Purification and characterization of a second form of acid lipase in human liver

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We describe here the purification and characterization of a form of acid lipase from human liver (designated ALII), which differed from the more abundant M_r -29000 form (ALI). ALII was solubilized from frozen human liver with Triton X-100 and purified 8500-fold by chromatography over concanavalin A-sepharose, CM-cellulose and finally h.p.l.c. over a Mono S column. ALII migrated as a single band on polyacrylamide-gel electrophoresis in both the presence and the absence of SDS. The M_r of ALII was estimated to be 58 500 by SDS/polyacrylamide-gel electrophoresis. Gel filtration on Sephacryl S-200 gave an apparent M_r of 69 000. 4-Methylumbelliferyl (4MU) palmitate, cholesterol oleate and triolein were substrates for ALII, with apparent V_{max} values of 5000, 1100 and 2500 nmol/min per mg respectively and K_m values of 1.0, 1.5 and 1.8 mM respectively. Cholesterol oleate and triolein were hydrolysed optimally by ALII at pH 4.5, whereas 4MU palmitate was hydrolysed optimally at pH 5.3. Antisera were raised against ALI and ALII and, on immunoblot analysis, no antigenic similarity was observed between ALI and ALII. Cellulose acetate electrophoresis followed by reaction with 4MU palmitate revealed two forms of lipase, corresponding to ALI and ALII. The two enzymes were also separated by hydrophobic chromatography. The activity of ALII was stimulated by several proteins and was partially inhibited by millimolar concentrations of NaCl, $CaCl_2$ and $MgSO_4$.

INTRODUCTION

Lysosomal acid lipase (EC 3.1.1.13) plays an important role in the metabolism of cholesterol esters and the modulation of intracellular cholesterol release (Goldstein *et al.*, 1975; Brown & Goldstein, 1976). Acid lipase catalyses the degradation of several substrates at acid pH, among which are the fatty acyl esters of cholesterol, triacylglycerols and 4-methylumbelliferone (4MU). Inherited deficiency of acid lipase in humans gives rise to Wolman's disease and cholesterol ester storage disease (Assman & Fredrickson, 1983). Individuals with Wolman's disease accumulate grossly abnormal amounts of both cholesterol esters and triacylglycerols in many tissues (Patrick & Lake, 1969), whereas patients with cholesterol ester storage disease exhibit a moderate course, with hepatomegaly and hypercholesterolaemia as the major clinical features (Burke & Schubert, 1972). It has been postulated that a relative deficiency of acid lipase leads to the accumulation of cholesterol esters within the arterial wall, thus promoting premature development of atherosclerosis (de Duve, 1974).

Acid lipase has been purified from several different sources, including human liver (Warner *et al.*, 1981), bovine thyroid gland (de Wolff *et al.*, 1975), rat liver (Teng & Kaplan, 1974; Brown & Sgoutas, 1980), rabbit liver (Imanaka *et al.*, 1984) and human fibroblast secretions (Sando & Rosenbaum, 1985).

Our laboratory has previously demonstrated the existence of an acid lipase from human liver with an apparent M_r of 29 000 $\pm$ 1000 (Warner *et al.*, 1981), which we designate ALI. In the present paper we demonstrate the existence of a second acid lipase (ALII) in

human liver with physical and immunological properties distinct from those of ALI. We have purified ALII 8500-fold and compared its molecular and kinetic properties with those of ALI.

EXPERIMENTS

Materials

Cholesteryl [1- 14 C]oleate (56 Ci/mol) and tri[1- 14 C]-oleoylglycerol (47 Ci/mol) were obtained from Amersham, Arlington Heights, IL, U.S.A. Cholesterol oleate and trioleoylglycerol were from Nu-Check prep, Elysian, MN, U.S.A. Con A-Sepharose 4B was from Pharmacia Fine Chemicals, Uppsala, Sweden. CM-cellulose (CM52) was from Whatman, Maidstone, Kent, U.K. Nitrocellulose was from Schleicher and Schuell, Keene, NH, U.S.A.

