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SEPARATION OF TWO MOLECULAR FORMS OF RAT ADIPOSE-TISSUE LIPOPROTEIN-LIPASE

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SUMMARY

Lipoprotein-lipase extracted from rat adipose tissue acetone-ether powders, was separated by gel filtration on Biogel A 1.5 M into two active fractions : lipoprotein-lipase "a" and lipoprotein-lipase "b" as named by Garfinkel et al. (1). Then each of these two fractions was again chromatographed on heparin-Sepharose column according to our own method (2). The lipoprotein-lipase "b" was eluted in the first fraction like the microsomal lipoprotein-lipase ; lipoprotein-lipase "a" was eluted in the second one like plasma membranes lipoprotein-lipase (3). We compared the properties of the two lipoprotein-lipases obtained by those two different chromatographic methods.

INTRODUCTION

Transfer of triglycerides from the blood stream across the cell membranes into the adipocytes requires prior hydrolysis of the triglyceride-rich lipoproteins by lipoprotein-lipase (LPLase) (4,5,6). Several works have shown that LPLase extracted from intact rat adipose tissue can be separated by gel chromatography into two major fractions LPLase "a" and LPLase "b" (1,7). The LPLase "a" ("high molecular weight form") would be extracellular, while the LPLase "b" ("low molecular weight form") would be intracellular (8). In a previous study (2), we found that the LPLase extracted from the whole adipose tissue can be fractionated also by heparin-Sepharose chromatography, namely into two fractions that we called fraction "1" and fraction "2"

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ABBREVIATIONS :

LPLase : lipoproteine-lipase EC 3.1.1.3

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according to their order of elution. More recently (3), we have shown that the microsomal LPLase contains the fraction "1" as its main component, while fraction "2" is mainly located in the plasma membranes. However, no direct experiments permitted until now a comparison of the different forms of the LPLase obtained by those two techniques. In order to make such a comparison, we rechromatographed separately the LPLase "a" and "b", previously obtained by gel filtration, on heparin-Sepharose column. The properties of the different fractions obtained are discussed in this paper.

MATERIALS AND METHODS

The epididymal fat pads of fed Wistar rats were defated as previously described by Salaman and Robinson (9). The acetone-ether powders were suspended in 0.05 M NH_4OH/NH_4Cl buffer pH 8.6 with shaking, at 4°, for 12 h. The supernatant containing the lipoprotein-lipase was obtained after centrifugation, at 3 000 g, for 15 min, at 4°C, and then immediatly applied to a column of Biogel A 1.5 M (1). The fractions eluted from this gel filtration were submitted to an affinity chromatography on a column of heparin-Sepharose (2). The LPLase activity was assayed according to Corey and Zilversmit (10) using glycerol [1 ^{14}C]-trioleate as substrate emulsified in the presence of triton X-100. Incubations were run for 30 min, at 37°C, and terminated by addition of the organic solvent system (9). The free fatty acids released were extracted by the conventional method (11), and radioactivity was assayed by liquid scintillation of Bray's solution. The LPLase activity was characterized by its sensitivity to serum activator, and its inhibition by 1 M NaCl.

RESULTS AND DISCUSSION

Lipoprotein-lipase, extracted with 0.05 M NH_4OH/NH_4Cl buffer from whole adipose tissue acetone-ether powders, can be separated into two fractions either by gel filtration or by affinity chromatography (1,2,7,12). One can ask : are there two or four different lipoprotein-lipases? Like Schotz and Garfinkel (1), we obtained by gel filtration of the whole lipoprotein-lipase extract, on Biogel A 1.5 M column, in a first peak eluted within the void volume the lipoprotein-lipase LPLase "a", and in a second peak the lipoprotein-lipase LPLase "b" (Figure 1). Etienne et al. (2) obtained by affinity chromatography of the same crude NH_4OH/NH_4Cl extract, on heparin-Sepharose column, in the first peak eluted the lipoprotein-lipase fraction "1" ("low

