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PURIFICATION OF RAT ADIPOSE TISSUE LIPOPROTEIN LIPASE BY AFFINITY CHROMATOGRAPHY

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Summary

Lipoprotein lipase (EC 3.1.1.3) from rat adipose tissue was purified by affinity chromatography with heparin-Sepharose. Elution was carried out with buffered solutions of increasing NaCl molarity. Proteins without affinity for heparin were eluted with 0.5 M NaCl, while lipoprotein lipase activity was eluted as two peaks with 1.16 M NaCl (In earlier work on human adipose tissue (Etienne et al. (1974) C.R. Acad. Sc. Paris 279, 1487—1490) two fractions with lipoprotein lipase activity were also obtained). Phospholipase activity was detected in the fraction eluted with buffered 0.5 M NaCl and containing proteins without affinity for heparin.

On feeding the fasting rats with fresh cream or glucose two peaks were also obtained, but the first peak had clearly increased while the second one had remained virtually unchanged.

Introduction

Lipoprotein lipase (EC 3.1.1.3) hydrolyses the circulating triglycerides of VLDL and chylomicrons and is present in extra hepatic tissues such as adipose, muscle and heart tissues and vascular endothelium. Affinity chromatography has made it possible to separate and purify lipoprotein lipase from various sources [1—11].

We are reporting here results obtained by separating rat adipose tissue with affinity chromatography on heparin-Sepharose: two peaks with lipoprotein

Abbreviations: VLDL: very low density lipoproteins; apo CII: one of the three major apoprotein constituents of VLDL.

lipase activity were found. These had similar physical and chemical characteristics when the animals were fasting. However feeding differences were observed.

Materials and Methods

Enzyme sources. Epididymal adipose tissue was obtained from Wistar strain rats (150—200 g) previously fasted for 15 h. Acetone/ether powders were prepared without delay and extracted with 0.025 M NH_4OH/NH_4Cl buffer (pH 8.5) [12].

In the feeding experiments, two groups of animals were used.

Group 1: 2 ml of fresh cream were given by gastric intubation to rats fasted for 15 h. 3 h later, the animals were killed and the epididymal adipose tissue rapidly excised.

Group 2: the animals were either allowed to drink 20% glucose solution ad libitum overnight and then killed (the average amount of glucose absorbed per animal was then 6 g per 12 h), or alternately fed 2 ml glucose solution (1 g/ml) by gastric intubation following a 15-h fast, and killed 3 h later.

Preparation of heparin-Sepharose column for affinity chromatography. Crude heparin (129 USP/mg, Wilson laboratories) was covalently bound to agarose gel (Sepharose 4B, Pharmacia Fine Chemicals, Uppsala, Sweden) activated by cyanogen bromide as described by Iverius [13] (7 mg of heparin/g of activated Sepharose). The crude enzyme extract was applied to a heparin-Sepharose column (7 cm × 1 cm) and eluted with 3 solutions of sodium chloride of increasing molarity (30 ml, 0.5 M; 50 ml, 0.7 M; and 50 ml, 1.16 M) in 0.005 M barbital buffer (pH 8.4) (see Table I).

5-ml fractions were collected after recording the absorbance at 280 nm. Each fraction was dialysed against a 0.005 M barbital buffer containing 0.157 M NaCl (pH 8.4).

TABLE I

SUMMARY OF THE SUCCESSIVE STAGES IN THE PURIFICATION OF RAT ADIPOSE TISSUE LIPOPROTEIN LIPASE

	Volume (ml)	Protein (mg)	Total enzyme activity (μm/h)	Specific activity (μm/h/mg protein)
NH_4Cl/NH_4OH extract of 300 mg acetone/ether powder (from 15 g rat epididymal fat pads)	15	75	75	1
Heparin Sepharose chromatography:				
applied	10	50	50	1
unbound proteins (elution with 0.5M NaCl)	30			
washing (elution with 0.7M NaCl)	50			
activity recovered (elution with 1.16M NaCl)				
peak 1	20	0.140	22.1	158
peak 2	20	0.080	17.6	220

Determination of enzyme activity. Lipoprotein lipase activity was measured by a semi-automatic technique [14]. Fatty acids were estimated after being released by incubating the enzyme with a synthetic triglyceride emulsion (Lipiphysan) at 37° C for 1 h. The rate of production of unesterified fatty acids was linear with time up to 60 min. (The substrate had previously been activated with fresh normal human serum). Enzyme activity was expressed in nmol of fatty acids released per h.