Purification of acid lipase from human liver

Fresh liver tissue was obtained at autopsy from male adults. Pathological examination determined the absence of liver disease. The tissue was sliced into 100 g portions (wet wt.) and frozen at -20°C . All purification steps were carried out at 4°C unless otherwise stated. Acid lipase activity was monitored during the purification step with 4MU palmitate as a fluorogenic substrate analogue and, after completion of the isolation procedure, with cholesterol [1- 14 C]oleate (1 mM; 0.56 Ci/mol) and tri[1- 14 C]oleoylglycerol (1 mM; 0.47 Ci/mol) as substrates as described by Warner *et al.* (1981). The apparent K_m and V_{max} values for the above substrates were determined by double-reciprocal plots of substrate-saturation curves.

Abbreviations used: 4MU, 4 methylumbelliferone; Con A, concanavalin A; TBS, Tris-buffered saline (10 mM-Tris/HCl, pH 7.4, 0.15 M-NaCl).

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Assays for determination of pH optima were performed as standard assays, except that citrate/phosphate buffer was substituted for sodium acetate buffer. Hydrolysis rates of 4MU substrates with various chain lengths were examined under standard assay conditions, with 4MU butyrate, 4MU palmitate, 4MU elaidate and 4MU nonanoate. Buffers used for the isolation of acid lipase contained proteinase inhibitors [antipain, chymostatin and leupeptin (1 $\mu\text{g}/\text{ml}$ each) and pepstatin (0.1 $\mu\text{g}/\text{ml}$)] in all steps except the final purification step. Protein concentration was determined by a modification of the method described by Lowry *et al.* (1951). The purification scheme presented here is a modified version of the procedure developed by Warner *et al.* (1981).

In a typical purification, 100 g of frozen liver was allowed to thaw in 300 ml of Buffer I [10 mM-sodium acetate (pH 5.0)/1 mM-2-mercaptoethanol] in a Waring blender. The tissue was homogenized for 3×1 min, allowing for 2 min between each surge to maintain the homogenate at 4 °C. The homogenate was then centrifuged at 48000 *g* for 40 min. The resulting supernatant was discarded, and the pellets containing acid lipase activity were removed. The pellets were suspended in 160 ml of Buffer I containing 16 mM-Triton X-100 (10 mg/ml) and homogenized as previously described. This homogenate was centrifuged at 48000 *g* for 40 min and the resulting supernatant was stored at 4 °C. The pellet was re-extracted and the supernatant was pooled with the initial Triton extract. These extracts were then gently stirred for 2 h in the presence of 50 ml of Con A-Sepharose 4B that was previously equilibrated in Buffer I containing 16 mM-Triton X-100. The resulting slurry was then poured into 50 ml polypropylene centrifuge tubes, and the Con A-Sepharose 4B was collected by centrifugation for 5 min at 1000 *g* in a clinical centrifuge; the lectin-Sepharose was then washed in a batchwise fashion, sequentially with 200 ml of Buffer II [20 mM-sodium acetate (pH 5.0)/10 mM-2-mercaptoethanol/8 mM-Triton X-100/500 mM-NaCl/33 % (v/v) ethylene glycol], 400 ml of Buffer III [20 mM-NaCl (pH 5.0)/10 mM-2-mercaptoethanol/33 % ethylene glycol], and 200 ml of Buffer III containing 375 mM- α -methyl mannoside. After each wash the lectin-Sepharose was collected by centrifugation at 1000 *g*, the supernatant was discarded and acid lipase activity was eluted by gently suspending the resultant pellet in Buffer III containing 1.6 mM-Triton X-100 and 375 mM- α -methyl mannoside at 37 °C. The slurry was then centrifuged at 1000 *g* and the supernatant was collected on ice. This process was repeated several times until the lectin-Sepharose had been eluted with 200 ml of the elution buffer. The pooled eluates were then passed over a small (1.0 cm $\times$ 2.0 cm) CM-cellulose column that had been previously equilibrated in Buffer III containing 1.6 mM-Triton X-100. The eluate was recirculated over this column three or four times in order to absorb up to 90 % of the acid lipase activity. The CM-cellulose column was then washed with 20 ml of Buffer III and was subsequently eluted with 10 ml of Buffer III containing 500 mM-NaCl. The concentrated eluate was then dialysed against 1 litre of Buffer III for 24 h.

