THE HEPATIC HEPARIN RELEASABLE LIPASE BINDS TO HIGH DENSITY LIPOPROTEINS

Gunilla BENGTSSON and Thomas OLIVECRONA
Department of Physiological Chemistry, University of Umeå, S-901 87 Umeå, Sweden

Received 22 August 1980

1. Introduction

Injection of heparin releases two lipases into the circulating blood. One, lipoprotein lipase, originates from extrahepatic tissues [1]. The physiological role of this lipase is to hydrolyze triacylglycerols and phospholipids in chylomicrons and very low density lipoproteins [1]. The other lipase comes from the liver [2]. It is generally assumed that also this lipase has an important role in lipoprotein metabolism, but it is now known what its physiological substrate is. We demonstrate here binding of this lipase to high density lipoproteins.

2. Materials and methods

Human postheparin plasma was obtained from male donors 10 min after intravenous injection of 100 I.U. heparin/kg body weight. The lipase of hepatic origin was partially purified by adsorption of the postheparin plasma with heparin-Sepharose [3]. It was further purified by adsorption to heparin which had been modified by partial *N*-desulfation followed by acetylation of exposed amino groups [4]. This step removed antithrombin from the lipase preparation. The specific activity of a typical preparation was 50 μmol fatty acids released/min × mg (pH 8.5, 25°C) against a gum arabic stabilized emulsion of [^{3}H]trioleoylglycerol.

Human high density lipoproteins were prepared by ultracentrifugation of normal plasma. Very low density lipoproteins and low density lipoproteins were first flotated by centrifugation at d 1.063 g/cm^3 for 24 h at 36 000 rev./min in a Beckman Ti 50 rotor, 15°C. High density lipoproteins were then prepared by centrifugation of the infranatant at d 1.21 g/cm^3 for 48 h at 36 000 rev./min. In one experiment a subfraction of high density lipoproteins isolated in the density range 1.125–1.21 g/cm^3 was used (high density lipoproteins$_3$). The lipoproteins were dialyzed against 0.1 M NaCl 10 mM Tris/Cl pH 8.5. The concentration of high density lipoproteins used in the experiments is expressed as protein determined by the Lowry method.

High density lipoprotein-Sepharose was prepared by mixing CNBr-activated Sepharose (Pharmacia, Uppsala, Sweden) with high density lipoproteins (10 mg protein/ml gel) in 0.1 M $NaHCO_3$, 0.5 M NaCl at 4°C over night. Remaining activated sites on the Sepharose were blocked by 0.1 M ethanolamine (pH 8). The gel was washed with the carbonate buffer, then with 0.1 M acetate pH 4 and stored in 10 mM phosphate, 0.1 M NaCl, pH 7.4 with 0.2% sodium azide. All experiments were carried out within two weeks after the preparation of the gel. Unsubstituted Sepharose 4B was used as a control. Human apolipoprotein $CIII_2$ was prepared from human very low density lipoproteins as described [5].

Intralipid 10% containing [^{14}C]oleic acid-labeled trioleoylglycerol was donated by AB Vitrum, Stockholm, Sweden. The triacylglycerol-rich particles were separated from the excess phospholipids by flotation through 20 mM Tris/Cl, 0.1 M NaCl, pH 8.5 by centrifugation for 20 min in a Beckman SW-50:1 rotor at 25 000 rev./min [5]. The top layer was recovered and dispersed in buffer. Albumin was a fraction V preparation from Sigma, St. Louis, MO., USA. The release of labeled fatty acids was determined as described [5]. Deoxycholate was from Merck, Darmstadt, F. R. G.

3. Results

The activity of the hepatic heparin-releasable lipase against a triacylglycerol emulsion was strongly inhib-

