Lipoprotein lipase moves rapidly between lipid droplets

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1. INTRODUCTION

The substrates of lipolytic enzymes are insoluble in water and form separate phases; lipid droplets, micelles or liposomes. The first step in the enzymatic action must be that the lipase adsorbs to the lipid–water interface [1]. This step has been studied most thoroughly with pancreatic lipase in monolayer systems. These studies have shown that the binding step is only slowly reversible, such that a lipase molecule once it has bound to the interface tends to remain there for hours [2,3]. We demonstrate here that for lipoprotein lipase in an emulsion system the situation is quite different; this lipase moves within seconds between the lipid droplets. The implications of this for how the enzyme acts on plasma lipoproteins in vivo are discussed.

2. MATERIALS AND METHODS

Lipoprotein lipase was purified from bovine milk as in [4]. Triglyceride-rich lipid droplets were isolated from Intralipid by centrifugation as in [5] but the flotation was repeated twice. The glycerol tri-[^{3}H]oleate-labeled Intralipid was a kind gift from AB Kabi-Vitrum (Stockholm). The bovine albumin was a fraction V preparation from Sigma (St Louis MO). Details of the conditions are given in the figure legend.

3. RESULTS

When lipoprotein lipase was added to the triglyceride emulsion hydrolysis commenced immediately without any discernible lag phase (fig. 1). This demonstrates that the on rate for binding of the lipase to the lipid droplets was high in relation to the time scale of the experiments (minutes). Under the conditions of these experiments virtually all of the lipase (> 80%) is associated with the lipid

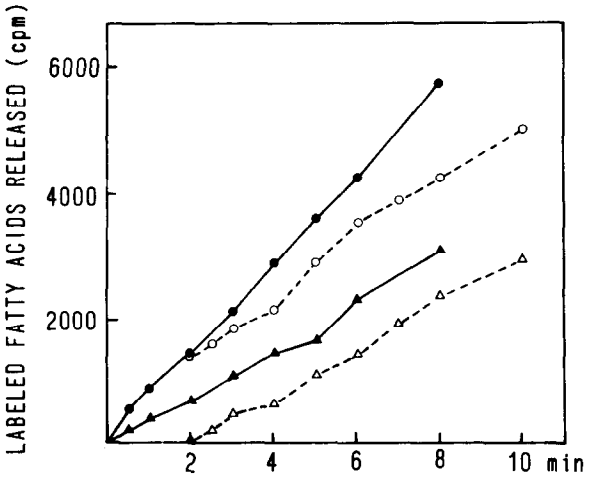

Fig. 1. Rapid movement of lipase to new substrate droplets added during incubation: The incubations were at 25°C in a medium at pH 8.5 containing in 2 ml total vol. 200 μmol NaCl, 300 μmol Tris– HCl, 120 mg bovine serum albumin and substrate as described below, 5 μg lipoprotein lipase was added at time 0. The reactions were followed by taking 200 μl aliquots at a series of times for extraction of free fatty acids [6]. In (●) the system contained 16 mg labeled triacylglycerols only. In (▲) the system contained the same amount of triacylglycerols, but it was a 1:1 mixture of labeled and unlabeled Intralipid particles. In (△) the reaction was started with 8 mg triacylglycerols from unlabeled Intralipid. At 2 min, 8 mg labeled Intralipid was added. In (○) the reaction was started with 8 mg triacylglycerols from labeled Intralipid and 8 mg unlabeled Intralipid was added at 2 min. In (●) 1 μmol free fatty acid corresponds to 1700 cpm.

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droplets as demonstrated by centrifugal separation of the medium [6]. Thus, the equilibrium favours binding. In accord with this, the rate of hydrolysis remained essentially the same when the triglyceride concentration was doubled (fig. 1). When the incubation was started with labeled triglyceride, and an equal amount of unlabeled triglyceride was added 2 min later, the rate of release of labeled fatty acids immediately decreased to half. Thus, the lipase molecules rapidly distributed equally among the old and the new substrate droplets. This demonstrates that the rate of dissociation of enzyme from the lipid droplets was high in relation to the time scale of our experiments. This is further demonstrated by the incubation which was started with unlabeled emulsion only (fig. 1). When labeled triglyceride was added at 2 min, release of labeled fatty acids at the expected rate started immediately and remained linear throughout the incubation. Similar experiments have been carried out at pH from 7.4–8.5 and at 25°C as well as 37°C with concordant results. Principally the same results were also obtained when the experiments were repeated with apolipoprotein CII present (not shown).