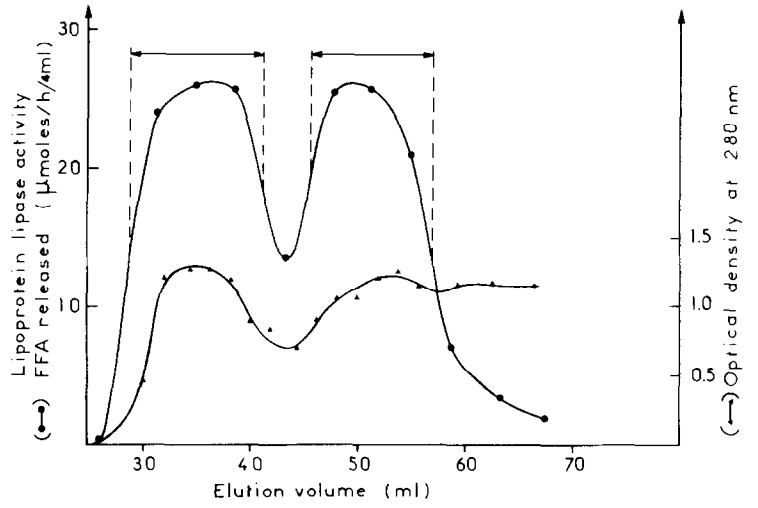

FIGURE 1 : Gel chromatography of LPLase from rat adipose tissue on Biogel A 1.5 M

The lipoprotein-lipase from adipose tissue acetone-ether powders (solubilized in 0.05 M NH_4OH/NH_4Cl buffer, pH 8.6) was applied to the column (100 x 1.5 cm) of Biogel A 1.5 M. The column was equilibrated with the same buffer containing glycerol 20 % v/v. Fractions eluted at a flow rate of 10 ml/h were assayed for LPLase activity and collected as indicated by the arrows. The recovery of the applied enzyme activities was 83 ± 6 % (mean ± SD). This elution curve is typical for six experiments.

affinity for heparin"), and in the second peak the lipoprotein-lipase fraction "2" ("high affinity for heparin").

In our previous work (3) we formulated the hypothesis that fraction "1" is similar to LPLase "b", and that fraction "2" is similar to LPLase "a". This hypothesis was based on a comparison of the properties of the different forms of lipoprotein-lipase : their presumed subcellular localization, their specific properties, and the effect of nutrition on the level of each lipoprotein-lipase activity. In the present work we have shown (Figure 2), by direct experiments, that, like fraction "2", LPLase "a" has high affinity for heparin and is eluted within an elution volume of 30 ± 4 ml (mean ± SD) of buffer containing 1.16 M NaCl. Like fraction "1", lipoprotein-lipase "b" has low affinity for heparin and is eluted within the void volume 7.2 ± 0.8 ml (mean ± SD). Iterative chromatographies do not change the elution pattern of each fraction, indicating that the chromatographical behavior of one form of the enzyme was not influenced by

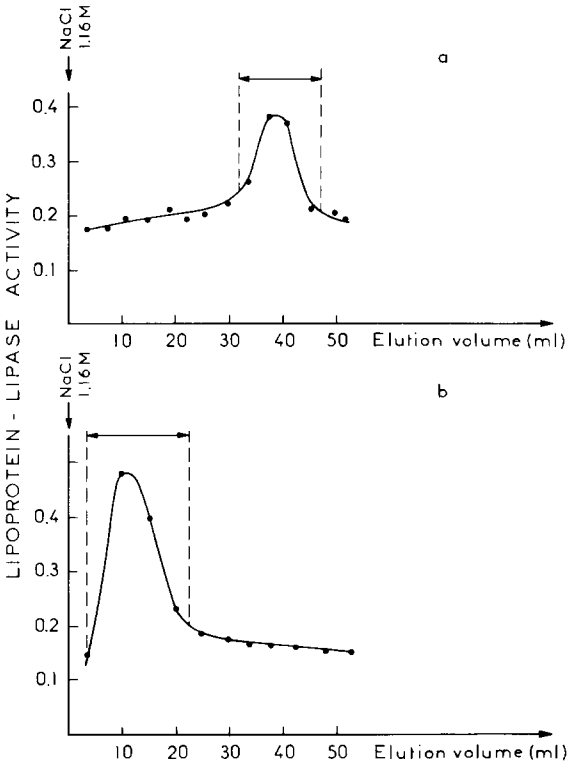

FIGURE 2 : Heparin-Sepharose chromatography of the LPLase fractions isolated by gel filtration

