HEPARIN-BOUND LIPOPROTEIN LIPASE IS CATALYTICALLY ACTIVE AND CAN BE STIMULATED BY APOLIPOPROTEIN CII

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1. Introduction

Lipoprotein lipase hydrolyses acylglycerols in chylomicrons and very low density lipoproteins at the vascular endothelium [1]. It has been proposed that the enzyme is held in place there via interaction with a heparin-like polyanion; e.g., heparan sulphate [2]. This theory has gained support from studies showing that enzymatic digestion of the heparan sulphate chains on cultured endothelial cell impedes the binding of lipoprotein lipase to the cells [3,4].

Lipoprotein lipase readily binds heparin [5]; this stabilizes the enzyme [6] and makes it more soluble [7]. However, heparin seems to have little or no effect on the catalytic activity of lipoprotein lipase. This is true both in crude systems with very low density lipoproteins as the substrate [8] and with triglyceride emulsions [9]. Since the heparin molecule has a strong negative charge and specifically affects the activity of several enzymes in the coagulation cascade the lack of effect on lipoprotein lipase was somewhat surprising. There were two possible explanations:

(i) Binding of heparin does not affect the function of those regions of the lipase molecule which are involved in catalysis; or
(ii) The complex between heparin and lipoprotein lipase dissociates when the enzyme binds to a lipoprotein.

In support of (ii), there is a report that apolipoprotein CII, the activator protein for lipoprotein lipase, and heparin compete for binding to the enzyme [10].

It was therefore important to investigate directly the relation between binding of the enzyme to heparin and its ability to carry out the catalytic reaction. We report here that lipoprotein lipase can hydrolyze acylglycerols while bound to heparin–Sepharose and that apolipoprotein CII can stimulate the hydrolysis without affecting the binding to heparin.

2. Materials and methods

Heparin–Sepharose was prepared as in [11]. Lipoprotein lipase was purified from bovine milk by chromatography on heparin–Sepharose [7]. Labeled rat very low density lipoproteins were prepared by injection of ^{3}H-labeled oleic acid to rats as in [12]. The very low density lipoproteins were isolated by centrifugation of the plasma in a Beckman Ti 50 rotor, 36 000 rev./min, 24 h at 10°C. Apolipoprotein CII was isolated from the delipidated protein moiety of human very low density lipoproteins by gel filtration in 6 M guanidinium–hydrochloride followed by chromatography on DEAE-cellulose in urea [13].

The monooleoylglycerol–Triton dispersion was prepared by sonication of 15 mg monooleoylglycerol containing [^{3}H]glycerol-labeled monooleoylglycerol (7.2×10^{6} cpm, prepared by Dr L. Krabisch, Institute of Physiological Chemistry, Lund) with 32 mg Triton X-100 in a total volume of 2 ml 0.4 M Tris–HCl (pH 8.5).

For the incubation experiments lipoprotein lipase was first bound to 100 μl heparin–Sepharose in Eppendorf plastic tubes. The total volume was 750 μl containing 0.1 M NaCl, 0.1 M Tris–HCl and 40 mg/ml bovine serum albumin (fraction V, Sigma, St Louis MO). The pH was 8.5. The tubes were gently shaken at 4°C for 1 h. They were then centrifuged 30 s in a Beckman Minifuge. The supernatants were discarded and the gels were washed twice with the buffer used for the subsequent incubation. After the second wash 650 μl incubation buffer (10°C) was added to the gels

followed by 250 μl substrate (very low density lipoproteins to a final concentration of 1 mg/ml triacylglycerol or the monooleoylglycerol–Triton X-100 dispersion to a final concentration of 5.9 μmol/ml monoacylglycerol). The tubes were then incubated at 10°C. The hydrolysis was followed by taking 200 μl aliquots from the tubes for extraction and determination of free fatty acids [14] or of free glycerol [15]. For this, the tubes were centrifuged for 30 s immediately before sampling. Time curves for the hydrolysis by gel-bound lipoprotein lipase required one incubation tube for each time point since the proportion of gel to substrate was altered after sampling. For the control experiments without gel the same tube could be used for all time points.

The protein concentrations in solutions of lipoprotein lipase and of apolipoprotein CII were measured by quantitative amino acid analysis. For all experiments apolipoprotein CII was dissolved in 3 M guanidinium–hydrochloride in 20 mM Tris–HCl (pH 8.5). Corresponding amounts of the guanidinium–hydrochloride buffer were added to incubations without apolipoprotein CII and were found to have little or no effect on the lipase activity.