The dialysed sample was then subjected to a second cation-exchange chromatography, on a Pharmacia Mono S column attached to a Varian 20 h.p.l.c. system. Acid lipase was eluted from the Mono S column with a 30 ml linear gradient of 0–500 mM-NaCl in Buffer III.

Protein eluted from the column was monitored by u.v. absorbance at 280 nm. Fractions of volume 1 ml were collected, and those containing approx. 80 % of the acid lipase activity eluted from the column were dialysed as separate fractions against 1 litre of Buffer III. The dialysed samples from these fractions were stored at –20 °C.

Electrophoresis

Polyacrylamide-gel electrophoresis in the presence of SDS was conducted in 12.5 % acrylamide gels as described by Laemmli (1970). Phosphorylase *b* (M_r 92500), bovine serum albumin (M_r 66200), ovalbumin (M_r 45000), carbonic anhydrase (M_r 31000), soya-bean trypsin inhibitor (M_r 21500) and lysozyme (M_r 14400) were run as M_r markers. Gels were fixed in 50 % (v/v) methanol/10 % (v/v) acetic acid for 15 min, stained in the fixing solution containing 0.1 % (w/v) Coomassie Brilliant Blue for 20 min and destained by diffusion in 10 % methanol/7 % acetic acid. Polyacrylamide-gel electrophoresis under non-denaturing conditions was modified from the method of Gabriel (1971). Stacking gels contained 2.5 % acrylamide, 0.63 % *NN*-methylenebisacrylamide, 0.1 % Triton X-100 and 1 % ethylene glycol; the last two were added to the lower gel. Electrophoresis was conducted with β -alanine/acetic acid buffer containing 1 % Triton X-100 (pH 4.5) at 4 °C. Migration was toward the cathode, and Methyl Green was employed to follow the progress of the electrophoresis. Acid lipase activity was detected by overlaying the gel with filter paper immersed in 4MU palmitate assay buffer (pH 5.2) and incubating at 37 °C for 10 min. Activity was observed under u.v. illumination, and the fluorescence was marked with small incisions in the gel. Gels were fixed, stained and destained as described above. The protein bands were then excised and eluted from the gel as described by Hunkapillar *et al.* (1983). Proteins corresponding to unique bands in the native gel were electrophoresed in the presence of SDS as described above. Cellogel electrophoresis was carried out as described by Warner *et al.* (1981).

Gel filtration for M_r determination

The native M_r of ALII was estimated by gel filtration on a Sephacryl S-200 column (2.0 cm $\times$ 90 cm) equilibrated in Buffer III by the method of Andrews (1970). The column was calibrated with Blue Dextran 2000 (M_r 2×10^6), haemoglobin (M_r 64500) and α -chymotrypsinogen (M_r 25400); 10 units of enzyme in 1 ml were applied to the column and 2.5 ml fractions were collected.

Antibody preparation

Rabbit antibodies to ALI and ALII were prepared. To produce a monospecific antibody to ALI, several preparative SDS/polyacrylamide gels were run and the protein band corresponding to ALI was excised from the gel and eluted as described above. ALII was treated in a similar manner for antibody production. Approx. 100 μg of each respective enzyme was emulsified in 500 μl of complete Freund's adjuvant. The rabbits were inoculated with the emulsified preparations at several injection sites and were boosted on day 28 with 50 μg of enzyme emulsified in 500 μl of complete Freund's adjuvant. Blood was collected from the ear vein on day 35, and the serum was immediately prepared. IgG was purified on a Protein A-Sepharose 4B column (1.0 cm $\times$ 1.5 cm). The

Table 1. Purification of ALII from 100 g of frozen human liver tissue

Activity is expressed in units (nmol of palmitic acid released/min).

