HUMAN C-APOLIPOPROTEINS PROMOTE HYDROLYSIS OF DIMYRISTOYL PHOSPHATIDYLCHOLINE BY SNAKE VENOM PHOSPHOLIPASE A_2

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1. Introduction

Lipoprotein lipase, the enzyme which metabolizes triglyceride-rich plasma lipoproteins, is activated by apolipoprotein CII [1]. That this activation is important for the physiological action of the enzyme is demonstrated by the massive hypertriglyceridemia in patients deficient in this apolipoprotein [2]. Whereas the activation of lipoprotein lipase by CII appears to be a specific effect and probably involves direct protein–protein interaction, there are also reports that other apolipoproteins activate or inhibit lipoprotein lipase [3–11] and/or the related heparin heparin-releasable lipase [12–14]. These effects are not well understood. The possibility that effects of the apolipoproteins on the organization of the lipid substrate are involved has not received much attention. Physical studies have demonstrated that these proteins have flexible conformations which allow them to bind rapidly and reversibly to lipid–water interfaces, and that this binding is associated with changes in the conformation of the proteins and in the organization of the lipid [15]. We demonstrate here that phospholipase A_2 from *Crotalus adamanteus* venom is stimulated several fold by human C-apolipoproteins.

2. Materials and methods

Phospholipase A_2 from *Crotalus adamanteus* venom (760 units/mg) was from Sigma (St Louis MO). Apolipoproteins CI, CII and CIII-2 were purified from human very low density lipoproteins. For this, the lipoproteins were isolated from normal plasma by ultracentrifugation, and were then delipidated in cold ethanol:ether (2:1). The proteins were separated by gel filtration on Sephacryl S-300 (Pharmacia, Uppsala) in 6 M guanidinium chloride followed by ion-exchange chromatography on diethylaminoethyl cellulose (Whatman, DE 52) in 5 M urea at 10°C as in [16]. Apolipoprotein CIII-2 was rechromatographed once on the diethylaminoethyl cellulose to remove any remaining traces of apolipoprotein CII. The protein fractions were desalted by gel filtration on Sephadex G-25 in 2 M acetic acid and were then lyophilized. The purity was checked by polyacrylamide gel electrophoresis in urea [17] and in sodium dodecylsulphate [18]. The apolipoproteins were dissolved (10 mg/ml) in 3 M guanidinium chloride, 20 mM Tris–HCl (pH 8.5). Protein concentrations in these solutions were determined by quantitative amino acid analysis.

The dimyristoyl phosphatidylcholine was from Sigma. A handshaken dispersion of the phospholipid was obtained by mixing 60 mg in 12 ml 0.1 M NaCl, 20 mM Tris–HCl, pH 8.5 at 37°C for 10 min on a Vortex test tube shaker. The final incubation system contained 1 ml total vol.: 600 μl dimyristoyl phosphatidylcholine dispersion, 2 μmol $CaCl_2$, 200 μmol Tris–HCl and 100 μmol NaCl, pH 8.5 at 25°C. The mixtures were preincubated for 15 min before the enzyme was added. The reactions were stopped by addition of organic solvents and the amount of fatty acid released was determined by titration [19]. Deoxycholate was from Merck (Darmstadt) Triton X-100 was from Packard Instr. (Downers Grove IL). Ovalbumin and bovine serum albumin (fraction V) were from Sigma.

3. Results

The activity of phospholipase A_2 from *Crotalus adamanteus* against liposomes of dimyristoyl phos-

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Table 1

Addition		Activity of phospholipase A_2 (μmol free fatty acid released/ml incubation mixture)
–		0.08
Deoxycholate	0.2 μmol	0.40
	2.0 μmol	0.95
Triton X-100	0.1 mg	0.30
	1.0 mg	0.90
Apolipoprotein	CI 50 μg	0.50
	CII 20 μg	0.65
	CIII-2 50 μg	0.50

Effects of detergents and some lipid-binding proteins on the hydrolysis of liposomes of dimyristoyl phosphatidylcholine by phospholipase A_2. Conditions were as in section 2. The substrate (total vol. 1 ml) was first incubated for 10 min at 25°C with the additions specified. Then 80 ng phospholipase A_2 from *Crotalus adamanteus* was added. After another 30 min the reaction was stopped and the fatty acids released were extracted and titrated

phatidylcholine under our basal conditions was low (table 1). The activity was enhanced by the anionic detergent deoxycholate and by the non-ionic detergent Triton X-100 (table 1). The activity was also enhanced by apolipoproteins CI, CII and CIII-2 (table 1). This enhancement followed a similar dose–response relation for all 3 proteins (fig.1). Amounts below 4 μg/ml had little or no effect, whereas with 20 μg/ml the activity was 4–6-times higher than in the absence of the proteins. At this amount of protein, the phospholipid/protein molar ratio is ~2000:1. The hydrolysis progressed roughly linearly with time both in the presence and absence of a stimulating protein (fig.2).