 Elsevier/North-Holland Biomedical Press

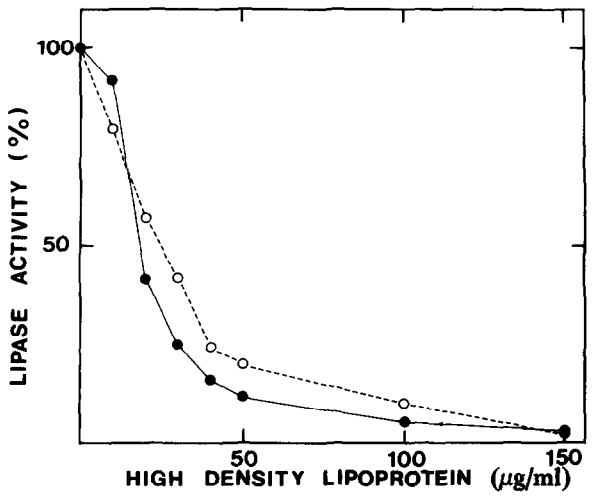

Fig.1. Inhibition of the lipase activity by high density lipoproteins. Triacylglycerol-rich particles from ^{14}C-labeled Intralipid corresponding to 4 mg triacylglycerol/ml, 0.1 M NaCl, 0.2 M Tris/Cl, 60 mg albumin/ml, pH 8.5. (•) 25°C, (○) 10°C. The indicated amounts of high density lipoproteins were added 10 min before the enzyme. Serial samples of 0.2 ml were withdrawn and the amount of free fatty acids determined. In all cases hydrolysis was linear with time.

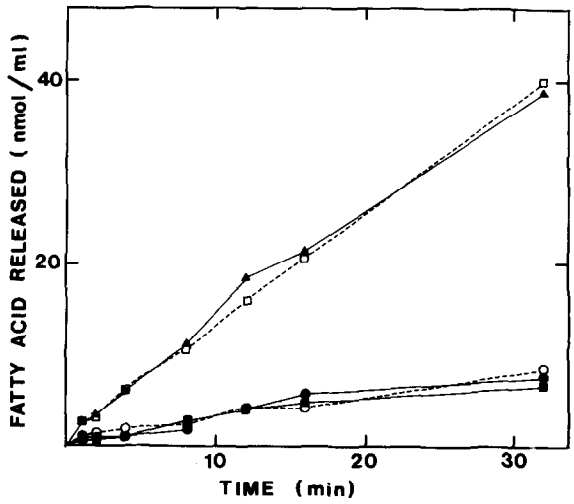

Fig.2. Relief of the inhibition by removal of the high density lipoproteins from the medium. Media of the same composition as in fig.1, but without albumin were incubated with high density lipoproteins or with apolipoprotein $CIII_2$ for 10 min at 25°C. To 2 ml of the media 100 mg sucrose/ml was added. These samples were then layered under 3 ml 0.1 M NaCl, 0.2 M Tris/Cl pH 8.5 and centrifuged in a Beckman SW 50:1 rotor at 30 000 rev./min for 20 min. The flotated lipid droplets were recovered after slicing the tubes and were suspended in the original volume of buffer. They were then used as substrate for incubations under the same conditions as in fig.1 (25°C). (○) medium preincubated with high density lipoproteins, no centrifugation. The final incubation medium contained 50 μg high density lipoproteins/ml. (□) Medium preincubated with high density lipoproteins, then separated by centrifugation. (•) Medium preincubated with apolipoprotein $CIII_2$, no centrifugation. The final incubation medium contained 50 μg $CIII_2$/ml. (■) Medium preincubated with apolipoprotein $CIII_2$, then separated by centrifugation. (▲) No addition during preincubation, then separated by centrifugation.

ited by high density lipoproteins (fig.1). Kinnunen and Ehnholm have shown that C-apolipoproteins inhibit the activity of the hepatic lipase against a gum arabic stabilized triacylglycerol emulsion [6]. Inhibition with apolipoprotein $CIII_2$ was obtained also with the present phosphatidylcholine stabilized trioleoylglycerol emulsion (fig.2). The mechanism of this inhibition is presumably that the apolipoprotein competes with the lipase for the same binding sites on the emulsion droplets. However, the inhibition by high density lipoproteins was obtained also with the subfraction high density lipoprotein$_3$ although these contain only small amounts of C-apolipoproteins (not shown). Furthermore, high density lipoproteins caused inhibition also at 10°C (fig.1) although this low temperature impedes the transfer of apolipoproteins [7]. When the emulsion droplets were preincubated with apolipoprotein $CIII_2$ and then separated from unbound apolipoprotein by centrifugation the inhibition persisted (fig.2). In contrast, the inhibition caused by high density lipoproteins was fully relieved when the droplets were separated from the lipoproteins by centrifugation (fig.2). Thus, the inhibition could not be accounted for by transfer of apolipoproteins from the high density lipoproteins to the emulsion droplets but required the presence of the high density lipoproteins themselves.

When the lipase was incubated with Sepharose-bound high density lipoproteins about two-thirds of the enzyme bound to the gel (table 1). In contrast only small amounts bound to unsubstituted Sepharose. The bound enzyme could be displaced by addition of free high density lipoproteins demonstrating that binding was indeed to the immobilized lipoproteins. The enzyme could also be eluted by deoxycholate.

