PURIFICATION OF ACID LIPASE FROM RABBIT LIVER

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1. Introduction

In [1] we demonstrated that acid lipase activity with 4-methylumbelliferyl oleate as substrate represented both acid cholesterol esterase and triacylglycerol lipase activity and that the enzyme might be associated with the lysosomal membrane or hydrophobic macromolecules in rabbit liver. The enzymes seems to be involved in catabolism of lipoproteins and to play an important role in the intracellular metabolism of cholesterol esters and triacylglycerols [2,3].

Purification of the enzyme has been attempted by several groups, but highly purified enzyme has not been obtained because of its instability and hydrophobicity [1,4–6]. The enzyme was purified 120-fold from rat liver [4] and 2500-fold from human liver [5]. Moreover, 1000–3000-fold purification of the enzyme from human placenta was reported in [6].

Here, using 4-methylumbelliferyl oleate as substrate, we purified lysosomal acid lipase from rabbit liver (21 000-fold) to a homogeneous form. This is the first report of highly purified acid lipase from lysosomal particles of rabbit liver.

2. Experimental

2.1. *Preparation of lysosomal acid lipase*

The livers of female white rabbits (3.0–3.5 kg) were homogenized in 4 vol. 0.25 M sucrose containing 1 mM EDTA and 0.1% ethanol (pH 7.4). All the media used for preparation of the enzyme contained protease inhibitors (leupeptin, chymostatin and antipain, 1 μg/ml each, and pepstatin, 0.1 μg/ml) except those for the final preparation. These inhibitors were obtained from the Protein Research Foundation (Osaka). The homogenate was centrifuged at 1000 × *g* for 10 min and the postnuclear supernatant was centrifuged at 3300 × *g* for 10 min. The resulting supernatant was centrifuged at 14 000 × *g* for 20 min and the precipitate (lysosome-rich fraction) was suspended in the same medium. The precipitate was washed by recentrifugation at 14 000 × *g* for 20 min, then homogenized in the same medium containing 0.5% (v/v) digitonin in a volume equivalent to 1/3rd that of the postnuclear supernatant. The mixture was stirred at 4°C for 40 min and then centrifuged at 50 000 × *g* for 30 min and the supernatant was collected.

2.2. *Assay of acid lipase*

Acid lipase was assayed with 4-methylumbelliferyl oleate (Koch-Light, Colnbrook) as substrate as in [1].

2.3. *Polyacrylamide gel electrophoresis*

Electrophoresis was carried out on 7.5% polyacrylamide slab gel at pH 8.9 as in [7]. The running gel and buffer solution contained 10% (v/v) ethylene glycol and 5 mM mercaptoethanol, added to stabilize the enzyme. In the case of preparative polyacrylamide slab gel electrophoresis, gel containing the enzyme was cut out and placed in a Posner Elution Recovery Device (Geluter; E-C Apparatus Corp., FL) as in [1,8]. Electrophoresis on SDS-gel was done as in [9]. Bovine serum albumin, M_r = 68 000; RNA polymerase (α-subunit), M_r = 39 000 and trypsin inhibitor, M_r = 21 500 (Boehringer, Mannheim) and ovalbumin, M_r = 45 000 (Sigma, St Louis) were run with the sample as molecular weight markers. Protein was stained with Coomassie brilliant blue.

2.4. *Determination of protein*

Protein was measured by the method in [10] with

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bovine γ-globulin as a standard (Bio-Rad Protein Assay Kit).

