DIFFERENTIAL RELEASE OF HEPATIC LIPOLYTIC ACTIVITIES

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Received June 21,1977

SUMMARY. Perfusion of rat liver with a heparin-containing medium results in a release of upto 95% of the total neutral (or alkaline) triacylglycerol hydrolase activity (EC 3.1.1.3). Of the monoacylglycerol hydrolase (EC 3.1.1.23), phospholipase A_1 (EC 3.1.1.32) and palmitoyl-CoA hydrolase (EC 3.1.2.2) activities only 17 to 4% were found to be heparin-releasable. Antiserum raised against heparin-releasable liver lipase inhibits triacylglycerol hydrolase, monoacylglycerol hydrolase, palmitoyl-CoA hydrolase and phospholipase A_1 activities in perfusates of isolated livers completely. Non-heparin-releasable monoacylglycerol hydrolase, palmitoyl-CoA hydrolase and phospholipase A_1 activities are not inhibited by the antiserum and are therefore catalyzed by different enzyme(s). The difference in substrate specificities of releasable and non-releasable liver enzymes is discussed in relation to their possible function in lipoprotein metabolism.

Various lipolytic activities are released from liver by heparin[1-9], of which triacylglycerol hydrolase at least may also be measured with palmitoyl-CoA as substrate[4,10]. Both heparin-releasable triacylglycerol and palmitoyl-CoA hydrolases were purified together, both were inhibited by the same antibody[4,10], were varied to the same extent by diet[10], the hormonal state[11] or drugs[12]. Therefore both activities may be catalyzed by the same enzyme. The possible existence of other heparin-releasable lipolytic enzymes in liver, however, may not be excluded[13]. It is the purpose of the present paper to identify heparin-releasable besides non-releasable lipolytic activities in liver and to discriminate the different proteins with the antibody prepared against heparin-releasable lipase [4,10], as well as by difference in substrate specificity.

METHODS AND MATERIALS. Fed, male Wistar rats of about 250 g were used. Livers were perfused at 30°C through the portal vein with a Krebs-Ringer bicarbonate buffer, containing 5 mM glucose at a rate of 20 ml/min. Release of lipase from the liver was obtained by per-

ISSN 0006-291X

fusion with the same medium, containing 1.2% bovine serum albumin (w/v; Sigma fraction V), as well as 5 I.U. heparin/ml, for a period of 6 min (TABLE 1) or 20 min (TABLE II). In a number of experiments (not shown) it was found that perfusion beyond 6 min released less than 6% more of the total heparin-releasable lipase activity. Phospholipase A_1 activity was measured with [1-^{14}C]-L-α-dipalmitoylphosphorylcholine (Applied Science Laboratories, Inc., State College, Pennsylvania, USA) as the substrate. Each test contained 0.5 mM phospholipid (1 mCi/μmole), 0.1 M Tris-HCl pH 8.5, 5 mM $CaCl_2$, 5 to 20 μl sample and water to a final volume of 55 μl. After incubation for 10 min at 30°C the reaction was stopped with 1 ml of a mixture of heptane, isopropanol and 10 N H_2SO_4 (400:400:1 by volume). Separation of phases was obtained with 200 μl water. After vigorous stirring, the mixture was centrifuged, the upper layer isolated and treated with about 5 mg silicic acid (100 Mesh, Mallinckrodt Chem. Works), to remove phospholipids. After centrifugation radioactivity was determined by liquid scintillation counting. Monoacylglycerol hydrolase activity was estimated with 2-[^{3}H]-glycerolmonooleate. Each test contained 2.5 mM glycerolmonooleate , 5 mg bovine serum albumin, 0.1 M Tris-HCl (pH 8.5), 20 to 200 μl sample and water to a final volume of 0.5 ml. After 10 min at 37°C the reaction was stopped with 0.5 ml 10% trichloroacetic acid. After centrifugation all glycerides are found in the pellet, while glycerol is present in the supernatant[14]. 0.2 ml of the supernatant was counted in "Instagel".
Triacylglycerol hydrolase activity was estimated with tri[1-^{14}C]-oleoylglycerol, emulsified with gum acacia as the substrate, essentially as described by Ehnholm et al.[12] with the exception that 1 M NaCl was omitted from the incubation medium. The lipid substrate was purified by thin layer chromatography before use.
Palmitoyl-CoA hydrolase activity was measured as described before[15].