3. Results

Initial experiments demonstrated that lipoprotein lipase bound to heparin–Sepharose could hydrolyze lipids in rat very low density lipoproteins. The rate was ~½ that with the heparin-bound enzyme as with a corresponding amount of free enzyme. Since most of the enzyme probably is attached inside the gel particles, the substrate lipoproteins must penetrate the gel and the products of lipolysis must diffuse out from the sites of lipolysis at a sufficiently high rate to allow the reaction to continue unhindered. In an attempt to limit the exclusion effects and also to be able to study the effects of apolipoprotein CII we used monooleoylglycerol dispersed in Triton X-100 as the substrate for the following experiments.

The monooleoylglycerol dispersion was hydrolyzed at about the same rate by the heparin-bound enzyme as by the free enzyme (fig.1). In most experiments with gels a lag-phase of 5–10 min was seen before the high rate was attained. When the heparin–Sepharose was separated from the incubation so that only free enzyme could act on the substrate, the rate of hydrolysis was much lower (fig.1). Thus, most of the hydrol-

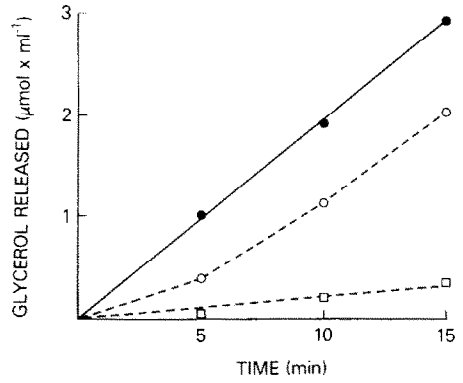

Fig.1. Hydrolysis of monoacylglycerols by lipoprotein lipase bound to heparin–Sepharose: lipoprotein lipase 20 μg was added to each tube; for details see section 2; (•) control without heparin–Sepharose (free enzyme); (○) lipoprotein lipase bound to heparin–Sepharose; (□) lipoprotein lipase bound to heparin–Sepharose but 10 s after the addition of substrate the gel was sedimented by centrifugation for 30 s. The supernatant was then transferred to another tube for continued incubation.

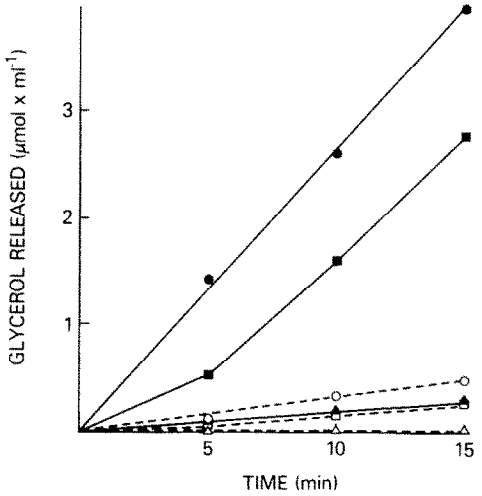

Fig.2. Stimulation of heparin-bound lipoprotein lipase by apolipoprotein CII: 4 μg lipoprotein lipase was added to each tube; after washes (section 2) a monooleoylglycerol–Triton dispersion with or without apolipoprotein CII was added; final concentration of CII was 22 μg/ml; free lipoprotein lipase (•) with and (○) without apolipoprotein CII. Lipoprotein lipase bound to heparin–Sepharose (■) with and (□) without apolipoprotein CII. Lipoprotein lipase bound to heparin–Sepharose but the gel was removed by centrifugation as in fig.1 (▲) with and (△) without apolipoprotein CII.

Table 1
Lack of effect of substrate and of apolipoprotein CII on the binding of lipoprotein lipase to heparin–Sepharose

Conditions	Lipoprotein lipase activity in the supernatant (% of control)		
	Time (min)		After addition of heparin
	1	20	
Without substrate	<5	<5	95
With substrate	<5	<5	88
With substrate + apolipoprotein CII	<5	<5	90

The gels were incubated as in fig.1 and 2 but with an unlabeled monooleoylglycerol–Triton dispersion. Aliquots (10 μl) of the supernatants were taken for assay of lipoprotein lipase activity after 1 min and 20 min, respectively. Then 1 mg heparin/ml was added to release the enzyme from the gels and a third 10 μl aliquot of the supernatant was taken 10 min thereafter (at 33 min). These aliquots were incubated in an assay medium with [^{3}H]trioleoylglycerol labeled Intralipid as substrate (a kind gift from A. B. Vitrum, Stockholm) and human serum as source of activator protein [14]. The activity of lipoprotein lipase in the supernatants are expressed as % of the activity in corresponding controls without heparin–Sepharose. There was no significant loss of activity in these controls during the course of the experiment

ysis in the complete system was by the heparin-bound enzyme. Apolipoprotein CII stimulated the heparin-bound enzyme at least to the same degree as to which it stimulated the free enzyme (fig.2).

