APOLIPOPROTEIN CII ENHANCES HYDROLYSIS OF MONOGLYCERIDES BY LIPOPROTEIN LIPASE, BUT THE EFFECT IS ABOLISHED BY FATTY ACIDS

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Received 21 August 1979

1. Introduction

Lipoprotein lipase is an enzyme that hydrolyzes triglycerides and phospholipids in lipoproteins. Several aspects of its mode of action are not well understood. One is why fatty acids strongly inhibit the activity [1]. Another is why monoglycerides do not accumulate during hydrolysis of triglycerides, although the rate of monoglyceride hydrolysis has been reported to be substantially lower than the rate of triglyceride hydrolysis [2–4]. We report here that under appropriate experimental conditions monoglyceride hydrolysis is enhanced several-fold by apolipoprotein CII and reaches rates exceeding those for triglyceride hydrolysis. We also demonstrate that fatty acids interfere with and at higher concentrations completely abolish the lipolysis-promoting effects of the cofactor protein. This previously unrecognized effect of fatty acids must be a major factor in their inhibition of acylglycerol hydrolysis.

2. Materials and methods

Lipoprotein lipase was purified from bovine milk as in [5]. Apolipoprotein CII was purified from human very low density lipoproteins by gel filtration and ion-exchange chromatography [6]. It was dissolved in 3 M guanidinium hydrochloride, the protein concentration was determined by quantitative amino acid analysis and was then adjusted to 8.8 mg/ml (1 μmol/ml). Tl protein was purified from egg yolk lipoproteins [7]. Bovine serum albumin (fraction V) and oleic acid were from Sigma, St Louis, MO. The albumin was dissolved in 0.5 M Tris–HCl at 200 mg/ml and was adjusted to pH 8.5. Intralipid® and heparin were from AB Vitrum, Stockholm.

Monoolein and [^{3}H]glycerol labeled monoolein were prepared by Lennart Krabisch, University of Lund. About 90% was the 1(3)-isomer. Sodium deoxycholate was from Merck, Darmstadt, and Triton X-100 was from Packard Instruments, Downers Grove, IL. To prepare the monoglyceride dispersion, 15 mg monoolein, containing 7.2×10^6 cpm in 4 ml 0.2 M Tris–HCl, 0.8% Triton X-100 (pH 8.5) were sonicated chilled in ice water. In some experiments the monoolein was dispersed in 6 mM deoxycholate instead of in Triton. The final concentrations in the assay medium were 5 μmol/ml monoolein, 0.18 M Tris–HCl, 0.05 M NaCl, 1 mg/ml heparin. In some incubations albumin was also present (30 mg/ml). All incubations were at 25°C. The reaction was terminated, glycerol extracted and its radioactivity determined, as in [8].

3. Results

Lipoprotein lipase readily hydrolyzed the monoolein–Triton dispersion. In some experiments there was a short lag phase during which the rate of hydrolysis increased but then a steady rate was reached. This lag phase was abolished by addition of oleate to the system, and was thus presumably due to some change in the organization of the lipid micells. The rate was linear with the amount of enzyme and was ~60 nmol/min and μg enzyme. It could not be substantially increased by changes in the concentra-

tions of monoolein and/or Triton X-100, and similar rates were obtained with monoolein dispersed in deoxycholate or in oleate. For comparison, the triglyceride emulsion Intralipid, which is often used for studies of lipoprotein lipases, was hydrolyzed at a rate of ~400 nmol fatty acid/min and μg enzyme by this preparation (at the same temperature and pH and with cofactor present).

Apolipoprotein CII increased the initial rate of hydrolysis in the monoolein–Triton system, but the rate decreased as hydrolysis progressed and when ~1 μmol oleate/ml had been released the rate with cofactor was no longer significantly higher than without (fig.1a). This suggested that the fatty acids interfered with the cofactor effect. To test this hypothesis, oleate was added to the medium before the enzyme. Whereas 0.2 nmol/ml CII enhanced the rate of hydrolysis 4-fold in the absence of oleate, it enhanced the rate only 2.4-fold when 0.5 μmol/ml oleate had been added and it had no effect when 2 μmol/ml oleate had been added (fig.2). Large amounts of oleate caused some inhibition also in the absence of cofactor; 5 μmol/ml decreased the rate by ~60% (fig.2). This is probably due to substrate dilution; at this point the oleate:monooleate molar ratio was 1:1. CII did not enhance hydrolysis of the

