hoppe and Theimer, 1989). Recent results of O'Sullivan *et al.* (1990) support these conclusions. Fractions from a hydroxylapatite column from which the 62 kDa protein had been removed still contained most of the lipase activity. However, this fraction was challenged with an antiserum containing lipase-inhibitory elements and an immunologically cross-reactive polypeptide of approximate 67 kDa was revealed.

In the present work we describe alternative strategies for the isolation of lipase from cotyledons of germinated rapeseeds (*Brassica napus cv.* Ceres). This enzyme is found mainly in the 100,000×*g* supernatant from homogenate probably occurring as protein/lipid aggregates or micelles which are presumably derived from lipid body appendages of germinating seedlings (Rosnitschek and Theimer, 1980; Wanner and Theimer, 1978). The solubilized enzyme is partially purified and characterized.

Materials and Methods

Reagents and chemicals

All reagents and chemicals used were of analytical grade and purchased from Fluka, Merck, Serva and Sigma. Polyethylene glycol 8000 was obtained from Aldrich. Chemicals for PAGE were purchased from BioRad. All solvents were distilled before use.

Plants

Rapeseed (*Brassica napus cv.* Ceres) was obtained from Saaten-Union GmbH, Norddeutsche Pflanzenzucht, Hohenlieth, Germany.

The seedlings were germinated at 25 °C in the dark on moist filter paper for 6 days.

Isolation of lipase

The isolation was carried out according to Hills and Mukherjee (1990). All operations for isolation were done at 4 °C.

About 10 g (fresh weight) of cotyledons from 6-day-old rape seedlings were homogenized in 30 ml of a grinding medium, containing 150 mM Tricine-NaOH buffer, pH 7.6, 400 mM sucrose, 10 mM KCl, 1 mM $MgCl_2$, 1 mM EDTA, 2 mM DTT and 0.5 mM PMSF. The homogenate was filtered through two layers of Miracloth and centrifuged for 15 min at 20,000×*g* yielding a fat layer, a supernatant liquid and a pellet. After removal of the fat layer with the aid of a spatula the supernatant was separated from the pellet by filtration through Miracloth, recentrifuged for 5 min at 20,000×*g*, filtered through one layer of Miracloth and diluted 5 times with dilution buffer consisting of 150 mM Tricine-NaOH buffer, pH 7.6, 10 mM KCl, 50 mM $MgCl_2$, 1 mM EDTA, 2 mM DTT and 0.5 mM PMSF. The resulting solution was centrifuged for 1 h at 100,000×*g* in a Beckman L8-70 ultracentrifuge.

PEG precipitation

The pellet obtained by ultracentrifugation was discarded and the proteins of the supernatant were precipitated by addition of 4% (w/v) PEG 8000. After 10 min of stirring the lipase was pelleted by centrifugation for 15 min at 10,000×*g*.

The supernatant was discarded and the pellet was resuspended in 10 ml buffer containing 20 mM Tris-HCl buffer, pH 8.5, 0.5 mM EDTA, 0.5 mM β-mercaptoethanol, 0.5 mM $MgCl_2$ and 0.5 mM PMSF. Then 100 mg deoxycholic acid was added.

Assay of lipase activity

Qualitative estimation: Modified according Höfelmann *et al.* (1983); a mixture of 0.4 g agar-agar, 0.5 g triolein, 200 µl 0.1% (w/v) Rhodamin B and 20 ml Tris-HCl buffer, pH 8.5, containing 2 mM DTT and 2 mM $CaCl_2$; was heated to 60 °C and sonified 2 min with a Branson Sonifier 250. Hot liquid agar substrate was poured onto a glass plate. After applying lipase the gel was incubated for 12 h at 37 °C. Light pink bands (fluorescing at 366 nm) were visible on the intensively red gel.

Quantitative estimation: Free oleic acid released from triolein was assayed. A stock of 0.1 M triolein and 5% (w/v) gum arabic (dissolved in water) was sonified for 2 min immediately before use. A mixture of 10 ml 0.05 M Tris-HCl buffer, pH 8.5, containing 2 mM DTT, 2 mM $CaCl_2$ and 500 µl triolein

stock solution, was incubated and stirred at 40 °C for 5 min. Addition of 100 µl enzyme suspension started the reaction. Controls without lipase were included.

After 10 min the reaction was stopped by addition of 1 ml 6 N HCl and subsequently the reaction mixture was kept at 90 °C for 10 min. The released oleic acid was extracted quantitatively into *n*-hexane. Phase separations were facilitated by centrifugation.

