Activation of lipoprotein lipase by apolipoprotein CII

Demonstration of an effect of the activator on the binding of the enzyme to milk-fat globules

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Received 3 September 1982

Lipoprotein lipase *Apolipoprotein CII* *Milk fat globule* *Lipolysis* *Binding effect* *Activation*

1. INTRODUCTION

Lipoprotein lipase catalyses the hydrolysis of triacylglycerols and phospholipids in chylomicrons and very low density lipoproteins. This enables transport of fatty acids and of monoacylglycerols from the lipoproteins into the tissue cells [1]. From in vitro experiments it is known that apolipoprotein CII, which is a constituent of these lipoproteins, is an activator for lipoprotein lipase [2,3]. That this activator has a function also in vivo is demonstrated by the fact that patients deficient in apolipoprotein CII accumulate large amounts of chylomicron-like lipoproteins in their blood [4].

The development of methods to purify both the lipase and the activator protein has made it possible to study the mechanism of activation in defined model systems. In these systems lipoprotein lipase usually has significant activity even in the absence of its activator, and the activator causes a < 10-fold stimulation of the reaction rate [3,5–12]. Studies using Intralipid [6], monolayers of diacylglycerols [11], triacylglycerol-coated glass beads [13], apolipoprotein CII-deficient very-low-density lipoproteins [14] and liposomes of dipalmitoyl phosphatidyl choline [15] have demonstrated that lipoprotein lipase binds to lipid–water interfaces. In these systems, the activator protein is not needed for the binding, and it has therefore been concluded that the activator acts after the enzyme has bound to the interface [6,11,14]. This view is supported by experiments with non-lipid binding fragments of apolipoprotein CII which under certain circumstances activate the enzyme [9,10,14,16]. There are, however, some studies which indicate that the activator can also affect the binding of the lipase. Fielding [17], as well as Posner and Morrison [18] have reported that a mixture of apolipoproteins, including the activator, lowers the apparent K_m of lipoprotein lipase for the lipid substrate. Schrecker and Greten found a lower apparent K_m for triacylglycerol droplets stabilized by apolipoprotein CII than for droplets covered by CI or CIII, and concluded that this was because CII enhanced the binding of the lipase to the lipid droplets [19]. Matsuoka et al. [20], Fitzharris et al. [21] and Catapano et al. [14] found in studies with CII-deficient very-low-density lipoproteins, that the main effect of CII is to lower the apparent K_m of lipoprotein lipase for its lipoprotein substrate.

Since both lipoprotein lipase and apolipoprotein CII are lipid binding proteins and since a direct protein–protein interaction between lipoprotein lipase and its activator has been implicated by several studies [22–24], it would be expected that one protein could affect the binding of the other under certain circumstances. These considerations suggest that the full effect of CII is not seen in those model systems where the enzyme itself binds well to the lipid droplets. It was known that milk-fat globules are almost completely resistent to lipolysis by lipoprotein lipase as long as their structure is intact; large amounts of lipoprotein lipase are present in bovine milk but little or no lipolysis takes place [25]. Only small amounts of this lipase

Published by Elsevier Biomedical Press
00145793/82/0000–0000/$2.75

are associated with the lipid droplets [26]. It has been demonstrated that addition of high-density lipoproteins induces rapid lipolysis in milk [27]. Here, we demonstrate that apolipoprotein CII causes an immediate activation of lipoprotein lipase in milk and that an important factor in this activation is to increase the binding of the enzyme to the milk fat globules.

2. MATERIALS AND METHODS

Milk was obtained from a dairy research farm in Umeå. The cows were gently milked by hand. Unless otherwise stated the milk was immediately cooled to 4°C. The milk was used for experiments within 6 h. Milk from several individual cows was tried without qualitative differences in the results. Lipoprotein lipase was purified from bovine milk as described [28], and was iodinated by the lactoperoxidase method as in [6]. Apolipoproteins CII, CI and CIII-2 were prepared from delipidated human very-low-density lipoproteins by gel filtration in 6 M guanidinium chloride followed by ion-exchange chromatography on diethylaminoethyl cellulose in 5 M urea [29]. Apolipoprotein A1 was prepared from delipidated human high-density lipoproteins [30]. The preparations were desalted by gel filtration in 2 M acetic acid and were then lyophilized. For the experiments the apolipoproteins were dissolved in 3 M guanidinium chloride, 20 mM Tris (pH 8.5). The protein concentration in these solutions was determined by quantitative amino acid analysis. In experiments without CII the same amount of guanidinium chloride buffer was added. T1 and T2 proteins from hen's egg yolk were prepared as in [31]. Triacylglycerol-rich particles from Intralipid and from Intralipid-containing [^{3}H]oleic acid labeled trioleoylglycerol (AB Vitrum, Stockholm) were isolated as in [6]. Bovine serum albumin (a fraction V preparation) and melittin were obtained from Sigma (St Louis MO). Conditions for the incubations are detailed in the legends.