	Total protein (mg)	Total activity (units)*	Specific activity (units/mg)	Purification (fold)	Yield (%)
Homogenate	12285	80750	6.6	—	100
Triton X-100 extract	1980	126100	64	9.7	156
Con A-Sepharose	300	90000	300	46	111
CM-cellulose	17.2	26000	1550	235	23
Mono S h.p.l.c.	0.40	21000	53000	8030	26

* The initial supernatant was assayed for activity on 4MU palmitate, and this activity was subtracted from the total activity of the homogenate to correct for a soluble neutral lipase previously characterized by Warner *et al.* (1980). The increased total activity in the Triton X-100 extract is apparently due to inhibition in the crude homogenate and stimulation by Triton X-100.

specificity of each antibody was examined by immunoblotting liver homogenates as described below.

Western blotting

Protein samples were subjected to SDS/polyacrylamide-gel electrophoresis as described above. After electrophoresis, the proteins were transferred to nitrocellulose sheets by electroblotting by the method of Burnett (1981). The blots were then equilibrated in TBS (pH 7.5) for 10 min, followed by equilibration in TBS (pH 7.5) containing 3 % (w/v) gelatin. This solution was then decanted and the blot was incubated for 4 h at room temperature in the presence of rabbit anti-lipase IgG added to TBS (pH 7.5)/3 % gelatin. This solution was decanted from the blot and stored for later use at -20°C . The nitrocellulose sheet was rinsed sequentially in TBS for 10 min, TBS containing 0.05 % Nonidet P40 for 5 min and again with TBS for 10 min. Approx. $7\ \mu\text{l}$ of peroxidase-conjugated goat anti-rabbit IgG was added to 15 ml of TBS (pH 7.5)/3 % gelatin. The blot was incubated in this solution for 1 h and the solution was decanted off. The nitrocellulose sheet was then washed as described above. The following solutions were prepared just before development of the blot: 60 mg of 4-chloronaphth-1-ol in 20 ml of cold methanol and $60\ \mu\text{l}$ of 30 % (v/v) H_2O_2 in 100 ml of TBS, pH 7.5. These two solutions were mixed together and incubated with the immunoblot for 5–10 min.

Immunoprecipitation

Antisera raised against ALI and ALII were used to precipitate acid lipase activity. Precipitation reactions were carried out as described by Hubert & O'Brien (1983).

Thermolability

Samples of purified ALI and ALII were incubated in a water bath at 52°C , and samples were removed from the bath periodically and stored on ice. Residual acid lipase activity in each sample was determined with 4MU palmitate as a substrate under standard assay conditions for the fluorogenic substrate.

RESULTS

Purification of ALII

Owing to the need for rapid, sensitive, assay, acid lipase activity was monitored throughout the isolation

procedure with 4MU palmitate. ALII appeared to be particularly susceptible to proteolytic degradation; thus, proteinase inhibitors such as chymostatin, leupeptin, pepstatin and antipain were included in all buffers, with the exception of the media used in the final purification step. A typical purification scheme for the isolation of ALII from 100 g of human liver is presented in Table 1. The enzyme was maximally solubilized with 1 % Triton X-100 as described by Warner *et al.* (1979); other non-ionic detergents such as Emulphogen were tested, but Triton X-100 proved to be a superior solubilizing agent.

The next step involved absorption to Con A-Sepharose 4B; approx. 90 % of the enzyme activity was adsorbed, and the immobilized lectin-Sepharose could be quickly and efficiently washed by suspending the slurry in buffer and gently inverting the suspension. Adsorption of the enzyme and subsequent washing of the absorbent in a conventional column was time-consuming and tedious, and failed to increase the yield significantly. ALII, like ALI, required a temperature of 37°C for maximal elution from Con A-Sepharose. The eluate was concentrated on a small ($1.0\ \text{cm} \times 2.0\ \text{cm}$) CM-cellulose CM52 column, and was re-applied to the column several times to adsorb 90 % of the enzyme activity. The presence of Triton X-100 was not necessary for elution of ALII, although we found that eluting the CM52 column with the above elution buffer containing as little as 0.01 % Triton X-100 increased the yield of ALII by as much as 20 %. In order to study the native physical properties of ALII, we did not include Triton X-100 in the CM52 elution buffer.