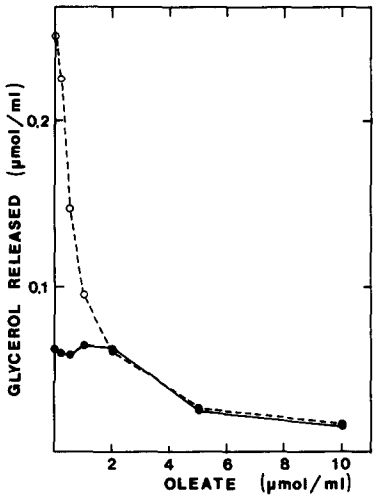

Fig.2. Effect of fatty acids on the stimulation of monoolein hydrolysis by apolipoprotein CII. CII (17.6 μg/ml) and the indicated amounts of oleate were added to the incubations 10 min before the enzyme (2 μg/ml). For each concentration of oleate time curves were obtained. In the presence of cofactor, the rate decreased as hydrolysis progressed, the values plotted are the amounts of glycerol released within the first 30 s. In the absence of cofactor there was a short lag phase in the incubations without oleate and with 0.2 μmol/ml oleate, the values plotted represent the steady rate then attained. (○) with CII; (●) without CII.

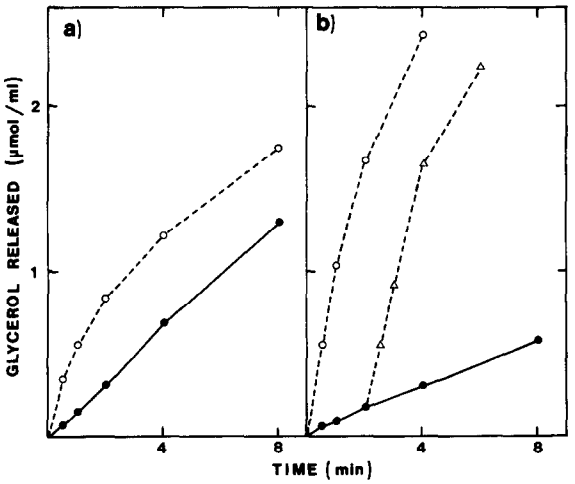

Fig.1. Effect of apolipoprotein CII on hydrolysis of monoolein by lipoprotein lipase. (●) No CII; (○) 17.6 μg CII/ml, added 10 min before the enzyme; (△) 17.6 μg CII/ml, added 2 min after the enzyme. 3 μg/ml lipoprotein lipase. In (b) the system also contained 30 mg/ml albumin.

monooleate–deoxycholate dispersion. Thus, deoxycholate had the same effect as oleate. Tl protein from egg yolk lipoproteins enhanced hydrolysis with the monoolein–Triton dispersion, but not with the monoolein–deoxycholate dispersion.

In the absence of cofactor, albumin decreased the rate at which the monooleate–Triton dispersion was hydrolyzed (compare fig.1a and 1b). In the presence of cofactor, however, the rates were at least as high with as without albumin. Furthermore, hydrolysis continued at the rapid rate, presumably because albumin bound the fatty acids so that they did not interfere with the cofactor effect. Addition of CII 2 min after the enzyme caused an immediate increase in the rate of hydrolysis (fig.1b). Thus, the interactions needed for its effect were rapidly established.

Figure 3 shows dose–response curves for stimulation of hydrolysis with the monoolein–Triton dispersion and with a phosphatidylcholine-stabilized triglyceride emulsion. Although the general shape of

