The Purification of a Lipoprotein Lipase from Human Adipose Tissue

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ABSTRACT. The purification of lipoprotein lipase from human adipose tissue was accomplished after successive chromatography on heparin-Sepharose and concanavalin A-agarose of an acetone-ether treated crude homogenate. The preparation obtained was purified 2,000-fold. A single protein with an apparent molecular weight of 60,000 daltons was obtained after solubilization of the protein with sodium dodecylsulfate in the presence of leupeptin before separation of the proteins by slab gel polyacrylamide electrophoresis. Omission of the protease inhibitor, leupeptin, resulted in breakdown of lipoprotein lipase into two small proteins of different size of 31,000 and 16,000 daltons.

Key words: purification — lipoprotein lipase — adipose tissue

Lipoprotein lipase, first detected by Hahn,[1] is important in the breakdown of the triglyceride of chylomicron and very-low-density (VLDL) lipoprotein in the plasma. We report the purification of human adipose tissue lipoprotein lipase (LPL) to homogeneity.

EXPERIMENTAL PROCEDURES

Source of Enzyme and Crude Enzyme Preparation — Human adipose tissue removed for obesity control, ordinarily discarded, was collected from several patients, frozen and stored at − 80℃ until used for enzyme preparation. Enzyme powder was prepared from pooled tissue specimen which were homogenized and extracted by the acetone-ether method previously described[2] before drying under vacuum on Whatman 1 mm filter paper. Enzyme was extracted from the resulting powder with a buffer solution consisting of 1.2 M NaCl, 0.005 M sodium barbital, 20% glycerol, 0.001 mM leupeptin, 1 mM EDTA, pH 7.0, using 1.0 ml per 50 mg powder at 4℃ while stirring for 4 hrs. The extract was then centrifuged at 30,000 g for 20 min at 4℃, the supernatant fraction removed and dialyzed against 2,000 volumes of 0.001 M sodium barbital, pH 8.5 at 4℃ for 2 hrs. Protein concentration in the dialyzed supernatant fraction was determined by the method of Lowry using bovine serum albumin (BSA) as the standard.

Purification of Human Adipose Tissue LPL — Enzyme extracts prepared as above were adjusted to 0.5 M with NaCl and applied to a heparin-Sepharose 6B column (12×1 cm) previously equilibrated with buffer containing 0.5 M NaCl, 0.005 M sodium barbital, 20% glycerol, 0.001 mM leupeptin, 1.0 mM EDTA, pH 7.0. The column was washed with the same buffer (1.0 ml/min.) until absorbance of the wash decreased to 0 nm at 280 nm. Using a stepwise NaCl gradient, the first protein peak eluted with 0.8 M NaCl and a second peak eluted with 1.5 M NaCl. LPL activity was associated with the second peak, Peak II. Peak II fractions in 1.5 M NaCl were pooled and applied directly to a column of concanavalin A-agarose (13×1 cm) previously equilibrated with buffer containing 1.0 M NaCl in 0.005 M sodium barbital, 20% glycerol, 0.001 mM leupeptin, 1.0 mM EDTA, pH 7.0 at the rate of 0.5 ml/min. The column was extensively washed with equilibrating buffer monitoring fractions for protein by absorbance at 280 nm. LPL was eluted with 0.5 M α methyl-D mannopyranoside in buffer. Fractions with protein as determined by absorbance were pooled, dialyzed against 2,000 volumes of 0.001 M sodium barbital, pH 8.5 at 4℃ for 2 hrs before assay for LPL activity. Another aliquot was dialyzed against 2,000 volumes of double distilled water for 6 hrs with two changes before lyophilization for SDS polyacrylamide gel electrophoresis (PAGE).

SDS Polyacrylamide Gel Electrophoresis — The experiment was based on that described by Laemmli.[3]

Lipoprotein Lipase Assay — The assay for LPL was based on that described by Schotz.[4]

RESULTS AND DISCUSSION

The elution pattern of human adipose tissue LPL from the heparin-Sepharose 6B column is shown in Fig. 1. Approximately 98% of the loaded protein appeared in the combined void volume, wash and eluate from the 0.5 M NaCl gradient. Protein was also eluted with 0.8 M NaCl which did not hydrolyze triolein after dialysis. The fraction eluted with 1.5 M NaCl (Peak II) exhibited LPL activity with a 40-fold higher specific activity than the enzyme in the preparation applied to the heparin-Sepharose 6B column (Table 1). The quantity of protein recovered was extremely small, 2.11% of the amount loaded, which, however, represented 90% of the enzyme percent in the original sample (Table 1).

TABLE 1. Purification of human adipose tissue LPL.

