PURIFICATION OF SALT RESISTANT LIPASE AND LIPOPROTEIN LIPASE FROM HUMAN POST-HEPARIN PLASMA

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1. Introduction

Two different lipase activities salt resistant lipase and lipoprotein lipase have been isolated from human post-heparin plasma [1–3]. These enzymes require different conditions to hydrolyze triglyceride emulsions with maximal activity. Apolipoprotein-C II is mandatory for the activity of LPL only. By contrast, a high salt concentration (1 M NaCl) affords inhibition of LPL and maximal activity of SRL. In spite of these functional differences it has recently been claimed that the enzymes have identical amino acid composition, identical terminal amino acid residues and very similar molecular weights. The only distinguishing feature reported is different carbohydrate moieties [3].

The putative similarity between LPL and SRL must be reassessed in view of the findings in the present work. It is demonstrated that earlier described purification procedure by means of affinity chromatography on heparin–Sepharose [4], yield enzyme fractions with a high content of antithrombin. Modified procedures are described in which this major contaminant is removed by another affinity chromatography on heparin–Sepharose with low affinity for antithrombin [5].

Abbreviations: Salt resistant lipase, SRL (also called hepatic lipase), Lipoprotein lipase (EC 3.1.1.3), LPL

2. Materials and methods

2.1. *Post-heparin plasma*

Healthy male students were injected intravenously with heparin (AB Vitrum, Stockholm, Sweden; 100 IU/kg body wt). Twenty minutes after injection, 500 ml plasma was obtained from each subject by plasmapheresis and then immediately chilled to 4°C. All subsequent fractionation procedures were carried out at the same temperature.

2.2. *Heparin–Sepharose*

Unfractionated heparin was isolated and attached to Sepharose CL-6B (Pharmacia Fine Chemicals AB, Uppsala, Sweden) as described previously [6,7]. Fractionated heparin of low affinity for antithrombin [5], a generous gift from Drs I. Björk, M. Höök and U. Lindahl, was also linked to Sepharose CL-6B by the same procedure.

2.3. *Anti-antithrombin–Sepharose*

Human antithrombin [8] was kindly supplied by Dr I. Björk. The antigen (1 mg/ml) was emulsified with equal vol. Freund's complete adjuvant and rabbits were injected with 0.1 ml aliquots into one lymph node on the backside of each thigh. Booster doses using the incomplete adjuvant were given twice at two week intervals by the same route. The rabbits were bled two weeks after the last injection

and IgG antibodies were isolated [9]. The immunosorbent was prepared by coupling the antibodies to cyanogen bromide activated Sepharose 4B [10].

2.4. *Protein determinations*

The Lowry method was used [11] with a bovine serum albumin standard. Alternatively, protein concentrations were estimated by measuring absorbance at 280 nm.

2.5. *Immunodiffusion*

Double [12] and radial [13] immunodiffusion were both carried out in 1% agarose gel containing 0.04 M Veronal buffer (pH 8.6). The gels were stained by amido black.

2.6. *Polyacrylamide gel electrophoresis*

The sodium dodecyl sulphate discontinuous buffer system of Neville [14] was employed. Staining was performed with amido black.

2.7. *Enzyme assays*

The assay medium of Iverius et al. [15] was used. Triglyceride emulsions were radioactivly labelled with glycerol-tri-[9,10-(*n*)-^{3}H]oleate (1.3 Ci/mol) triglyceride) and used at a concentration of 2.5 mg/ml. Appropriate ionic strength was achieved by varying the sodium chloride concentration.

LPL activity was determined at ionic strength 0.16 using a 20:1.2 (w/w) soybean oil–egg lecithin emulsion (Intralipid 20%; generously provided by Vitrum AB, Stockholm, Sweden). Human serum (20%, v/v) was the activator.

SRL was assayed at ionic strength 1.0 without any serum added. Intralipid was employed for screening of chromatograms. A 1:1 (w/w) trioleate–gumarabic emulsion [16] was the substrate when the specific activity of purified SRL was determined.

Samples for extraction of fatty acids by the method of Belfrage and Vaughan [17] were taken in duplicates at the beginning and at the end of the 30 min incubation period. Aliquots (0.2 ml) were mixed with 5 ml Instagel (Packard Instrument AB) and counted in a Beckman LS-250 liquid scintillation spectrometer. Recoveries of the extraction procedures were obtained by means of a [^{3}H]oleic acid standard. Radioactivity counts were converted to enzyme activity by using the specific radioactivity of the emulsion triglyceride fatty acids.

3. Results

3.1. *Affinity chromatography on heparin–Sepharose*

Post-heparin plasma (1000 ml) was mixed with solid NaCl to a final concentration of 0.4 M [3] and then equilibrated with 120 ml heparin–Sepharose for 30 min. The gel was washed six times with 200 ml 0.4 M NaCl–30% (v/v) glycerol–0.01 M sodium phosphate (pH 7.5) and packed into a column (2.5 × 20 cm). After further washing with 3 bed vol. buffer, the column was eluted with a linear salt gradient from 0.4–1.5 M NaCl. Two peaks of lipase activity, representing SRL and LPL were resolved (fig.1). Peak fractions were pooled and concentrated by 3.6 M ammonium sulphate–0.01 M sodium phosphate (pH 6.5) as described [7]. Protein precipitates were dissolved in 1 ml 50% (v/v) glycerol–0.01 M sodium phosphate (pH 7.5) and stored at –20°C [7]. Double immunodiffusion (fig.2) showed that both enzyme fractions contained antithrombin. It corresponded to more than 90% of the total protein content in both enzyme fractions as determined by radial immunodiffusion.