3. RESULTS

In accord with [25] there was no measurable lipolysis in milk which had been collected under gentle conditions, but rapid lipolysis was induced by sonication of the milk (fig.1). Several other manipulations/additions were tried but did not induce lipolysis. These included cooling the milk to 4°C for 2 h and rewarming it to 37°C, addition of 60 mg albumin/ml, addition of 0.1 mg heparin/ml, addition of 1 μg purified lipoprotein lipase/ml or adjustment of the pH of the milk to 8.5. Addition of apolipoprotein CII caused rapid lipolysis even in milk which had not been sonicated (fig.1). The rates obtained by sonication only or by CII only were similar, but combination of the two resulted in a higher activity (fig.1). The dose–response relation for activation by CII is shown in fig.2. Similar amounts were needed with sonicated as with non-treated milk. In terms of fold stimulation, however, the results were more dramatic with the

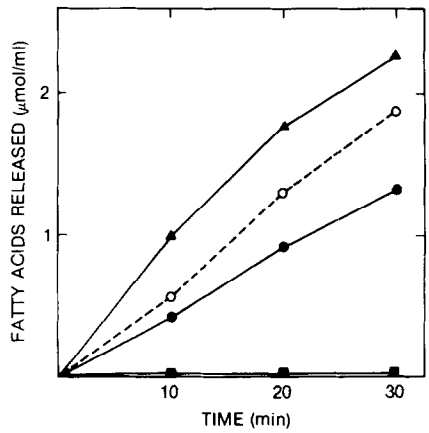

Fig.1. Induction of lipolysis in fresh bovine milk by addition of apolipoprotein CII and/or by mechanical treatment. Tubes containing 0.7 ml milk and 0.3 ml 0.15 M NaCl were incubated at 37°C. After the times indicated the reaction was stopped by addition of organic solvents and the released fatty acids were determined by titration [31]. The amounts of fatty acids in corresponding blank samples (extracted at 0 min) were subtracted. Untreated milk (▪). Milk sonicated for 2 min with a Branson sonifer immediately before the incubation (•). Milk to which 17.6 μg apolipoprotein CII/ml was added immediately before the incubation (○). Milk which was sonicated for 2 min and to which 17.6 μg apolipoprotein CII/ml was then added (▲). For this experiment the milk was collected from the cow into a thermos flask and was then kept at ~30°C until the incubations were started, ~1 h. Similar results were obtained if the milk had been cooled to 4°C before the experiment or if heat-inactivated milk was used to which purified lipoprotein lipase was added.

untreated milk because of the low activity without CII.

When incubated with a synthetic lipid emulsion such as Intralipid, lipoprotein lipase binds to the lipid droplets and the complex can be flotated by centrifugation ([6] and table 1). In contrast, no lipase bound to the milk-fat globules (table 1). This was not because some other component in the milk prevented the binding; when Intralipid was added to the milk, 70% of the ^{125}I-labeled lipoprotein lipase and 65% of the enzyme activity floated on centrifugation. Thus, the lack of binding to the milk fat globules must relate to their structure. Addition of apolipoprotein CII to the milk caused the enzyme to bind to the milk-fat globules. This is illustrated by the experiment in table 1, where 16% of the radioactive enzyme and 12% of the enzyme activity was recovered with the lipid droplets when the system contained CII compared to < 1% in the absence of CII. Centrifugation of sonicated milk demonstrated lipase radioactivity and enzyme activity in the fat layer, but quantification of the binding was not possible since some of the lipid did not flotate under the conditions used. Similar experiments were carried out with human apolipoproteins AI, CI and CIII-2, with porcine colipase

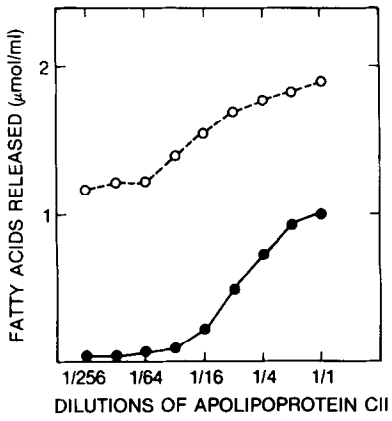

Fig.2. Dose–response curves for the stimulation by apolipoprotein CII of lipolysis in untreated milk and in sonicated milk, respectively. For this experiment the lipoprotein lipase in the milk was first inactivated by heating the milk for 15 min at 60°C. Then one part of this milk was chilled in ice-water and sonicated for 4 min with a Branson sonifer. For the incubations 5 μl apolipoprotein CII (4.4 mg/ml) or logarithmic dilutions of CII in 3 M guanidinium chloride was added to 1 ml of milk. After 5 min at 25°C, the reactions were started by addition of 2.5 μg purified lipoprotein lipase. Then the reactions were carried out for 10 min at 25°C; (•) not sonicated milk, the activity without apolipoprotein CII was 0.03 μmol fatty acid/ml; (○) sonicated milk, the activity without CII was 1.23 μmol fatty acid/ml.