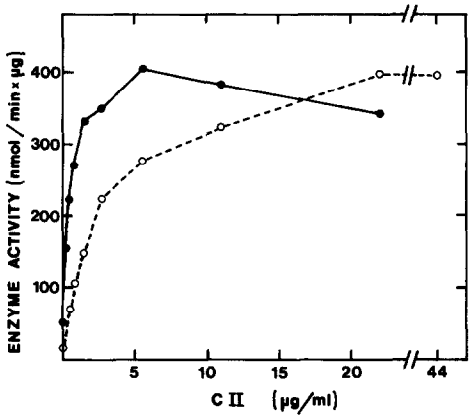

Fig.3. Dose–response curves for stimulation of monoglyceride and of triglyceride hydrolysis by apolipoprotein CII. Monoglyceride hydrolysis (○) was studied with the monoolein–Triton X-100 dispersion as in section 2, with 30 mg/ml albumin added immediately before the enzyme (0.3 µg/ml). The total volume was 200 µl and incubation was for 5 min at 25°C. To study triglyceride hydrolysis Intralipid was used (●). It is an emulsion of soy bean triglycerides and egg yolk phosphatidylcholine, manufactured for parental nutrition. It was first separated by centrifugation to remove excess phospholipid. For this, 10% (w/v) sucrose was added and the emulsion layered under 0.15 M NaCl in 5 mM veronal buffer (pH 7.4) and centrifuged 30 min at 25 000 rev./min in a SW 50:1 rotor in a Beckman L2-50 centrifuge. The tubes were sliced 1 cm from the top and the triglyceride-rich particles resuspended in 0.15 M NaCl, 5 mM veronal (pH 7.4). Final concentrations in the assay medium were: 4 mg/ml triglyceride; 60 mg/ml albumin; 1 mg/ml heparin; 0.1 M NaCl; 0.2 M Tris–HCl (pH 8.5). The albumin was added immediately before the enzyme (0.3 µg/ml). The total volume was 1 ml. Incubation was for 10 min at 25°C. The reaction was stopped, fatty acids extracted and titrated as in [9]. In both systems 10 µl/ml 3 M guanidinium hydrochloride containing the indicated amount of CII was added to the assay medium 10 min before the enzyme. This amount of guanidinium hydrochloride caused <10% inhibition in the absence of CII.

the curves were similar, two differences were noted. The ratio between the activity without cofactor to the activity with large amounts of cofactor was ~0.15 with the triglyceride emulsion but only ~0.05 with the monoglyceride dispersion. In other words cofactor enhanced hydrolysis ~7-fold with the emulsion but almost 20-fold with the monoglyceride dispersion. Half-maximal stimulation required 0.038 ± 0.004 nmol/ml with the emulsion (5 expt) but almost 10-times more with the monoglyceride dispersion (0.34 ± 0.04 nmol/ml, 6 expt).

4. Discussion

A major conclusion of this work is that proteins with cofactor activity for lipoprotein lipase, such as human apolipoprotein CII and Tl protein from egg yolk lipoproteins, enhance the action of this enzyme on monoglycerides. Thus, the effect of the cofactor proteins is not restricted to triglyceride emulsions. Under our conditions the monoglyceride concentration was not rate-limiting and it was not possible to substantially increase the rate by any other change in the composition of the system than addition of cofactor. This suggests that its effect was exerted directly on the enzyme. The apparent dissociation constant for enzyme–CII interaction from the amount needed for half-maximal stimulation was 0.34×10^{-6} M. However, experiments with a phosphatidylcholine–triglyceride emulsion yielded a value almost an order of magnitude stronger. One possible explanation is that CII adsorbs more completely to the emulsion droplets, giving a higher local concentration. A further possibility which may explain the apparent difference in affinity is suggested by the general theory of lipase action discussed [10]. According to their model lipases can exist in more or less active forms, designated E^x and E. One possible role for a cofactor protein would be to bind preferentially to E^x, thus changing the equilibrium in its favour. If so, the amount of cofactor needed would depend on the equilibrium between E^x and E in the particular system studied. In the present case, the emulsion was apparently more favourable for E^x than the dispersion, since the relative activity of the enzyme in the absence of cofactor was higher with the emulsion.

Another major conclusion is that fatty acids interfere with the cofactor effect. It was previously known that lipoprotein lipase is strongly inhibited by fatty acids, but this inhibition had not been adequately explained. In systems where cofactor enhances hydrolysis many-fold, loss of its effect must be a major factor in the product inhibition. There is indirect evidence that the enzyme binds fatty acids [11,12]. Direct evidence that it binds deoxycholate has been obtained in our laboratory by equilibrium dialysis and by charge-shift electrophoresis (G. B., T. O., unpublished observations). However, the relationship between the site(s) on the enzyme which bind fatty

acids and deoxycholate and the site which binds cofactor is not known.

Our observations also explain why previous investigators have not found any effects of cofactor on monoglyceride hydrolysis. In their systems the monoglycerides were dispersed with deoxycholate [2–4] or fatty acids [3], agents which now turn out to abolish the cofactor effect. The optimal rates for monoglyceride hydrolysis had been reported to be less than for triglyceride hydrolysis, ~20%. Yet monoglycerides have not been found to accumulate to any large extent during triglyceride hydrolysis unless excess albumin [1,13] or higher density lipoproteins [14] are present to sequester them from the action of the enzyme. This discrepancy is underlined by a kinetic analysis of the data in [1], which assigns a higher rate constant to monoglyceride than to triglyceride hydrolysis (D. M. Forster and M. Berman, personal communication). This apparent discrepancy is resolved by the present demonstration that with cofactor present monoglyceride hydrolysis reaches rates at least as high as those for triglyceride hydrolysis.

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