3.2. *Affinity chromatography on heparin–Sepharose with low affinity for antithrombin*

Four preparations of SRL obtained as described

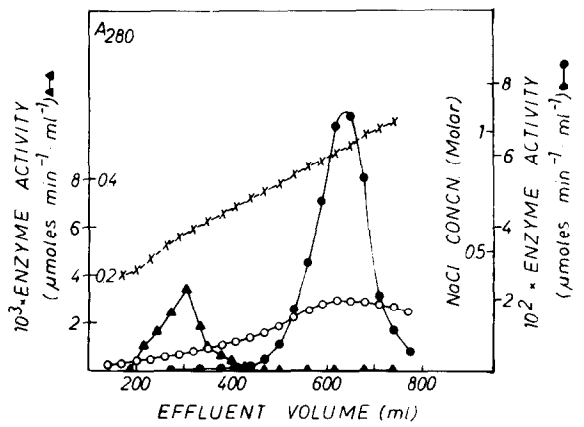

Fig.1. Affinity chromatography on heparin–Sepharose. The column (2.5 × 20 cm) was eluted with a 700 ml linear gradient of 0.4–1.5 M NaCl in 30% (v/v) glycerol–0.01 M phosphate (pH 7.5) at a flow rate of 1 ml/min. The fractions (5 ml) were analysed for salt resistant lipase activity (▲ - ▲), lipoprotein lipase activity (● - ●), absorbance at 280 nm (o - o) and sodium chloride concentration (× - ×).

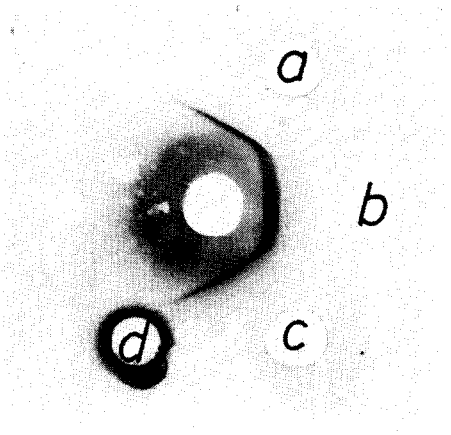

Fig.2. Immunodiffusion analysis of antithrombin. The peripheral wells contained (a) salt resistant lipase fraction (fig.1, first peak; 10 μg), (b) human antithrombin (7 μg), (c) lipoprotein lipase fraction (fig.1, second peak; 10 μg) and (d) bovine milk lipoprotein lipase (7 μg). The central well was loaded with anti-human antithrombin.

above were pooled and NaCl was added to a final concentration of 0.4 M. The sample was applied to a heparin–Sepharose column (1.6 × 5 cm) with low affinity for antithrombin. After washing with 160 ml 0.4 M NaCl–30% (v/v) glycerol–0.01 M sodium phosphate (pH 7.5), the enzyme was eluted with the same buffer containing 0.8 M sodium chloride (fig.3A).

A similar procedure was used for LPL. However, the sodium chloride concentration of the buffer was 0.5 M for sample application and column washing and 1.2 M for elution of the enzyme (fig.3B).

3.3. *Adsorption to C_γ-aluminium hydroxide gel*

This purification step was employed for LPL only and carried out as described [7]. The enzyme fraction obtained after the second heparin–Sepharose chromatography was adsorbed to 2 ml sedimented gel. After washing and elution, the enzyme fraction was collected by dialysis against ammonium sulphate, dissolved in 1 ml 50% (v/v) glycerol–0.01 M sodium phosphate (pH 7.5) and stored at −20°C.

3.4. *Chromatography on anti-antithrombin–Sepharose*

The final purification step for both LPL and SRL was removal of trace amounts of antithrombin by anti-antithrombin–Sepharose. The samples were passed through columns (1.5 × 1.5 cm) of the immunosorbent in the presence of 0.5 M NaCl–30% (v/v) glycerol–0.01 M sodium phosphate (pH 7.5). Active fractions were pooled, dialyzed against ammonium sulphate and stored at −20°C in the presence of glycerol as described above.

3.5. *Properties of purified salt resistant lipase and lipoprotein lipase*

The purification procedures of SRL and LPL are summarized in tables 1 and 2, respectively.