For estimation of oleic acid by HPLC, the *n*-hexane was evaporated under a stream of nitrogen. Samples were taken up in 200 µl dichloromethane and analyzed on a Shimadzu LC-10 AD using a method by Chang and Rhee (1989). Detection was carried out at 214 nm with a UV spectrophotometric detector SPD-10 A.

Protein content

A modification of the procedure of Bradford (1976) was used to estimate protein contents. The absorbance of a mixture of the Coomassie solution and of the appropriate sample was measured at 595 and 465 nm. The ratio of the optical densities at 595 nm and 465 nm was determined. Bovine serum albumin was used as standard.

Esterase assay

Esterase activity was tested with 1.2 mM *p*NPA in 0.05 M Tris-HCl buffer, pH 8.5. This *p*NPA stock solution (8 ml) and 200 µl lipase suspension were incubated at 40 °C for 30 min and the absorbance was immediately measured at 405 nm. Blank samples of 8 ml *p*NPA stock solution and 200 µl 0.05 M Tris-HCl buffer, pH 8.5, and of 8 ml 0.05 M Tris-HCl buffer, pH 8.5, and 200 µl lipase suspension were estimated.

Extraction and analysis of lipids from lipase

The lipase suspension was extracted two times with a 2-fold volume of the appropriate solvent (*n*-hexane, diethyl ether, dichloromethane, chloroform). The resulting emulsion was centrifuged for 5 min at 6000×*g* until two phases resulted. The organic phase was used for further purification steps and investigated qualitatively with TLC. Delipidation of the PEG pellet was carried out with acetone at −20 °C.

TLC

The lipids were fractionated by TLC on silica gel G 60 containing 2% boric acid using petroleum ether/diethyl ether/acetic acid (90/10/1, v/v/v) as solvent in order to separate the different lipid fractions, which were detected by charring after spraying with concentrated sulphuric acid.

Ion exchange chromatography

A Mono Q HR 5/5 (Pharmacia) anion exchange column was equilibrated with 20 mM Tris-HCl buffer, pH 8.5, and operated at a flow rate of 1 ml/min. Aliquots (1 ml) of lipase solution (770 µg/ml), extracted with *n*-hexane and filtered through a 0.45 µm Sartorius filter, were applied to the column.

The column was eluted with the same buffer using a NaCl gradient (0–1 M NaCl) and the absorbance was monitored at 280 nm, using a single path monitor UV-1 with optical and control unit (Pharmacia). Fractions containing lipase activity were collected.

Size exclusion chromatography

The molecular mass of lipase was determined with a HiLoad 16/60 Superdex 200 prep grade column, equilibrated with 20 mM Tris-HCl buffer, pH 8.5, and 150 mM NaCl. Partially purified lipase solution (500 µl) from ion exchange chromatography, concentrated with Centricon 10 kDa from Amicon, was applied onto the column and eluted at a flow rate of 1 ml/min. Fractions were collected and the absorbance was monitored at 280 nm.

The void volume was determined with blue dextran 2000 kDa. Column calibration was carried out with proteins of different molecular masses (catalase: 240 kDa, lactate dehydrogenase: 140 kDa, bovine serum albumin: 67 kDa, ovalbumin: 45 kDa, horse myoglobin: 17.8 kDa) in 150 mM NaCl in 20 mM Tris-HCl buffer, pH 8.5.

SDS polyacrylamide gel electrophoresis

Proteins were precipitated by adding solid trichloroacetic acid to about 5% (w/v) of acid. The samples were centrifuged for 5 min at 6000×*g*. The precipitate was carefully washed 3 times with acetone and once with ether. The solvent residue was removed for 20 min under vacuum. The precipi-

tate was resuspended in SDS sample buffer (50 mM Tris-HCl buffer, pH 6.8, 5% DTT, 0.003% EDTA, 2.5% SDS, 0.1% bromophenolblue). The samples were incubated for 2 min at 100 °C. SDS-PAGE was performed on gradient gels (ExcelGel SDS gradient 8–18% with buffer strips, Pharmacia), according to the manufacturer's instructions, in a Pharmacia Multiphor II electrophoresis apparatus at 10 °C. Samples (5 µg protein) and Pharmacia Low Molecular Weight Calibration Kit standards were run.

The gels were stained either with Coomassie R-250 or by silver staining (Heukeshoven and Dernick, 1985).