ALII was eluted from the Mono S column at a NaCl concentration of 240–300 mM, whereas ALI was eluted at 350–475 mM. The Mono S column provided a fast, efficient and reproducible purification step, and approx. 8 mg of protein could be applied; larger amounts of protein applied to the column led to decreased resolution. In a typical preparation the central portion of the enzyme, representing about 80 % of the activity eluted from the column, was examined by SDS/polyacrylamide-gel electrophoresis, and this fraction was found to be homogeneous when silver-stained (Fig. 1): This analysis localized acid lipase activity corresponding to ALII to a specific band on a gel.

The specific activity of the final enzyme preparation was 8000 times that of the homogenate, and enzyme recovery was greater than 25 %. The purification scheme

Fig. 1. SDS/polyacrylamide-gel electrophoresis of ALII

Fractions (1 ml) representing 100 % of the 4MU-palmitate-assayable activity eluted from the Mono S column were each concentrated to 50 μ l and subjected to SDS/polyacrylamide-gel electrophoresis on a 10 % gel containing 0.1 % SDS. Protein bands were observed by silver staining as described by Oakley *et al.* (1980). Tracks: (1) Fraction 15; (2) fraction 16; (3) fraction 17; (4) fraction 18; (5) fraction 19; (6) fraction 20; (7) fraction 21; (8) M_r standards.

Table 2. Kinetic values for ALI and ALII

The values for ALI have been published previously (Warner *et al.*, 1981). All ALII assays were performed under conditions identical with those for ALI. Abbreviation: ND, not determined. Units: K_m , mM; V_{max} , nmol/min per mg.

Substrate...	Cholesterol oleate			Triolein			4MU palmitate		
	K_m	V_{max}	pH optimum	K_m	V_{max}	pH optimum	K_m	V_{max}	pH optimum
ALI	0.8	1400	4.4	0.8	5400	4.4	ND	ND	4.4
ALII	1.5	1100	4.5	1.8	2500	4.5	1.0	5000	5.3

was reproducible, and approx. 400 μ g of ALII, of specific activity 53 000 units/mg of protein, was obtained from 100 g of human liver tissue. ALII was stable at -20°C in the presence of 10 mM-2-mercaptoethanol and 33 % ethylene glycol for up to 1 month.

Molecular properties

The apparent M_r of purified ALII was estimated to be 58 500 by SDS/polyacrylamide-gel electrophoresis (Fig. 1). The M_r of the native enzyme was judged to be 69 000 by chromatography over a Sephacryl S-200 column in the presence of 33 % ethylene glycol.

Both ALI and ALII were hydrophobic, as determined by running purified samples through small (1.0 cm $\times$ 2.0 cm) columns of octyl- and phenyl-Sepharose 4B. Neither enzyme was adsorbed in the presence of 33 % ethylene glycol, but when the ethylene glycol concentration was decreased to 10 % both enzymes were adsorbed. ALI was eluted from octyl-Sepharose with 80 % ethylene glycol and from phenyl-Sepharose with 60 % ethylene glycol, whereas ALII could not be eluted from octyl-Sepharose even in the presence of 80 % ethylene glycol containing 0.1 % Triton X-100. ALII was eluted from phenyl-Sepharose only in the presence of 80 % ethylene

glycol containing 0.1 % Triton X-100. These results indicate that both enzymes are hydrophobic, and ALII is more hydrophobic than ALI. Both enzymes were adsorbed to Con A-Sepharose and eluted with 375 mM- α -methyl mannoside, indicating that they are glycoproteins.

Catalytic properties

The specificity of ALII towards 4MU palmitate, cholesterol oleate and triolein was studied and compared with previously published kinetic data on ALI by using assay conditions identical with those published (Warner *et al.*, 1981), except that bovine serum albumin was not included in our final purified preparation. ALII hydrolysed 4MU palmitate over the pH range 4.0–6.4, with an optimum at pH 5.3. ALII hydrolysed both cholesterol oleate and triolein optimally at pH 4.5; its optimum for cholesterol oleate was sharp, whereas that for triolein was relatively broad.