RESULTS AND DISCUSSION

It has been shown earlier by us[11] that post-heparin serum may be separated into two clearcut activity peaks on a Sepharose-heparin column. The first peak, eluted with 0.7 M NaCl, contained the bulk of the palmitoyl-CoA hydrolase activity and about half of the triglyceridase activity, while the second peak, eluted with 1.2 M NaCl, contained very little palmitoyl-CoA hydrolase activity as well as the other half of total triglyceridase activity. It was further observed[11] that triglyceride emulsions were hydrolyzed completely to fatty acids and glycerol (molar ratio of 3:1), while the second peak yielded relatively much partial glycerides (molar ratio of fatty acid to glycerol 8:1). This indicated to us, since a separate diglyceridase has never been found, that the first peak should contain considerable monoglyceridase activity. An antibody against the purified protein, giving one band on polyacrylamide gel electrophoresis, was raised in rabbits[4,10,11] and was used in the present study.

TABLE I

RELEASABILITY OF DIFFERENT LIPOLYTIC ACTIVITIES FROM RAT LIVER BY HEPARIN

Rat livers were perfused with a heparin-containing medium as described under METHODS. During 6 min the perfusate was collected. Then the liver was homogenized in 0.25 M sucrose. In the perfusate and in the homogenates (10% w/v) lipolytic activities were estimated as described under METHODS. Mean liver weights were 10 g. Results are expressed as the activities found in a whole liver after perfusion or in the total perfusates. The activities are given as μmoles free fatty acids produced/min ± S.D. When monooleate and palmitoyl-CoA were the substrates, glycerol and coenzyme A formed were measured respectively. Number of perfusions was 4.

Substrates	Activity in homogenates	Activity in perfusates	% of total activity released
Trioleate	0.5±0.02	2.6±0.2	85
Palmitoyl-CoA	74.2±10	3.0±0.2	4
L-α-dipalmitoyl glycero-phosphocholine	1.15±0.22	0.17±0.03	15
Monooleate	340±46	57±9	17

It can be seen from TABLE I that almost all of the neutral triglyceridase is released by perfusion of isolated livers with heparin, while the other hydrolytic activities were only partially released. This suggests heterogeneity of enzyme pattern.

Similar enzyme activity ratios were found in the homogenates after heparin perfusion, whether homogenates were tested directly (TABLE I) or after delipidation by making an acetone powder prior to extraction (not shown). TABLE II shows that 95% of the triglyceridase activity was removed by heparin and the the antiserum against releasable hepatic lipase inhibits both releasable and non-releasable triglyceridase activities completely. This suggests that the variable small amount of triglyceridase that is not released with heparin in 6 min may be the same enzyme but not (readily) accessible to the high molecular heparin. The other activities of which the bulk is not released by heparin (TABLE II) are only sensitive to the antibody insofar the activities may be released by heparin. Therefore it is most likely that heparin-releasable liver lipase(s) may act on a variety of substrates and is (are) immunologically

TABLE II

INFLUENCE OF ANTISERUM AGAINST RELEASABLE LIVER LIPASE ON THE HEPARIN-RELEASABLE AND NON-RELEASABLE ACTIVITIES

Rat livers were perfused with a heparin-containing medium as described under METHODS. During 20 min the perfusates were collected. Then the livers were homogenized in cold 0.25 M sucrose and 5 volumes of acetone (-20°C) added. After 10 min stirring at 0°C, followed by centrifugation, the pellet was reextracted with cold acetone, again centrifuged and n-butanol (-20°C) added to the pellet instead of acetone. The butanol was removed by washing twice with acetone and finally 2 washings were carried out with cold diethylether, after which the preparation was dried. Extraction of the acetone powder was carried out with 0.05 M NH_4OH/NH_4Cl buffer of pH 8.5 for 60 min. The extracts were incubated overnight with antiserum. After centrifugation during 4 min at 15,000 g the remaining activity was estimated with different substrates as described under METHODS.