Lipiphysan contains 10 g of triglycerides: cotton seed oil, (1% C_{14}, 22.5% C_{16}, 1.6% C_{18}, 23.7% $C_{18:1}$, 50% $C_{18:2}$, 1% $C_{18:3}$) and the following emulsifying agent: 1.5 g of soja bean lecithins and 5 g of sorbitol per 100 ml.

Phospholipase activity was detected by incubation with [U-^{14}C]phosphatidyl glycerol.

The [U-^{14}C]phosphatidyl glycerol was extracted from *Staphylococcus aureus* cultured in presence of [^{14}C]sodium acetate. It was prepared and ultrasonically disintergrated according to the method formerly described [15]. Its specific activity was 170 000 cpm per μg of phosphorus. After incubation of the enzyme with [U-^{14}C]phosphatidyl glycerol, the lipids were extracted and separated by thin layer chromatography: phosphatidyl glycerol, lysophosphatidyl glycerol and fatty acids were then located by autoradiography as previously described [15].

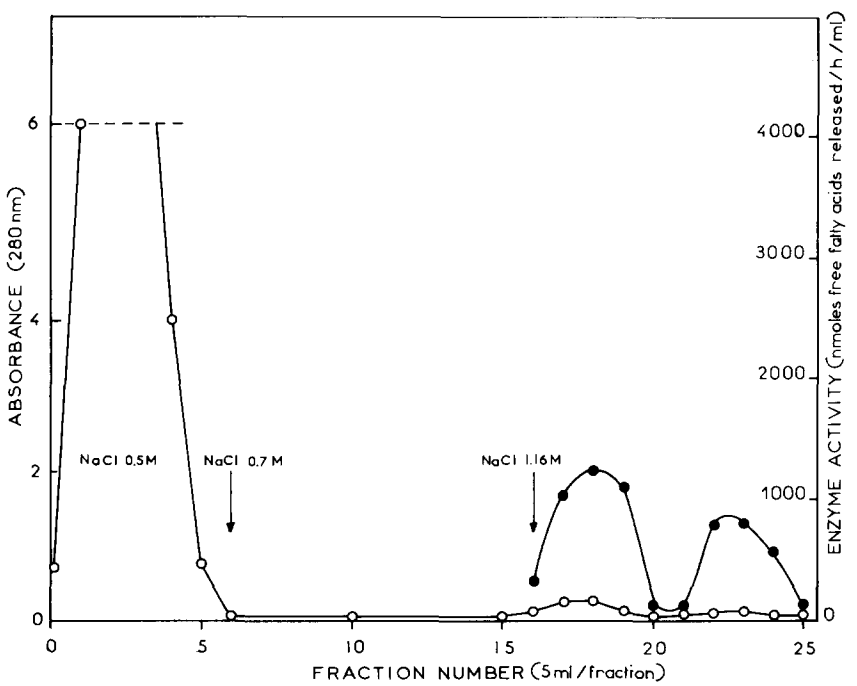

Fig. 1. Heparin-Sepharose chromatography of fasting rat adipose tissue lipoprotein lipase. A 10-ml NH_4Cl/NH_4OH extract of an acetone powder (200 mg) was applied to a 7 cm × 1 cm column as described under Materials and Methods. Lipoprotein lipase activity-containing fractions were eluted with buffered 1.16 M NaCl. Enzyme activity is expressed as nmol of fatty acids released per h per ml of enzyme solution. ●——●, lipoprotein lipase activity (10 experiments); ○——○, absorbance at 280 nm.

Measurement of proteins. For the calculation of the specific activities, proteins were measured by the method of Lowry et al., using bovine serum albumin as a standard [16].