4. DISCUSSION

The Intralipid particles used here as substrate are large lipid aggregates which contain several million molecules of triglycerides. In our incubations the molar ratio of lipase to triglyceride was about 1:300 000; corresponding to several lipase molecules/lipid droplet. If a lipase molecule once it had absorbed to a lipid droplet remained on that droplet until all the triglycerides were degraded, we would have seen no movement of lipase molecules in our experiment when additional substrate was added during the incubation. Initially, the added triglycerides would have been hydrolyzed very slowly and the rate would then have increased gradually as the original substrate was consumed and lipase molecules were freed. In contrast, our data show that the lipase molecules became equally distributed between the original and the newly added substrate droplets in <30 s. During this time only $\sim 0.5\%$ of the ester bonds have been hydrolyzed. This leads to a picture of rapidly reversible lipase–particle binding where each interaction leads to hydrolysis of only a small proportion of the lipids, but where the equilibrium between soluble and lipid-bound lipase favours the latter.

The turnover number for a lipoprotein lipase dimer [7] with human very low density lipoproteins as the substrate is $\sim 650\ s^{-1}$ at pH 7.4, 37°C. A very low density lipoprotein contains $<15\,000$ triglyceride molecules [8]; they could be hydrolyzed to monoglycerides and free fatty acids by a single lipase molecule in <1 min. This is much less than the residence time for these lipoproteins in the circulation, which in man is several hours [8]. Their degradation may well be accomplished by many short-lived interactions with lipase molecules, analogous to how the reaction takes place in the in vitro model system studied here. A corollary is that a very low density lipoprotein particle spends only a short fraction of its life-time in contact with lipoprotein lipase at the endothelium. Chylomicron metabolism, however, can not be accounted for in this way. Chylomicrons are large particles which contain several million molecules triglyceride [9]. Their residence time in the circulation is <10 min [10]. During this time $\geqslant 90\%$ of the triglycerides are hydrolyzed. The clearing of a chylomicron containing 3×10^6 molecules triglyceride would require at least 5×10^6 hydrolytic events. To accomplish this in 10 min, 15 lipase molecules would have to act continuously on the particle. Since the particle spends much of its time in the circulating blood the number of lipase molecules which act on the particle during the time it is at the endothelium must be higher. The cooperative effect of many simultaneous interactions with lipase molecules anchored to the endothelium may hold a large chylomicron at the endothelium for a relatively long time.

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REFERENCES

[1] Verger, R. (1980) Methods Enzymol. 64, 340–392.
[2] Rietsch, J., Pattus, F., Desnuelle, P. and Verger, R. (1977) J. Biol. Chem. 252, 4313–4318.

[3] Dervichian, D.G., Préhu, C. and Barque, J.P. (1973) CR Acad. Sci. Paris 276, 839–841.
[4] Bengtsson, G. and Olivecrona, T. (1977) Biochem. J. 167, 109–119.
[5] Bengtsson, G. and Olivecrona, T. (1980) Eur. J. Biochem. 106, 549–555.
[6] Belfrage, P. and Vaughan, M. (1969) J. Lipid Res. 10, 341–344.
[7] Iverius, P.-H. and Östlund-Lindqvist, A.-M. (1976) J. Biol. Chem. 251, 7791–7795.
[8] Eisenberg, S. (1979) Progr. Biochem. Pharmacol. 15, 139–165.
[9] Smith, L.C. and Scow, R.O. (1979) Progr. Biochem. Pharmacol. 15, 109–138.
[10] Grundy, S.M. and Mota, H.Y.I. (1976) Metabolism 25, 1225–1239.