4. Discussion

This study demonstrates that the hepatic heparin

Table 1
Binding of the lipase to high density lipoprotein-Sepharose

	High density lipoprotein-Sepharose		Unsubstituted Sepharose
	Exp. 1	Exp. 2	
	Hepatic lipase activity (%)		
Not bound	30	30	78
Wash 1	3	5	10
Wash 2	2	1	0
HDL 1	30		0
HDL 2	18		0
HDL 3	4		0
HDL 4	1		0
Wash 3	1		0
Wash 4	1		0
Deoxycholate 1	2	44	1
Deoxycholate 2	0	5	0

200 μl of the gels were equilibrated in test tubes with 0.1 M NaCl, 20 mM Tris/Cl pH 8.5 containing 10 mg bovine serum albumin/ml. The tubes were centrifuged for 1 min at 4°C in a Beckman Minifuge and the supernatants were then removed. 50 μg hepatic lipase in 0.8 ml of the equilibration buffer was added to the gels and the tubes were gently shaken at 4°C for 30 min. After centrifugation the supernatants were removed and the lipase activity not bound was determined using a gum arabic stabilized emulsion of labeled trioleoylglycerol [3]. The gels were washed twice with 0.8 ml of the equilibration buffer (wash 1 and 2). Then, 0.8 ml of the equilibration buffer containing 5 mg high density lipoprotein/ml was added to the gels and the tubes were shaken for 30 min. After centrifugation the supernatants were removed and their content of lipase activity was determined (HDL 1). The elution with high density lipoprotein was repeated 3 times (HDL 2-4). Then the gels were washed twice with 0.8 ml equilibration buffer (wash 3 and 4). Finally the gels were eluted with 2 successive 0.8 ml volumes of 5 mM deoxycholate in 20 mM Tris/Cl pH 8.5 (deoxycholate 1 and 2). In experiment 2, the elution with high density lipoprotein was omitted. The total lipase activities in the supernatants are expressed as per cent of the lipase activity initially added to the gels

releasable lipase binds to high density lipoproteins. The inhibition of the lipase activity against synthetic emulsions seen in this study with high density lipoproteins and in many previous studies with whole plasma is thus due to a competition for the enzyme between high density lipoproteins and the emulsion droplets. When the lipoproteins were removed from the incubation medium, hydrolysis of the emulsion droplets was again rapid demonstrating that the inhibition was dependent on the presence of the lipoprotein particles.

In vitro the hepatic lipase is able to hydrolyze acylglycerols in all major classes of lipoproteins [8–10]. However, from several lines of evidence it has been concluded that in vivo this enzyme acts mainly on high density lipoproteins [2,11]. The higher affinity of the hepatic lipase for high density lipoproteins than for triacylglycerol-rich particles demonstrated in the present study may be the mechanism by which the enzyme selects its physiological substrate from the various types of lipoproteins which circulate in plasma.

Acknowledgements

This work was supported by the Swedish Medical Research Council (13X-00727) and the Swedish Margarine Industry Fund for Research on Nutrition. We thank Miss Ulla Nordmark and Miss Solveig Nilsson for their skilful technical assistance.

References

[1] Nilsson-Ehle, P., Garfinkel, A. S. and Schotz, M. C. (1980) Annu. Rev. Biochem. 49, in press.
[2] Nikkilä, E. A., Kuusi, T., Harno, K., Tikkanen, M. and Taskinen, M. R. (1980) in: Atherosclerosis V (Gotto, A. M., Smith, L. C. and Allen, B. eds.) pp. 387–392, Springer-Verlag.
[3] Hernell, O., Egelrud, T. and Olivecrona, T. (1975) Biochim. Biophys. Acta 381, 233–241.
[4] Bengtsson, G., Olivecrona, T., Höök, M., Riesenfeld, J. and Lindahl, U. (1980) Biochem. J. 189, 625–633.
[5] Bengtsson, G. and Olivecrona, T. (1980) Eur. J. Biochem. 106, 549–555.
[6] Kinnunen, P. K. J. and Ehnholm, C. (1976) FEBS Lett. 65, 354–357.
[7] Eisenberg, S. (1978) J. Lipid Res. 19, 229–236.
[8] El-Maghrabi, R., Waite, M. and Rudel, L. L. (1978) Biochem. Biophys. Res. Commun. 81, 82–88.
[9] Musliner, T. A., Herbert, P. N. and Kingston, M. J. (1979) Biochim. Biophys. Acta 575, 277–288.
[10] Van Tol, A., van Gent, T. and Jansen, H. (1980) Biochem. Biophys. Res. Commun. 94, 101–108.
[11] Jansen, H., van Tol, A. and Hülsman, W. C. (1980) Biochem. Biophys. Res. Commun. 94, 53–59.