Results

(1) Lipoprotein lipase activity

Proteins without affinity for heparin were eluted with 0.5 M NaCl and the enzyme eluted as two peaks with 1.16 M NaCl (see Fig. 1).

Characteristics of the two peaks with lipoprotein lipase activity. The usual criteria for the identification of lipoprotein lipase from a tissue are: the presence of a serum peptidic factor (apolipoprotein CII or apo Glu [17]) for maximum activity, the inhibition of this activity by 1 M NaCl and a pH optimum of about 8.6.

The activity of the first peak was clearly inhibited when serum was omitted, and decreased when adding 1 M NaCl; the optimum pH was 8.5.

As to the second peak, maximum activity was obtained when serum was present and was only slightly inhibited by 1 M NaCl (see Table II). The optimum pH was 8.5.

(2) Phospholipase activity

On using radioactive phosphatidyl glycerol as substrate, phospholipase A activity was detected in the crude enzymatic extract before it was applied to the column and in the fraction eluted with buffered 0.5 M NaCl and containing proteins without affinity for heparin. No phospholipase A activity was detected in the fraction containing lipoprotein lipase.

(3) Effect of feeding

Total lipoprotein lipase activity. Total lipoprotein lipase activity per gram of adipose tissue was higher in the fed (15 ± 5 μmol/h/g) than in the fasting rats (5 ± 2 μmol/h/g).

Activity of the 2 lipoprotein lipase fractions. Each time, an increase of

TABLE II

EFFECT OF OMITTING SERUM OR OF ADDING 1 M NaCl ON RAT LIPOPROTEIN LIPASE PURIFIED BY HEPARIN-SEPHAROSE CHROMATOGRAPHY

Fractions representing the first lipoprotein lipase peak (see Fig. 1 legend) were pooled. The same for peak 2. The two fractions were dialysed and lipoprotein lipase activity measured as described under Materials and Methods (= 100% activity). In the above experiments, either serum was omitted or 1 M NaCl added. Results are expressed as a percentage of optimum activity.

% Activity	Lipoprotein lipase	
	Peak 1	Peak 2
Absence of serum	26% ± 3 (4) [a]	63% ± 4 (4)
Addition of 1 M NaCl	42% ± 5 (4)	90% ± 5 (4)

[a] number of experiments.

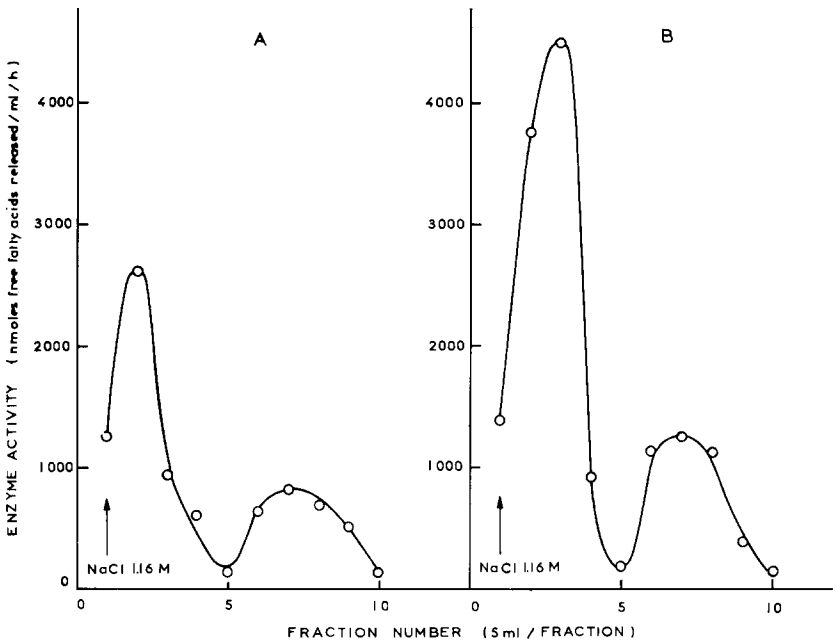

Fig. 2. Heparin-Sepharose chromatography of lipoprotein lipase from rat adipose tissue (for details see Fig. 1). Fasted rats fed with (A) fresh cream (3 experiments), (B) glucose (3 experiments).

enzyme activity was found in peak 1 only, that of peak 2 remaining the same, irrespective of diet.