The purified SRL was active at ionic strength 1.0 and did not require any serum activator for maximum activity. In accordance with earlier findings [18] Intra-

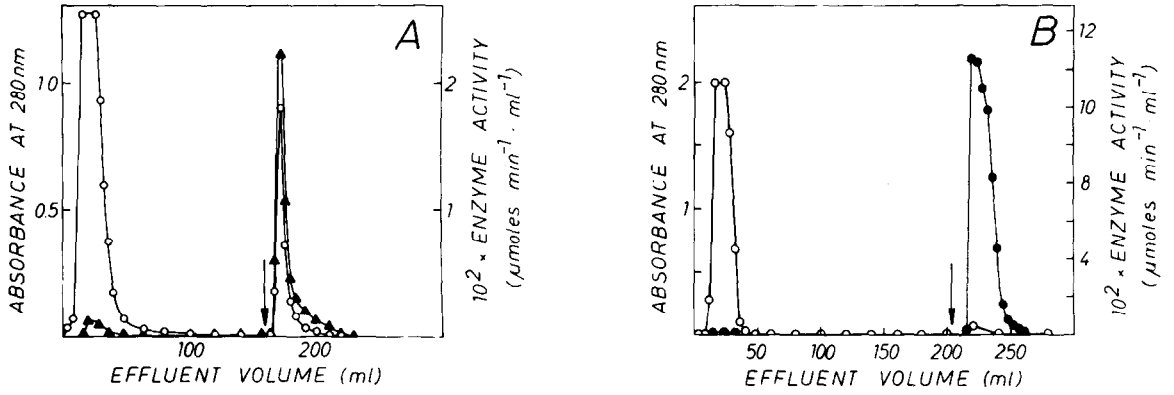

Fig.3. Affinity chromatography of (A) the salt resistant lipase fraction and (B) the lipoprotein lipase fraction on heparin–Sepharose with low affinity for antithrombin (see Materials and methods). Fractions of 4 ml were analyzed for salt resistant lipase activity (▲ - ▲), lipoprotein lipase activity (● - ●) and absorbance at 280 nm (○ - ○).

Table 1
Purification of salt resistant lipase from post-heparin plasma

	Volume (ml)	Protein[a] (mg)	Specific activity[b] (μmol/min mg)	Yield (%)
Post-heparin plasma	4000	240 000	0.004	100
Heparin–Sepharose	800	42.5	2	13
Ammonium sulphate 3.6 M (dialysis)	–	–	–	–
Heparin–Sepharose with low affinity for anti-thrombin	35	0.86	44	4
Anti-antithrombin–Sepharose	–	–	–	–
Ammonium sulphate 3.6 M (dialysis)	2.5	0.03	120	0.3–0.4

[a]The Lowry method
[b]Assayed with a triglyceride-gum arabic emulsion (see Materials and methods)

Table 2
Purification of lipoprotein lipase from post-heparin plasma

	Volume (ml)	Protein[a] (mg)	Specific activity[b] (μmol/min mg)	Yield (%)
Post-heparin plasma	4000	240 000	0.002–0.003	100
Heparin–Sepharose	740	150	2	50–60
Ammonium sulphate 3.6 M (dialysis)	–	–	–	–
Heparin–Sepharose with low affinity for anti-thrombin	30	1.2	100–150	20–30
C_γ-aluminium hydroxide gel	–	–	–	–
Ammonium sulphate 3.6 M (dialysis)	1	0.09	250	3–5
Anti-human antithrombin–Sepharose	–	–	–	–
Ammonium sulphate 3.6 M (dialysis)	1.5	0.01	400–450	1–2

[a]The Lowry method
[b]Assayed with a triglyceride–phospholipid emulsion activated by human serum (see Materials and methods)

lipid as the substrate resulted in low lipolytic activities with this enzyme.

The purified LPL required serum or apolipoprotein-C II for maximal activity and was completely inhibited at ionic strength 1.0. Furthermore, activity curves, obtained by varying the Intralipid concentration in the presence of constant amount of apolipoprotein-C II, had a biphasic shape which is similar to results obtained with highly purified bovine milk LPL [19].

Electrophoretic analysis of both enzyme fractions showed several components (fig.4).

Fig.4. Sodium dodecyl sulphate polyacrylamide gel electrophoresis of SRL (A) (20 μg) and of LPL (B) (10 μg).

4. Discussion

Affinity chromatography on heparin–Sepharose has been widely used for the purification of post-heparin plasma lipases [1–3,20,21]. The present work demonstrates that this procedure alone yields lipase fractions of which a major constituent is antithrombin. This finding is not surprising, since antithrombin also has a high affinity for heparin [5] and is present in plasma at a concentration of about 0.2 mg/ml.

Recently it has been shown that LPL from bovine milk binds to heparin–Sepharose of low affinity to antithrombin [5,22].

The present method of removing antithrombin results in human plasma lipase fractions of far higher specific activities (tables 1 and 2) than described earlier [2,20,21]. Yet, neither the SRL nor the LPL is pure (fig.4).

The degree of purity of the SRL is hard to assess, since it is not known which component is the enzyme protein. However, the specific activity obtained by the present procedure (table 1) is more than 5-fold higher than the highest specific activity so far reported [2].

The specific activity of the LPL fraction (table 2) is about 70% of that of highly purified LPL from bovine milk [7]. Assuming that both the bovine and the human have specific activities of a similar order, the human LPL may be more than 50% pure. Indeed, the major component of the LPL fraction (fig.4) has a mobility similar to LPL from bovine milk.

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