Table 1

Effect of apolipoprotein CII on the binding of lipoprotein lipase to milk-fat globules

Lipid source	Apolipoprotein CII	Radioactivity (%) in top phase	Enzyme activity (%) in top phase
Milk	–	< 1	< 1
	+	16	12
Intralipid	–	92	70
	+	85	72
+ Milk	–	70	65
	+	72	61

1 ml heat-inactivated milk (see fig. 2 legend) or 1 ml 0.15 M NaCl was mixed with 0.45 ml bovine serum albumin (100 mg/ml) in 0.5 M Tris–HCl buffer (pH 8.0) in a total volume of 1.5 ml. As indicated some samples contained 8 μg apolipoprotein CII and/or centrifuged Intralipid corresponding to 6 mg triacylglycerol. Sucrose (150 mg) was dissolved in each sample. A trace amount of ^{125}I-labeled lipoprotein lipase (15 000 cpm, 30 ng) and 1.5 μg unlabeled lipoprotein lipase was added. Each sample (1 ml) was layered under 4 ml 0.15 M NaCl, 10 mM Tris–HCl (pH 8.0) containing 1 mg bovine serum albumin/ml in tubes for ultracentrifugation. The tubes were centrifuged 25 000 rev./min, 20 min at 15°C in a Beckman SW50.1 rotor. The top 1 ml layer containing a thin lipid cake was recovered after slicing the tubes. The lipoprotein lipase activity in the top phases was determined by incubation of 10 μl samples in an assay mixture containing ^{3}H-labeled Intralipid with human serum as source of activator protein [6]. The enzyme activities are expressed as % of the activity in the non-centrifuged starting mixture. The radioactivity in the top phases is expressed as % of the total radioactivity in the tube. The total recovery of radioactivity after centrifugation and tube-slicing was >85%. Blank samples were run without lipid substrates. They gave typically ~3% of the ^{125}I-radioactivity and no measurable lipase activity in the top phase. These blank values were subtracted

and with melittin. None of these proteins caused any lipolysis. However, both T1 and T2 proteins from egg-yolk lipoproteins caused lipolysis. Thus, the effect seems to be specific for proteins known to activate lipoprotein lipase in model systems.

4. DISCUSSION

We conclude that with milk-fat globules as the substrate, the main factor determining the activity of lipoprotein lipase is the extent to which the enzyme can bind to the lipid droplets, and that apolipoprotein CII facilitates the binding. These conclusions are in apparent contrast to several recent studies which have emphasized that lipoprotein lipase binds almost completely to lipid–water interfaces even in the absence of activator protein [6,11,15]. These studies have led to the view that the effect of the activator protein is to enhance the catalytic efficiency of enzyme already bound to the lipid–water interface [6,9,11,16]. These data do not invalidate this conclusion but demonstrate another aspect of the CII–lipoprotein lipase interaction. The binding effect and the effect on the catalytical efficiency are not mutually exclusive. Which aspect dominates depends on the nature of the substrate being used. With intact milk-fat globules there was virtually no binding and no activity of lipoprotein lipase in the absence of apolipoprotein CII. The dramatic stimulation was probably due to a dual effect of the activator protein, enhancing both binding and catalytic efficiency. When the native structure of the milk-fat globules was disrupted by sonication, rapid lipolysis ensued and the activator caused only 2-fold further increase in the rate. This modest effect of the activator was probably due to stimulation of the catalytic efficiency only, as has been demonstrated in the model systems [6,11].

It is of interest to consider the parallel to pancreatic lipase. In this system, research has concentrated on the effect of the activator protein, colipase, on the binding of the lipase to the substrate droplets [33,34]. This has led to the view that colipase serves as an anchor for binding of the lipase to the lipid droplets [33,34]. However, there are indications that colipase can also enhance the catalytic efficiency of pancreatic lipase at the interface [35]. Thus, although research has this far led to rather different views on the mechanism by which the activator proteins enhance the activity of pancreatic lipase and of lipoprotein lipase, respectively, both mechanisms may operate in both systems and the analogies may be much closer than realized.

We cannot yet define what is the property that prevents binding of lipoprotein lipase to the milk-fat globules. However, it is apparent that the resistance of the milk-fat globules to lipolysis is biologically advantageous since it prevents breakdown of the droplets within the mammary gland. It may well be generally true that the need for the activator apolipoprotein restricts the activity of lipoprotein lipase to those plasma lipoproteins which carry the activator and prevents the enzyme from acting on other tissue lipids such as intracellular triglyceride droplets.

ACKNOWLEDGEMENTS

We thank Miss Ulla Nordmark for her skillful technical assistance. The work was supported by a grant from the Swedish Medical Research Council, project 13x-727.

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