INTERACTION OF TWO FRACTIONS OF HEART LIPOPROTEIN LIPASE WITH NATURAL AND SYNTHETIC SUBSTRATES

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Received 22 September 1981; revision received 30 October 1981

1. Introduction

Triglyceride moieties of circulating chylomicrons and very low density lipoproteins (VLDL) are natural substrates for lipoprotein lipase (LPL) (EC 3.1.1.34), an enzyme functional at the vascular endothelial surface [1,2]. A synthetic substrate, triolein emulsified in glycerol [3], has gained widespread use. We had employed this latter substrate to compare heparin-releasable LPL with the remaining, non-releasable LPL in the perfused rat heart [4]. The 2 fractions (releasable and non-releasable LPL) displayed a major difference in their app. K_m-values. We associated the high affinity nature of the 'releasable' fraction with the functional, endothelial-bound lipase. The low affinity nature of the 'non-releasable' fraction was compatible with its presumed role as precursor of the functional lipase and its suggested location in the parenchymal cells of the tissue [1,5,6].

In [7], LPL activity within isolated heart cells could not be demonstrated using a natural substrate, VLDL. These findings were in contrast to [8–10] where synthetic substrates were employed and in which substantial LPL activity was detected within the cells. Thus, we have employed a natural substrate, chylomicrons present in chyle, to determine:

(i) If indeed the non-releasable fraction in the perfused heart is capable of hydrolyzing chylomicron triglyceride;
(ii) Whether the releasable and non-releasable fractions display differences in K_m-values of the natural triglyceride substrate.

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2. Materials and methods

Male Sprague-Dawley rats (Hilltop Lab), 200–300 g, were fasted overnight. The preparation of the lipase fractions from perfusate and residual heart tissue has been described [4]. The natural substrate used was chyle, collected from the thoracic duct of rats pre-fed with 1–2 ml evaporated milk containing 100 μCi [1-^{14}C]palmitate (Rose Chemical Co.). More than 96% of the radioactivity was incorporated into the chylomicron fraction as revealed by isolation of the latter by centrifugation at 30 000 × g for 45 min at 16°C [11]. The triglyceride content was determined enzymatically [12]. Synthetic substrate was prepared by emulsifying triolein in glycerol with lecithin as detergent [3,4].

For the lipase reaction, 100 μl of the substrate emulsion was added to 100 μl of the enzyme fraction, containing either releasable or non-releasable LPL pre-purified by heparin–Sepharose chromatography [4]. One milliunit (mU) of enzyme activity represents 1 nmol free fatty acid released/min. For K_m determinations the chyle was serially diluted with 0.13 M Tris–HCl (pH 6.8) containing 2% (w/v) albumin, to final triglyceride levels of 0.023–0.9 mM. The dilution of the synthetic substrate for the K_m determinations has been described [4].

3. Results and discussion

Triglyceride present in chylomicrons was found to be a better substrate for both releasable and non-releasable LPL fractions, when compared to the synthetic emulsion (table 1). A Lineweaver-Burk double

Published by Elsevier/North-Holland Biomedical Press
00145793/81/0000–0000/$02.75

Table 1
Michaelis constants of LPL determined with natural and synthetic substrates

Enzyme source	Apparent K_m		Synthetic
	Natural	Synthetic	Natural
	(mM triglyceride)		
Releasable	0.05	0.44	8.8
Non-releasable	0.26	4.35	16.7

Values shown are the mean of 2 individual experiments

reciprocal plot of the 2 lipase activities against the natural substrate clearly shows that the non-releasable fraction has a higher K_m-value than the releasable enzyme (fig.1). As reported in [4], and as confirmed here (table 1), this difference in the K_m-values was observed also with the synthetic substrate.

We have suggested [4] that the non-releasable LPL represents the precursor for the endothelial-bound (releasable) lipase in the heart. This precursor might have a different conformation than the releasable

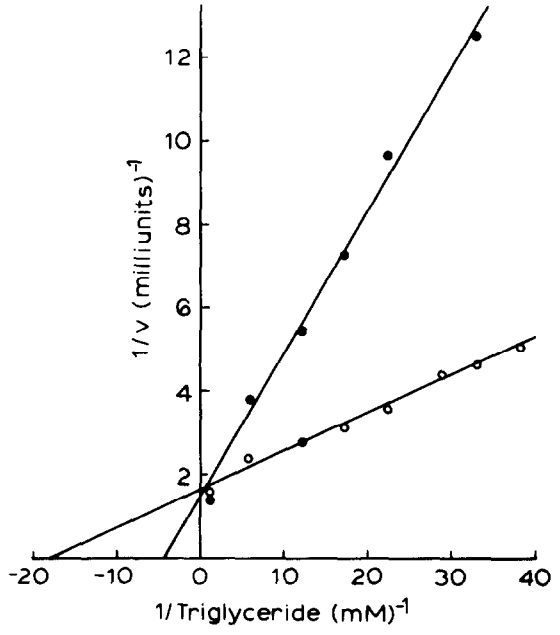

Fig.1. Lineweaver-Burk double reciprocal plot of releasable and non-releasable LPL activity with natural substrate. Releasable (o——o) and non-releasable (•——•) lipase fractions were eluted from heparin–Sepharose columns and assayed with varying concentrations of chyle, as in section 2. Each point is the mean of duplicate analyses. Similar Lineweaver-Burk plots employing the synthetic substrate have been shown in [4].

enzyme, hindering access of the substrate to the active site. This possible inaccessibility could be even more pronounced when synthetic substrate is used, hence the increased K_m ratio of synthetic/natural substrate in the non-releasable fraction (table 1).

Release of the membrane-supported LPL by heparin in heart perfusion did not change its kinetic properties [13]. The heparin-releasable fraction here was purified by heparin–Sepharose chromatography and displayed a K_m-value similar to that in [13] (0.05 mM *vs* 0.07 mM). Thus, purification of the heparin-releasable enzyme did not alter its K_m-value.

It is of interest to compare our data with reported K_m studies of the heparin-releasable and non-releasable fractions of LPL. In adipose tissue, no significant difference was found in K_m-values between the heparin-releasable fraction and the LPL from acetone powder extracts [14]. In other studies of adipose tissue [15], K_m of 0.23 mM for the heparin-releasable fraction was reported. This value is very close to that reported here for the non-releasable LPL in heart tissue (0.26 mM). Based on these kinetic similarities, it is possible that heart non-releasable LPL and the adipose LPL (releasable and non-releasable) are identical enzymes. The strikingly lower K_m-value of the heart releasable enzyme may reflect a unique activation process which occurs in heart. This activation could occur prior to, or during the transportation of the LPL from its cellular location to the endothelial site. The activation process, resulting in the high affinity for the circulating substrate, could, as suggested [15,16], assure adequate triglyceride uptake by the heart even at very low substrate concentrations.

Acknowledgements

This study was supported in part by research grants from the National Institutes of Health (HL16577), the American Heart Association, Greater Los Angeles Affiliate (492IG8) and the Veterans Administration. The authors are grateful to Dr K. Wishner for preparing the chyle and Dr M. Pedersen for assistance in the heart perfusions.

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