(a) Rats having received fresh cream: lipoprotein lipase activity in the first fraction was clearly increased after fresh cream feeding (increased 3—5 times according to the experiments), while activity in the second fraction remained similar to that found in the fasting rats in all experiments (see Fig. 2A).

(b) Rats having received glucose: similarly glucose enhanced the lipoprotein lipase activity of the first peak while peak 2 remained similar to that of the fasting rats (see Fig. 2B). The results were similar when glucose was given to the rats by gastric intubation 3 h before they were killed, or when they drank a 20% glucose solution freely for 12 h before being killed.

Discussion

Affinity chromatography on heparin-Sepharose enabled two peaks with lipoprotein lipase activity to be isolated from rat adipose tissue. In the first peak the presence of serum produced optimal activity and 1 M NaCl inhibited it, thus characterising this enzyme as a lipoprotein lipase. The enzyme activity of the second peak was less sensitive to the absence of serum or to the presence of 1 M NaCl.

The fact that the activation by serum produced in fraction 1 was not the same as that produced in fraction 2 might suggest that these fractions still

contained different concentrations of activator contaminants.

Recently, several authors have purified, (by affinity chromatography) lipoprotein lipase from cow's milk [2—4], human [5], dog [6] and pig [7] post-heparin plasma, pig [8], hen [9] and rat [10,11] heart or adipose tissue.

In all cases, only one peak with lipoprotein lipase activity was obtained when eluting with buffered 1.16 M NaCl.

From post-heparin plasma, Bensadoun et al. [7], Greten et al. [5,6] isolated 2 peaks with lipoprotein lipase activity with solution of increasing NaCl molarity. The first peak, obtained by elution with a 0.65 M NaCl solution, was identified as hepatic lipase, (now known to be also present in post-heparin plasma [18,19]) while the second peak obtained by elution with 1.2 M NaCl corresponded to lipoprotein lipase of adipose origin.

Using agarose gel chromatography and not affinity chromatography, Garfinkel and Schotz [20] (on biogel A 1.5 M) as well as Davies et al. [21] (on Sepharose 6B) isolated two peaks with lipoprotein lipase activity from rat adipose tissue.

It is possible that the two peaks we obtained correspond to the two peaks a and b described by Schotz and by Davies.

In intubation experiments when the time of food intake could be determined accurately, an increase in the activity of the first peak was observed after 3 h. It might be interesting to find out if the relatively precocious increase in activity of the second peak recorded by Garfinkel and Schotz [22] could also be demonstrated here using rats fasted for 24 h prior to refeeding.

In an earlier work [1] with human adipose tissue, we had separated two fractions with lipoprotein lipase activity by using affinity chromatography on heparin-Sepharose.

These results show that two peaks with lipoprotein lipase activity are found in rat as well as in human adipose tissue. However, there is a difference in the total activity of the two species: it is much lower in man that in rat. When fasting, the mean value is 0.6 μmol/h/g in man and 5 μmol/h/g in rat.

Lipoprotein lipase activity varies with the nutritional state of the animal: like other authors [22—25], we found that total lipoprotein lipase activity in rat adipose tissue fell when the animal was fasting and rose again after feeding.

Our results show there is an increase in activity in peak 1 only, after intake of lipids or glucose by rats. (The fact that the animals were killed 3 h after being given glucose by gastric intubation or were left to absorb it freely overnight made little difference to the results). In contrast, peak 2 remained virtually unchanged irrespective of diet.

It is not known whether the rise in lipoprotein lipase activity in the first fraction is due to an increase in synthesis or to an activation of one of the two forms of the enzyme. It would also be of interest to know if the two forms are interconvertible. The effect of other substances such as heparin and adrenaline, in vitro as well as in vivo, should also be studied.

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