Isoelectric focusing

The PEG pellet of lipase was treated with acetone at −20 °C. The supernatant was decanted and the delipidated lipase pellet was suspended in resuspension buffer. Then solid deoxycholate was added to 1% (w/v). The solubilized lipase was dialyzed against distilled water for 2 h at 4 °C and then centrifuged at 6000×*g* for 5 min.

Samples (20 µl) and Isoelectric Focusing Calibration Kit pH 3–10 standards (Pharmacia) were applied onto a Ampholine PAG plate, pH 3.5–9.5, according to the manufacturer's instructions on a Multiphor II apparatus. For the following electrophoresis the gel was cut into three pieces, each containing the same samples. One piece was stained with Coomassie R-250. For the detection of lipase activity, the second piece was incubated with 1% Tween 60, 0.05 M Tris-HCl, pH 8.5, and 2% $CaCl_2$ for 24 h at 37 °C. The gel was rinsed 3 times with distilled water, incubated in 2% $Pb(NO_3)_2$ for 5 min, washed 3 times with water and stained in a saturated H_2S solution. A black-brown precipitate of lead sulphide was visible. This procedure is an adaptation of a histochemical screening of lipase activity (Chayen and Bitensky, 1991).

The third piece of gel was blotted on an agar-agar gel to detect lipase activity qualitatively as described before.

Results

Delipidation of rape lipase

The lipase was isolated from the cotyledons of rape seedlings using centrifugation steps, followed by PEG precipitation. The protein/lipid ratio of the PEG precipitate of rape lipase was approximately 2:1. The lipid caused problems during lipase purification by anion exchange chromatography. Therefore a delipidation step was necessary for removing about 90% of lipid of sample preparation.

Fig. 1 demonstrates that the extraction with *n*-hexane, diethyl ether, dichloromethane, chloroform and acetone causes a decrease of lipase activity. *n*-Hexane yielded the highest activity after extraction and was therefore used for delipidation prior to further purification steps. Analysis of the extracted lipids using TlC (data not shown) revealed that most of the extracted lipids consist of phospholipids and free fatty acids.

Hydrolysis of trioleoylglycerol by rape lipase

The kinetics of hydrolysis of trioleoylglycerol are shown in Fig. 2. A reaction time of 10 min was used for the estimation of the specific activity and a reaction time of 30 min was used for all further experiments screening lipase activity.

The pH optimum of lipase activity was found to be pH 8.5 using 0.1 M Tris-HCl buffer, containing 2 mM DTT and 2 mM $CaCl_2$. In accordance with Rosnitschek and Theimer (1980) it was found that sodium deoxycholate stimulated the activity of the enzyme.

In contrast to Hills and Mukherjee (1990) we found that the isolated *Brassica napus* lipase did

Fig. 1. Influence of lipid extraction by different organic solvents on the activity of rape lipase. Lipase suspension was extracted two times with a 2-fold volume of the appropriate solvent. The resulting emulsion was centrifuged for 5 min at 6000×*g* until phase separation. a) The PEG precipitate of rape lipase was delipidated with acetone at −20 °C.

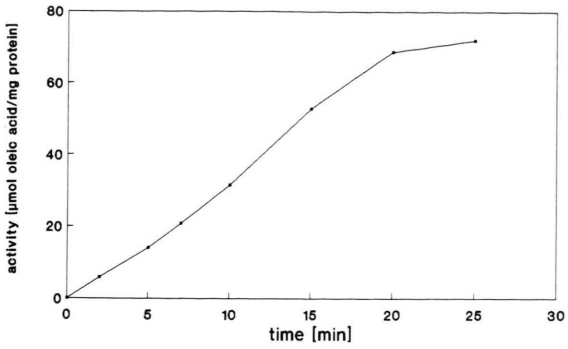

Fig. 2. Kinetics of trioleoylglycerol hydrolysis by delipidated lipase isolated from cotyledons of rape seedlings at day 6 of germination, determined by hydrolysis of triolein in 0.1 M Tris-HCl buffer, pH 8.5, containing 2 mM DTT and 2 mM $CaCl_2$.