	Protein	Protein % recovery	Specific activity	Total activity	Purification	Total activity % recovery
	mg	%	n mol free fatty acid released/mg protein/min	n mol free fatty acid released/min		%
Human adipose tissue	4200	100	9.7	40740	1	100
Heparin-Sepharose 6B chromatography	88.77	2.11	416.3	36954.9	43	90.7
Concanavalin A-agarose chromatography	1.19	0.028	20785.5	24734.7	2143	60.7

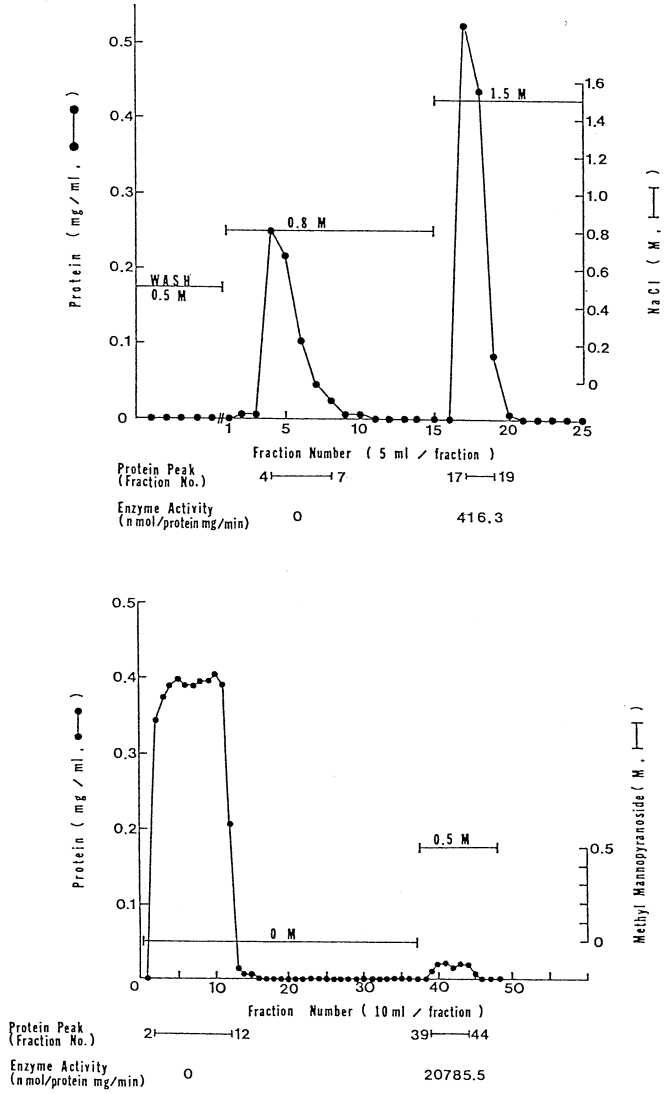

Fig. 1. Heparin-Sepharose 6B chromatography (above) and concanavalin A-agarose chromatography (below)

Peak II from the heparin-Sepharose 6B fraction was then applied to a concanavalin A column. Inactive protein was eluted during the wash with equilibrating buffer. LPL was eluted with 0.5 M α methyl-D mannopyranoside (Fig. 1). The specific activity of the enzyme in the pooled eluate was nearly 2,000 times higher than the enzyme in the initial extract of the acetone-ether treated adipose tissue loaded on the heparin-Sepharose column (Table 1). The quantity of protein recovered was 0.03% of the starting material but the total activity recovered was 60% (Table 1). This enzyme had an absolute requirement used in these assays, had an alkaline pH optimum, and was totally inhibited by protamine (2 mg/ml).

A progressive reduction in the number of protein bands from over a dozen,

to two, to one major component can be seen as the enzyme is taken through the several steps selected for purification (Fig. 2). The single band found after concanavalin A-agarose chromatography was calculated to be 60,000 daltons in apparent Mr by comparison with the migration of the purified standards used. Socorro and Jackson[5] indicated that the protease inhibitor phenylmethane sulfonyl fluoride (PMSF) was effective in inhibiting the breakdown of LPL during purification of this enzyme from bovine milk. PMSF was not effective in protecting human adipose tissue LPL as indicated by breakdown of 60,000 dalton proteins to two smaller sized bands of 31,000 and 16,000 daltons as shown in lane E in Fig. 2 in contrast to leupeptin which protected LPL effectively as indicated by the single band of 60,000 daltons seen in lane D. The selection of leupeptin instead of PMSF is, thus, considered, to be preferable in the purification of LPL from human adipose tissue.

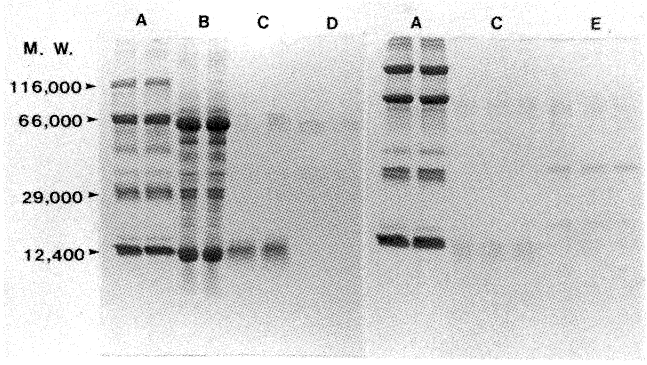

Fig. 2. SDS polyacrylamide gel electrophoresis of human adipose tissue LPL at each purified step
A : Molecular weight markers are β-galactosidase (116,000), bovine serum albumin (66,000), carbonic anhydrase (29,000) and cytochrome C (12,400)
B : Crude LPL
C : After Heparin-Sepharose column
D : After concanvalin A-agarose column with leupeptin
E : After concanavalin A-agarose column with PMSF

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