3. Results and discussion

In [1] we found that acid lipase was associated with the lysosomal membrane or hydrophobic macromolecules. Therefore, we prepared the enzyme from rabbit liver lysosomes and solubilized it by the treatment with 0.5% digitonin. In this way >80% of the activity was recovered in the soluble fraction. The solubilized enzyme was concentrated by ultrafiltration (Amicon, PM-10), and applied to a Bio-Gel A-1.5 m column (4 × 100 cm) and eluted with 0.05 M Tris–HCl buffer (pH 8.3) containing 5 mM mercaptoethanol and 0.05% NaN_3. In the following column chromatographic procedure, all solutions contained 5 mM mercaptoethanol and 0.01% NaN_3. About 1/3rd of the total protein applied was recovered in the void volume and acid lipase activity was eluted in a volume of ~2-times the void volume, a position corresponding to the second protein peak. The enzyme, recovered from the Bio-Gel A-1.5 m column, was applied to a 4 × 80 cm DEAE-Bio-Gel A column and eluted with a linear gradient of 0–0.25 M NaCl in 0.05 M Tris–HCl buffer (pH 8.3) (total vol. 2 l). Acid lipase was eluted with 0.15 M NaCl and concentrated by ultrafiltration in the presence of 40% (v/v) ethylene glycol. It was then applied to a 1.5 × 24 cm column of phenyl–Sepharose and eluted with a linear gradient of 40–90% (v/v) ethylene glycol in 0.01 M sodium phosphate buffer (pH 6.8) (total vol. 320 ml). Fig.1 shows the elution profile from the phenyl-Sepharose column. The acid lipase activity was eluted with 56% (v/v) ethylene glycol. Ethylene glycol was necessary to stabilize the enzyme after phenyl–Sepharose column chromatography. The enzyme fraction was concentrated by ultrafiltration and applied to preparative polyacrylamide slab gel (6 mm thick, 15 cm long). After electrophoresis, the enzyme was eluted from the slab gel as in section 2. The eluate containing enzyme activity was concentrated by ultrafiltration and applied to a 1.5 × 25 cm Sephacryl S-200 column. The enzyme was eluted with 70% (v/v) ethylene glycol in 0.05 M Tris–HCl buffer (pH 8.3). Fig.2 shows the elution profile obtained by Sephacryl S-200 column chromatography. The enzyme had affinity for this resin and was dissociated by 70% (v/v) ethylene glycol. The enzyme fraction recovered

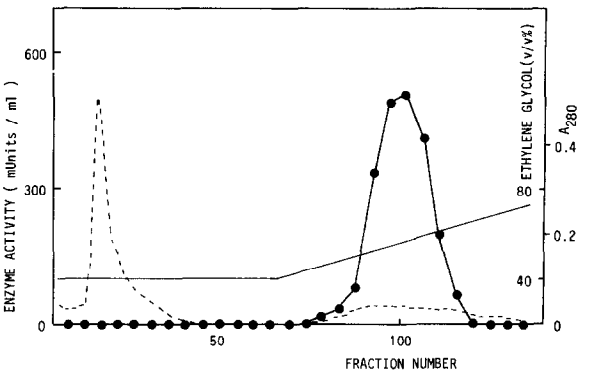

Fig.1. Elution profile on phenyl–Sepharose column chromatography. Details of the procedure are described in the text. The flow rate was 8 ml/h, (●——●) acid lipase activity; (– – –) absorbance at 280 nm; (——) ethylene glycol concentration.

from the Sephacryl S-200 column gave a single protein band on acrylamide slab gel in either the presence or absence of SDS (fig.3), suggesting that it was a single homogeneous protein. However, it may still have contained <5% of protein contaminants, judging from its profile on SDS–polyacrylamide gel electrophoresis. The M_r-value of the enzyme, calculated by SDS–polyacrylamide gel electrophoresis, was ~42 000. This value is different from those for the enzyme of rat liver (M_r = 58 000) [4,11] and human liver (M_r = 29 000) [5], possibly because of species differences, differences in purity of the preparations and the effect of surfactants.

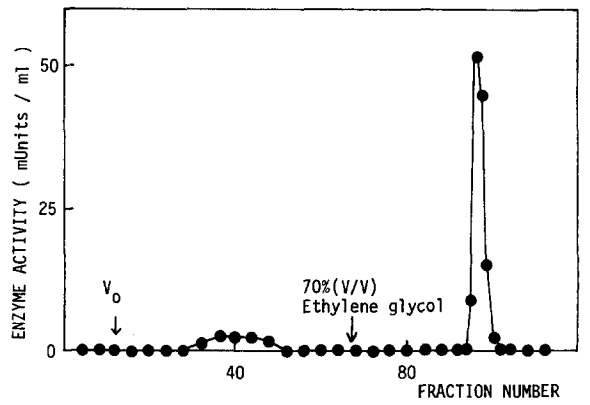

Fig.2. Elution profile on Sephacryl S-200 column chromatography. The flow rate was 8 ml/h. (●——●) acid lipase activity. The arrows show the positions of the void volume (V_o) and the start of elution with 70% (v/v) ethylene glycol.