The apparent K_m values of ALII for 4MU palmitate, cholesterol oleate and triolein were determined to be 1.0, 1.5 and 1.8 mM, and the apparent V_{max} values were determined to be 5000, 1100 and 2500 nmol/min per mg of protein respectively. The apparent K_m for cholesterol oleate was almost identical with that of triolein, but the

Fig. 2. Extent of immunological cross-reactivity between ALI and ALII, as examined by Western blots

In both panels lane 1 represents 10 μ g of a partially purified acid lipase preparation containing predominantly ALII, and lane 2 corresponds to 10 μ g of ALI purified by chromatography over octyl-Sepharose. The preparations were electrophoresed through a 12.5% polyacrylamide gel in 0.1% SDS and electrophoretically transferred to nitrocellulose sheets as described by Burnett (1981) and subsequently cut in half. The sites that had not reacted were blocked with 3% gelatin and made to react with antibody directed against ALI (a) and antibody directed against ALII (b) for 4 h. The sheets were then washed, made to react with horseradish-peroxidase-conjugated goat anti-(rabbit IgG) and chromogen, and developed as described in the text.

$V_{\max}$ of cholesterol oleate was about half of that for triolein.

A comparison of compiled kinetic data for ALI and ALII is summarized in Table 2. The pH at which optimal activity is observed is nearly identical for the two enzymes. The apparent K_m value of ALII for cholesterol oleate is about twice that of ALI, whereas the $V_{\max}$ value for the two is nearly identical. Comparison of kinetic values for ALI and ALII with triolein as substrate reveals a K_m for ALII that is twice that of ALI and an apparent $V_{\max}$ one-half that of ALI.

We also investigated the hydrolysis rates for synthetic 4MU derivatives ester-linked to various fatty acyl groups. ALI hydrolysed the 4MU derivatives tested, in order of decreasing rate 4MU butyrate > 4MU nonanoate > 4MU palmitate > 4MU elaidate, in contrast with ALII, which hydrolysed the above substrates in order of decreasing rate butyrate > elaidate > nonanoate > palmitate. ALII hydrolysed 4MU elaidate at a rate similar to that of butyrate, whereas ALI hydrolysed this substrate at a very slow rate.

Although we did not add protein to standard assays to stabilize the activity of ALII, we did observe that several proteins stimulated the hydrolysis of 4MU palmitate by ALII. Haemoglobin, ovalbumin and bovine serum albumin all appeared to increase the activity of ALII at concentrations of 200 μ g/ml, whereas at concentrations of 2 mg/ml the above proteins were inhibitory. At a

concentration of 200 μ g/ml, γ -globulin increased ALII activity to twice the basal value.

The addition of NaCl (100 mM) to the 4MU palmitate standard assay inhibited ALII activity by 25%, as did addition of CaCl_2 or MgSO_4 at 10 mM.

Antigenic characteristics of ALI and ALII

Antibodies directed against ALI cross-reacted on a Western blot with a single protein band in the liver homogenate corresponding to the M_r of ALI, and no reactivity against ALII was detected (Fig. 2). One preparation of antisera to ALI precipitated 75% of the activity on 4MU palmitate in purified preparations of ALI. Antibodies directed against ALII reacted strongly with a protein band corresponding to the M_r of ALII on Western blots, and with much decreased intensity to several protein species possessing a faster anodal migration in the liver homogenate immunoblot, but not to purified ALI. ALII appears to be particularly susceptible to proteolytic degradation, and we assume that the lower- M_r reactants are products of ALII proteolysis. Antiserum to ALII was able to precipitate 100% of the activity on 4MU palmitate found in purified ALII preparations. These experiments demonstrate that denatured ALI and ALII are antigenically different.

Electrophoretic characteristics

When partially purified ALI from a CM52 column was run alongside highly purified ALII on a cellulose acetate gel, they migrated differently; ALII migrated as a single band towards the anode at pH 5.8, but ALI failed to migrate.

Polyacrylamide-gel electrophoresis in the absence of SDS was performed on purified preparations of ALI and ALII. The two enzymes both migrated cathodally, but ALII had a slightly faster mobility. Both enzymes were observed as single bands of protein after staining, with mobilities in the same location as that represented by activity on 4MU palmitate.