Each of the two fractions of LPLase, isolated on Biogel A 1.5 M column, was applied separately to a column (9.5 x 0.9 cm) of Sepharose 4 B covalently linked to heparin (15) : a) LPLase "a", b) LPLase "b". The column was equilibrated with 0.005 M veronal buffer, pH 8.6, containing 0.5 M NaCl and 20 % glycerol (v/v). Elution was carried out, at 4°C, with a discontinuous NaCl gradient of increasing molarity (20 ml of 0.5 M ; 50 ml 0.75 M ; 50 ml 1.16 M) as described by Etienne (2). The fractions, eluted with Veronal buffer 1.16 M NaCl, contained the LPLase activities. They were collected, as indicated by the arrows. 29 % of LPLase "a" and 34 % of LPLase "b" activities applied were eluted with 0.5 M NaCl buffer; with 0.7 M NaCl buffer, respectively further 5 % and 3 % of the activities applied were eluted. The rest of the enzyme activities were eluted with 1.16 M NaCl buffer. The elution curves are typical for six experiments.

the presence of the other one. By those chromatographic methods lipoprotein-lipase extracted from adipose tissue of rat can be fractionated into two different fractions only. These findings can be compared with results obtained on other tissues which also lead to the idea of, at least, two forms of lipoprotein-lipase (13).

Table 1 : Properties of the eluted fractions of the lipoprotein-lipase.

	Sample applied	Elution * volume (ml) (name of the fraction)	% Activity °	
			Absence of serum	Présence of 1 M NaCl
Chromatography on BIOGEL A 1.5 M	Crude NH_4OH/NH_4Cl extract	30 (LPLase "a")	40	54
		45 (LPLase "b")	20	30
Chromatography on HEPARIN - SEPHAROSE elution with 1.16 M NaCl	Crude NH_4OH/NH_4Cl extract (ref. 2)	7.5 (fraction "1")	26	42
		32 (fraction "2")	63	90
	LPLase "a" **	32	40	90
	LPLase "b" **	7.5	25	42

* : as defined by Garfinkel (1)

° : per cent of optimum lipoprotein-lipase activity in presence of serum and in absence of 1 M NaCl. Standard deviation was less than 10 %.

** : previously eluted from Biogel A 1.5 M column

LPLase "a" and fraction "2" not only have the same elution pattern, but also display similar properties concerning inhibition by 1 M NaCl and activation by serum (Table 1). The same conclusion can be drawn from a comparison of the elution patterns and properties of LPLase "b" and of fraction "1" (Table 1). However, this latter molecular form displays the "usual" properties of the lipoprotein-lipase to a greater extent than LPLase "a" and fraction "2".

LPLase "a" has a constant and specific elution pattern on heparin-Sepharose column, both before (2) and after gel filtration (Table 1). Furthermore, previous data suggest that LPLase "a" and "b" have different subcellular localization (3,8) : LPLase "a" (fraction 2) is almost absent from heparin-Sepharose chromatography of the microsomal fraction, although it is the main enzyme form of the plasma membrane fractions. Moreover it may be releasable by heparin (13). These results, as well as the influence of the nutritional state on this subcellular distribution (2,3) seem to indicate that the LPLase "a" is present as such in the rat adipose tissue and is not formed during gel filtration.

Ashby et al. suggested that LPLase "a" migh be an association of lipoprotein-lipase with "a particulate material" (12). This material might be membrane fragments to which LPLase "a" would be associated in the cell. The particulate material would modify the enzyme properties and the immunological reactivity explaining thereby the results obtained by Schotz et al. (14), namely that 93 % of LPLase "b" bind to pure antibodies against lipoprotein-lipase, but that only 10 % of LPLase "a" bind to the same antibodies.

However one can not exclude the possibility that LPLase "a" is not such an association but an other form of the enzyme, different from LPLase "b", which would have specific immunological and enzymatical properties.

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