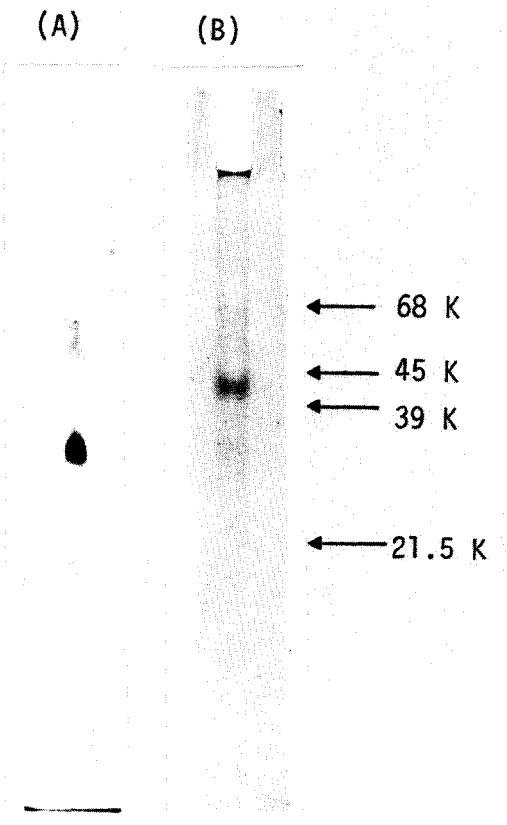

Fig.3. Polyacrylamide gel electrophoresis of purified acid lipase in the absence (A) and presence (B) of SDS: (A) Acid lipase (10 μg) was run on 7.5% polyacrylamide gel and stained with Coomassie brilliant blue; (B) Acid lipase (10 μg) was heated at 100°C for 3 min in 1.0% SDS and 1.0% mercaptoethanol. The sample was run on 7.5% polyacrylamide gel and stained with Coomassie brilliant blue. Arrows indicate the position of M_r markers: bovine serum albumin, M_r = 68 000; ovalbumin, M_r = 45 000; RNA polymerase (α-subunit), M_r = 39 000; trypsin inhibitor, 21 500.

The purity and recovery of the acid lipase at different stages of purification are summarized in table 1. The enzyme recovery in the final step was 4.83%. Recovery was low after the step of Sephacryl S-200 column chromatography, because the enzyme was unstable. The enzyme was purified 21 321-times from the postnuclear supernatant fraction. A 120-fold purification of the enzyme from rat liver was reported in [4], a 2500-fold purification of that from human liver in [5] and a 1000–3000-fold purification of that from human placenta in [6]. Thus our preparation seems to be the most highly purified and homogeneous, although there are probably differences in the enzyme in different species and tissues.

The highly purified enzyme was very unstable and half its activity was lost in 24 h at 4°C and in 2 weeks at −70°C in the presence of 40% (v/v) ethylene glycol. Some properties of the purified enzyme from rabbit liver were studied. Its optimal pH was 5.0 and it hydrolyzed both cholesterol oleate and triolein. It acted specifically on long-chain fatty acid ester, and did not hydrolyze 4-methylumbelliferyl propionate. The enzyme was adsorbed on phenyl–Sepharose and con A–Sepharose and dissociated from them by ethylene glycol and α-methylmannoside, respectively, suggesting that it was a hydrophobic glycoprotein. We are now studying other properties of the purified enzyme and the results may help to elucidate the physiological function of acid lipase in lysosomes.

Acknowledgement

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Table 1
Purification of lysosomal acid lipase

	Protein (total mg)	Activity (total units)[a]	Specific (units/mg)	Purity	Yield (%)
PNS	13 231	103.3	0.0078	1	100
Digitonin (Sup.)	480.5	121.1	0.252	32.3	117
Bio-gel A–1.5 m	159.8	108.1	0.676	86.7	105
DEAE-Bio-gel A	60.4	84.2	1.40	179	81.5
Phenyl–Sepharose	2.39	48.6	20.3	2603	47.0
Slab gel	0.18	24.9	138.3	17 730	24.1
Sephacryl S-200	0.030	4.99	166.3	21 321	4.83

[a] μmol 4-methylumbelliferone released/min

Abbreviation: PNS, postnuclear supernatant

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