DISCUSSION

By catalysing the cleavage of the ester bond inherent to cholesterol esters transported endogenously to the lysosome by lipoproteins, acid lipase plays a central role in cellular cholesterol metabolism. The cholesterol liberated by this catalysis acts as a second messenger that is responsible for suppressing further synthesis of cholesterol esters (Gil *et al.*, 1985; Lusky *et al.*, 1983). Thus acid lipase indirectly mediates the catabolism of cholesterol esters. Decreased catalytic activity of acid lipase has been implicated in several pathological disease states such as Wolman's disease (Patrick & Lake, 1969) and cholesterol ester storage disease (Assman & Fredrickson, 1983), and in the accumulation of atheroma (Fowler *et al.*, 1978). The purification and subsequent analysis of the molecular properties of acid lipase represent the first step in elucidating the mechanisms responsible for the above-mentioned disease states.

Human liver was chosen as a tissue source because through subcellular-fractionation studies it has been well documented that lysosomes contain within their confines the majority of hydrolytic enzymes possessing an acidic pH for optimal catalytic activity (Takano *et al.*, 1974;

Nilsson *et al.*, 1973; Stokke, 1972). Warner *et al.* (1980) devised a technique to solubilize acid lipase activity from human liver by using Triton X-100. This technique was employed here because it provided a speedy and reproducible means to extract acid lipase activity.

The M_r of ALII estimated by SDS/polyacrylamide-gel electrophoresis was about 58 500, whereas the value estimated by gel filtration through a Sephacryl S-200 column was approx. 69 000. This discrepancy could be the result of ALII being glycosylated and an affinity for Sephacryl S-200 as described (Imanaka *et al.*, 1982). There is disagreement in the literature as to the correct M_r of mammalian acid lipase. Some of the discrepancy has been attributed to species differences and to preparations that have not been sufficiently purified. We have isolated two distinct forms of highly purified acid lipase from human liver which differ in a multitude of properties, including subunit M_r (32 000 versus 59 000), pH optima (ALI more acid), hydrophobicity (ALI more hydrophobic), isoelectric point (ALII more anodal) and kinetics (ALI cleaves cholesteryl oleate and triolein at a lower K_m than does ALII). ALI and ALII do not cross-react immunologically, indicating that they do not share a precursor-product relationship and are separately coded polypeptides. The presence of two distinctive acid lipases in human liver may explain some of the discrepancies in the literature.

The subunit M_r of acid lipase from human tissue has differed markedly in previous reports. The human enzyme has been characterized from four separate tissues or secretions: liver, placenta, fibroblasts and gastric juice. The reported subunit M_r values were 29 000, 30 500 (estimated by gel-permeation chromatography), 49 000 and 44 000 respectively (Warner *et al.*, 1981; Burton & Mueller, 1980; Sando & Rosenbaum, 1985; Tiruppathi & Balasubramanian, 1982). Acid lipases have also been isolated from rabbit and rat liver, with demonstrated subunit M_r values of 42 000 and 69 000 respectively (Imanaka *et al.*, 1984; Burrier & Brecher, 1983).

Two groups have demonstrated the presence of soluble lipase with an anodal migration in human liver (Warner *et al.*, 1980; Cortner *et al.*, 1976). This soluble lipase, however, possesses a neutral pH optimum, has a specificity only for 4MU substrates (its natural substrates are unknown), and is considerably more thermolabile than ALI or ALII. Also, because the neutral lipase is soluble, it is discarded during our initial purification step. It can therefore be concluded that ALII is not this soluble neutral lipase.

We have observed a large variance in the concentration of ALII when autopsied human liver samples were compared. As indicated above, ALII appeared to be particularly susceptible to proteolysis and low activities may reflect autolysis *post mortem*.

More information is required to determine whether ALI and ALII are coded by separate genetic loci. It is clear that a mutation at a single autosomal locus leads to nearly complete loss of lipase activity in humans (Wolman's disease, cholesterol ester storage disease), implying a genetic association of ALI and ALII. It is important to reach an understanding of the genetics of ALI and ALII to elucidate the role they play in Wolman's disease, cholesterol ester storage disease and atherosclerosis.

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