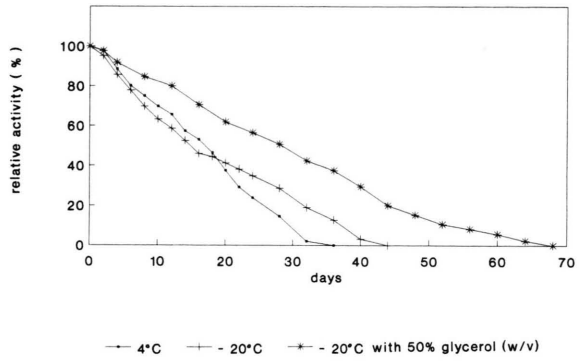

Fig. 3. Stability of crude rape lipase stored in resuspension buffer containing 20 mM Tris-HCl buffer, pH 8.5, 0.5 mM EDTA, 0.5 mM β-mercaptoethanol, 0.5 mM $MgCl_2$ and 0.5 mM PMSF.

not show esterase activity using *p*NPA as substrate.

Effect of temperature on stability of rape lipase

The stability of rape lipase was estimated during storage in resuspension buffer. All experiments were carried out with lipase of a single preparation. It can be seen in Fig. 3 that the enzyme activity decreased rapidly during storage at 4 °C. After one month activity was no longer detectable.

In a second experiment the enzyme was stored at −20 °C and frozen as well as thawed within 1 min. This process first caused a general initial decrease of activity, but the enzyme kept its activity for longer than one month.

In the third experiment 50% glycerol was added to the resuspension buffer and the enzyme preparation stored at −20 °C. After two months the lipase activity was less than 5%.

Ion exchange chromatography

Major contaminating proteins were removed by elution of the lipase from a Mono Q HR 5/5 with a step gradient of NaCl (Fig. 4). Lipase was eluted with 0.55 M NaCl. The collected fractions were qualitatively screened for lipase activity before the active fractions were assayed quantitatively. The yield of protein in the active fractions was found to be 23% of injected sample, but the increase of specific activity was low which could be explained by the relatively high salt concentration of purified lipase fractions. After dialysis for 2 h against resuspension buffer the specific activity increased to 10 µmol oleic acid/min/mg protein.

The active fractions were combined and concentrated by ultrafiltration in a Centricon-10 cartridge. 5% of the proteins, with molecular masses below 10 kDa, were removed by this step.

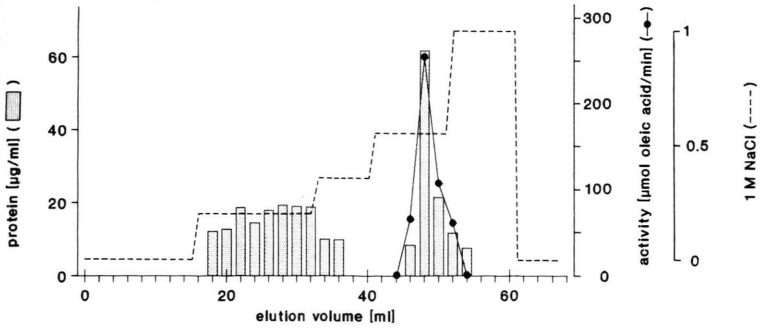

Fig. 4. Anion exchange chromatography of *Brassica napus* lipase on Mono Q HR 5/5. Lipase in resuspension buffer (1 ml), extracted with *n*-hexane, was applied to the column. Equilibration buffer was 20 mM Tris-HCl buffer, pH 8.5, and elution was performed by a NaCl step gradient (0–1 M NaCl) with a flow rate of 1 ml/min. Fractions were assayed for protein content and lipase activity.

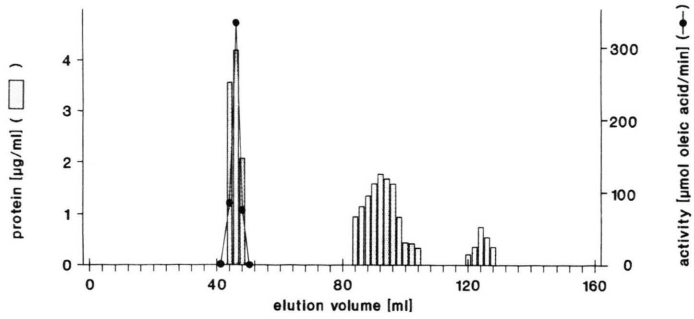

Fig. 5. Size exclusion chromatography of *Brassica napus* lipase on a HiLoad 16/60 Superdex 200 prep grade. Active fractions from ion exchange chromatography were concentrated and 500 µl concentrate (30 µg protein) was applied to the column. Concentration was carried out with Centricon 10 kDa. Elution buffer was 20 mM Tris-HCl buffer, pH 8.5, and 150 mM NaCl at a flow rate of 1 ml/min. Fractions were assayed for protein content and lipase activity.