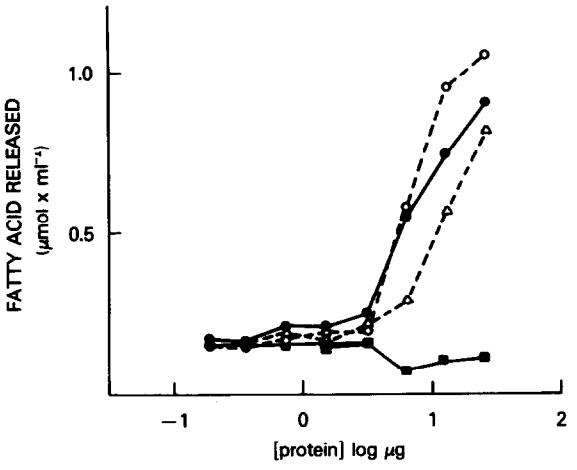

Fig.1. Dose–response curves for the stimulation of phospholipase A_2 by human C-apolipoproteins: 80 ng phospholipase A_2 from *Crotalus adamanteus* was incubated with liposomes of dimyristoyl phosphatidylcholine for 30 min. Dilutions of the respective proteins were made in 3 M guanidinium chloride in 20 mM Tris–HCl (pH 8.5) so that a total of 5 μl was added/ml incubation medium: apolipoproteins CI (△), CII (●), CIII-2 (○), ovalbumin (■).

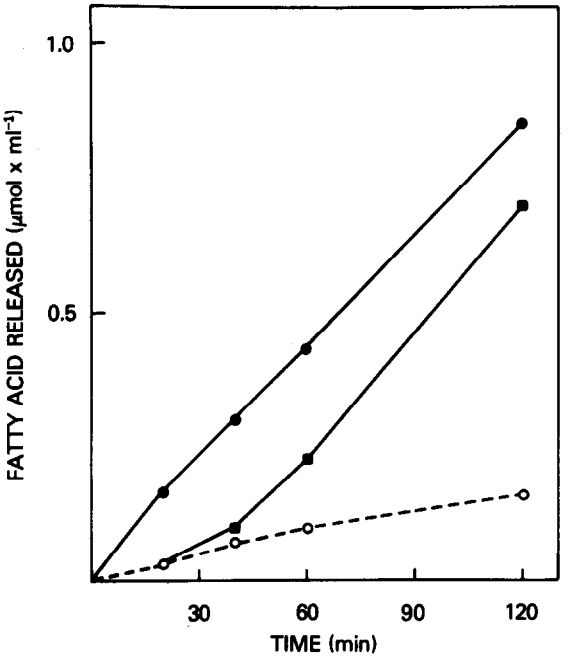

Fig.2. Time course for the hydrolysis with or without apolipoprotein CII: 40 ng phospholipase A_2 was incubated with liposomes of dimyristoyl phosphatidylcholine with (●) or without (○) apolipoprotein CII (10 μg/ml). In (■), 10 μg apolipoprotein CII was added after 10 min.

Thus, there was a true increase in the rate of hydrolysis and the effect cannot be ascribed to a shortened lag time. Similar results were obtained also when serum albumin (30 mg/ml) was included in the system as acceptor of the lipolytic products. When apolipoprotein CII was added 15 min after the enzyme, instead of before the enzyme, it caused an increase in the rate of hydrolysis to about the same level as when added before the enzyme.

4. Discussion

The major finding in this research was that low amounts of several apolipoproteins stimulated the activity of a phospholipase which has no relation to lipoprotein metabolism. It seems unlikely that the effects could be due to specific protein–protein interactions; it appears more likely that they were due to effects of the apolipoproteins on the organization of the lipid substrate. In this sense the effect would be analogous to the well-known effect of detergents on the activity of phospholipases [20], exemplified here by the stimulation by deoxycholate and by Triton X-100. The effect was seen at protein:phospholipid ratios as low as 1:5000. Others have demonstrated that at these low levels the apolipoproteins bind to the phospholipid liposomes without breaking them up into smaller lipid–protein structures as occurs with higher amounts of the apolipoproteins [21,22]. The observation that lipid-binding proteins can enhance the activity of lipid hydrolyzing enzymes is not new; this was shown for the bee venom protein mellitin with several phospholipases [23], for synthetic peptides with lecithin–cholesterol acyl transferase (LCAT) [24,25] and for the protein activators of some glycolipid hydrolyzing enzymes [26]. Among the best substrates for pancreatic phospholipase are the phospholipids of egg yolk lipoproteins [27]. These lipoproteins contain low M_r apoproteins analogous to human apolipoprotein CII [28].

With lipoprotein lipase, apolipoprotein CII enhances the activity against dimyristoyl phosphatidylcholine by as much as 50-fold ([22], unpublished), whereas with triglyceride emulsions the stimulation is usually <10-fold, often only 2–5-fold [5,6,8,29]. Detergents such as deoxycholate and Triton X-100 stimulate the activity of lipoprotein lipase against dimyristoyl phosphatidylcholine (unpublished) demonstrating that the enzyme profits from a reorganization of the liposomes. There is evidence that apolipoprotein CII forms a complex with lipoprotein lipase at lipid–water interfaces [29–31]. This would localize its effects on the lipid structure to a region close to the enzyme, adding to any direct effects the apolipoprotein may have on the enzyme.

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