To study whether substrate might dissociate the enzyme from heparin, the degree of binding of lipoprotein lipase to the heparin–Sepharose was measured during the course of the reactions (table 1). In several such experiments the amount of enzyme in the supernatant varied from close to zero up to ~15%, but there was no systematic effect of substrate. In particular, no substantial release of enzyme occurred during hydrolysis. In contrast, addition of 1 mg heparin/ml at the end of the incubation released ~90% of the enzyme (table 1) demonstrating that the binding was readily reversible. Though apolipoprotein CII caused a 10-fold stimulation of the enzyme's catalytic activity (fig.2) it did not cause any significant dissociation of its binding to heparin (table 1).

To further explore the possible relation between binding to heparin and activation by apolipoprotein CII the effect of CII on hydrolysis by soluble enzyme in the presence of a large amount of heparin was compared to its effect in the absence of heparin (fig.3). No difference was found.

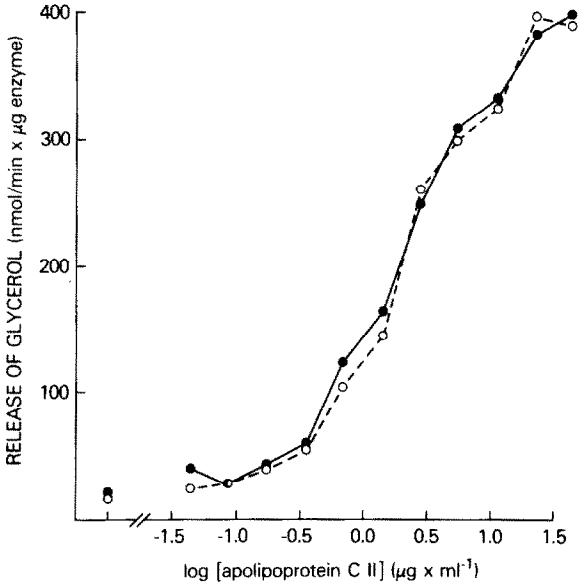

Fig.3. Effect of heparin on the stimulation of lipoprotein lipase by apolipoprotein CII. Conditions: 0.18 M Tris–HCl, 0.05 M NaCl, 30 mg bovine serum albumin/ml and 5.9 μmol monoolein/ml; (pH 8.5); total volume for each incubation was 200 μl; 2 μl of a serial dilution of apolipoprotein CII in 3 M guanidinium–hydrochloride was added to each incubation. A corresponding amount of guanidinium–hydrochloride was added to the incubation without CII (data point in lower left part of the figure). The incubations were started by addition of 0.25 μg lipoprotein lipase and were carried out for 5 min at 25°C: (○) with 1 mg heparin/ml; (●) without heparin.

4. Discussion

In addition to its active site, lipoprotein lipase has ⩾3 other functional regions [14]: a lipid binding region (interface recognition site); a region for interaction with apolipoprotein CII; and a region for interaction with heparin and other polyanions. The topography and the communications between these regions are presently not known. We show here that binding of heparin to the enzyme does not affect the function of the other 3 regions:

(i) There was no evidence for a direct effect of heparin on the catalytic efficiency of the enzyme, i.e., on its active site. The effects of heparin sometimes seen in cruder systems can probably be

ascribed to effects on the stability and/or the solubility of the enzyme as concluded in [6].

(ii) Adsorption of the enzyme to the lipid–water interface is presumably a prerequisite for its action on lipoproteins and on emulsion droplets [16]. Thus, the lack of effect of heparin on the enzyme's activity implies that it did not markedly affect the function of the 'interface recognition site'. In fact, lipoprotein lipase readily adsorbs to lipid emulsions both in the presence and absence of heparin [14,17].

(iii) The enzyme bound to heparin–Sepharose was stimulated by apoliprotein CII, and the stimulation of soluble enzyme by a wide range of CII concentrations was the same whether heparin was present or not. Thus, despite a report to the contrary [10], there does not seem to be any close relation between the interactions of lipoprotein lipase with heparin and with apolipoprotein CII.

It is not known what conformational changes, if any, binding of heparin causes in the lipoprotein lipase molecule. The implication of this work is that no major conformational changes are propagated to the 3 other functional regions, as if binding of heparin is to a conformationally rigid part of the molecule, or to a separately folded domain. This is in accord with the hypothesis that the heparin-binding site is involved in the attachment of the enzyme to the capillary endothelium [2], rather than in the actual catalytic function of the enzyme.

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