Size exclusion chromatography

For purification and determination of the native molecular weight of rape lipase size exclusion chromatography was carried out. The elution profile, given in Fig. 5, shows 3 major protein peaks. Lipase elutes in the void volume, indicating a molecular mass in excess of 2000 kDa.

The active fractions were pooled and examined electrophoretically.

In total, the entire purification procedure resulted in 337-fold purification (Table I).

SDS polyacrylamide gel electrophoresis

The protein composition of fractions obtained during lipase purification was determined by SDS-PAGE (Fig. 6). The SDS-denatured proteins of 20,000×*g* and 100,000×*g* particulate fractions were distributed over a wide molecular mass range. In the PEG precipitate and the ion exchange fraction, proteins with molecular weights ranging between 10 and 40 kDa were mainly eliminated, while some proteins larger than 60 kDa were enriched. These proteins were also found in size exclusion chromatography fractions, while the majority of the remaining proteins disappeared. At least two major bands of proteins representing lipase or lipase subunits could be detected in the size exclusion fraction with molecular weights of 62 and 64 kDa.

Isoelectric focusing

Isoelectric focusing was carried out to estimate the isoelectric point of rape lipase. The delipidated and dialyzed lipase and 0.8% Ampholine, pH 3–10, were applied to the gel at different positions. After electrophoresis, Coomassie staining showed different protein bands. Lipase activity was examined using an adaption of a histochemical assay (Chayen and Bitensky, 1991). Black-brown precipitates of lead salt were visible, showing lipase activity. The lipase did not move in the electric field. A second test was a diffusion blotting on a agar-agar gelt. Both assays confirmed the finding, that lipase in the sample did not migrate during isoelectric focusing.

Discussion

We have developed an alternative procedure (O'Sullivan *et al.*, 1990; Weselake *et al.*, 1989) for

Table I. Protein yield and specific activity in delipidated fractions obtained during purification of rape lipase (*Brassica napus cv.* Ceres). About 10 g of cotyledons from 6-day-old seedlings were used for the purification of *Brassica napus* lipase.

Fraction	Total protein [mg]	Total activity [µmol oleic acid min^{-1}]	Specific activity [µmol oleic acid mg $protein^{-1}$ min^{-1}]	Purification factor
100,000×*g* supernatant	479	134	0.28	1
PEG precipitate	7.7	24.2	3.14	11
Mono Q HR 5/5	0.17	0.7	4.36	16
Superdex 16/60	0.007	0.66	94.7	337

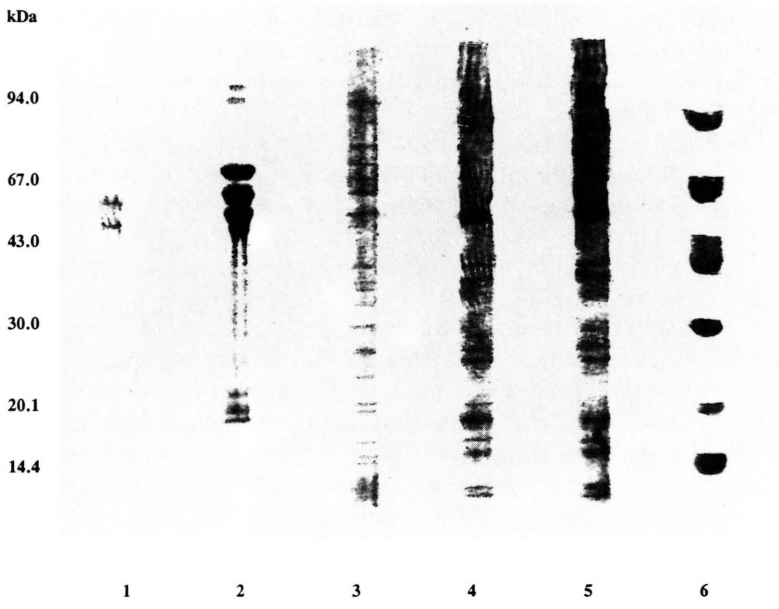

Fig. 6. SDS-PAGE of various fractions obtained during purification of *Brassica napus* lipase. Electrophoresis was performed on gradient gels (8–18% polyacrylamide), which were stained with Coomassie R-250. Lane 1: HiLoad 16/60 Superdex 200 prep grade fraction with peak activity; lane 2: Mono Q HR 5/5 fraction with peak activity; lane 3: PEG 8000 fraction; lane 4: 100,000×*g* particulate fraction; lane 5: 20,000×*g* particulate fraction; lane 6: standard proteins (α-lactalbumin, 14.4 kDa; trypsin inhibitor, 20.1 kDa; carbonic anhydrase, 30.0 kDa; ovalbumin, 43.0 kDa; albumin, 76.0 kDa; phosphorylase b, 94.0 kDa).

partial purification of *Brassica napus* lipase and have determined some characteristics of the enzyme.

In the current study it was found that delipidation of rape lipase preparation is a required step for separating active lipase by ion exchange chromatography. For this reason organic solvents were used to extract lipids. For the maintenance of lipase activity it is important that a layer of water molecules remains around the enzyme (Empie and Gross, 1988).

Lipases retain their activity in apolar organic solvents such as *n*-hexane (Zaks and Klibanov, 1986). It is considered that more polar organic solvents, such as diethyl ether, chloroform, dichloromethane and acetone, remove water molecules from the enzyme (Zaks and Klibanov, 1988; Rodionova *et al.*, 1987). Thereby the native conformation of the enzyme sample is changed and the lipase loses its activity. We showed that the lipase was inactivated almost completely after extraction of the enzyme with dichloromethane (Fig. 1). It was found by Meusel *et al.* (1992) however, that dichloromethane did not denature the activity of porcine pancreas lipase irreversibly. This aspect needs further investigations.

After lipid extraction from the lipase suspension with *n*-hexane 92% of its activity could still be measured. The 8% decrease of activity can be due to the presence of rape lipase suspension in *n*-hexane, or to the inactivation of lipase by *n*-hexane. Furthermore it appears that delipidation of lipase suspension with *n*-hexane removes various lipids such as triacylglycerols, phospholipids and free fatty acids completely.

Previous reports on the pH optimum of *Brassica napus* lipase are not consistent. The lipase suspension in our study exhibited a pH optimum for triolein hydrolysis in Tris-HCl buffer of 8.5, which was similar to the optimum of pH 8.0 as found by Lin and Huang (1983) using N-methylindoxylmyristate as substrate, and to the pH optimum of 9.0 as found by Theimer and Rosnitschek (1978) using a titrimetric assay.

The storage of lipase at the stage of PEG precipitation is favourable at −20 °C in the presence of 50% glycerol.

The purification of rape lipase by ion exchange chromatography produces a rather low enrichment of specific activity. This appears to be due to the ionic strength of the elution buffer (salt out effect), since the specific activity increased after dialysis. During size exclusion chromatography *Brassica napus* lipase exhibited a high apparent molecular weight which may due to the formation of lipase molecule aggregates. Interestingly, after purification by size exclusion chromatography the specific activity of lipase was enhanced to 95 μmol

oleic acid/min/mg protein. A summary of the purification procedures given in Table I shows that a final purification factor of 337 was achieved.

It has been observed (Hills and Murphy, 1988; Lin and Huang, 1983; Rosnitschek and Theimer, 1980) that detergents may inhibit as well as stimulate the activity of lipases. Weselake *et al.* (1989) used Triton X-100 for solubilization of lipase. This detergent inhibits lipase activity, but Maeshima and Beevers (1985) found that this effect is reversible during chromatography of castor bean alkaline lipase. We used sodium deoxycholate, because of its ability to release lipase from the microsomal membrane and maintain it in solution. It is likely that the presence of sodium deoxycholate causes the formation of protein/detergent aggregates. Despite this drawback the use of deoxycholate was warranted, because of its stimulating effect on enzyme activity.

We have successfully used two methods for direct detection of lipase activity on gels which may be useful in future studies. However, it was not possible to determine the isoelectric point of *Brassica napus* lipase with isoelectric focusing on polyacrylamide gel, because of the high molecular weight of lipase aggregates. The lipase did not migrate in the relatively small pores of polyacrylamide gel in the electric field. The use of self prepared agarose gels with large pores could be helpful.

The molecular weight of the polypeptides in the lipase containing fraction collected by size exclusion chromatography was determined by SDS-PAGE. Two main protein bands at 62 kDa and 64 kDa were found. Our results are similar to the findings based on immunoreactive studies with an antiserum to inhibit lipase carried out by O'Sullivan *et al.* (1990). In that paper, immunoblots revealed an immunologically cross-reactive polypeptide of approximately 67 kDa. Probably, refering to Hoppe and Theimer (1989) and O'Sullivan *et al.* (1990), in our experiment the 62 kDa polypeptide is the malate synthase and the SDS molecular weight of *Brassica napus* lipase is 64 kDa.

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