Substrate	Non releasable mU/total liver		Releasable mU/total liver	
	Control	Antiserum	Control	Antiserum
Triglyceride	315	0	5710	0
Monoglyceride	89754	84390	29693	0
Palmitoyl-CoA	16725	14967	2183	0
Phospholipid	42	42	203	0

different from the non-releasable lipases. Antibody titration curves[4,10,11] are presented in Fig. 1. The lower curves represent the activities of liver perfusates. They are all readily inhibited by the antibody. The upper curves are obtained with post-heparin serum of hepatectomized rats[11]. These extrahepatic activities were not inhibited by the antibody (Fig. 1).

In TABLE III apparent K_m and V_{max} values calculated from Lineweaver Burk plots of experiments not presented, are shown. Since Sepharose-heparin chromatography purifies both triglyceridase and monoglyceridase activities 240-fold (TABLE III), it is tempting to conclude that both activities are catalyzed by one enzyme, with a higher affinity and a higher velocity with the monoglyceride substrate. Hence the enzyme could be denominated as monoglyceridase, were it not that considerable activity with tri- (and di-) glyceride is also present. Hence the name glycerolester lipase may be more

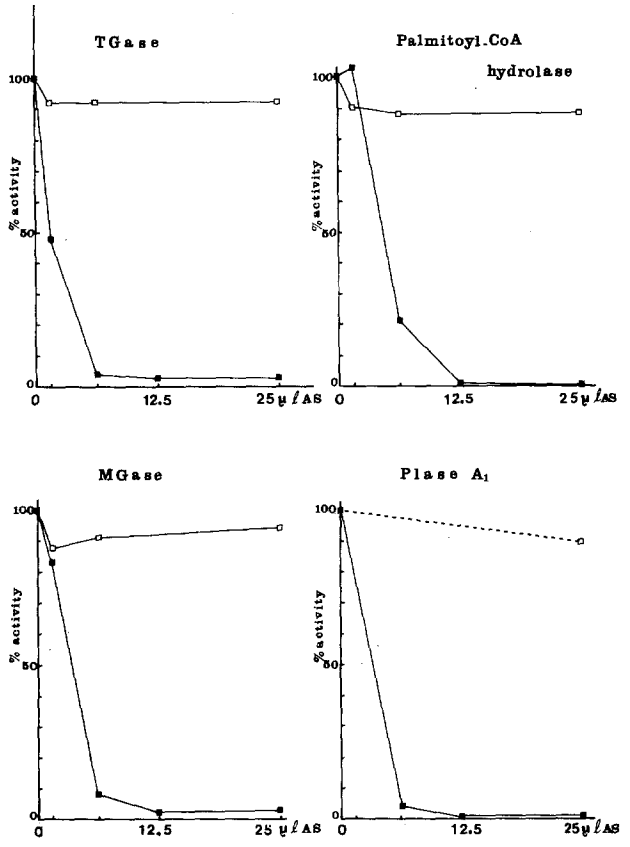

Fig. 1. Inhibition of triacylglycerol hydrolase (TGase), monoglyceridase (MGase), palmitoyl-CoA hydrolase and phospholipase A_1 (PLase A_1) activities in heparin containing perfusates of rat liver (closed symbols) or post-heparin serum of hepatectomized rats (open symbols) by antiserum (AS) raised against heparin-releasable rat liver lipase[4]. 500 µl liver perfusate or 50 µl post-heparin serum of hepatectomized rats was incubated overnight with various amounts of AS (as shown) and/or control rabbit serum to match the amount of protein used. Overnight incubation did not cause inactivation. After centrifugation, the remaining activities were determined as described under METHODS.

appropriate. Heparin-releasable phospholipase (A_1, since A_2 activity could not be detected - not shown) activity was purified almost 160-fold (TABLE III), so that the activity may partly be due to the same glycerolester lipase. A comparison of this enzyme with the heparin-releasable phospholipase A_1 activity of liver plasma membranes, described by Waite and Sisson[5], employing the antibody used in the present study, is being undertaken.

The high affinity and low velocity with phospholipid may be the reason why the heparin-releasable liver lipase activity is

TABLE III

K_m AND V_{max} VALUES OF HEPARIN-RELEASABLE LIVER LIPASE MEASURED WITH DIFFERENT SUBSTRATES

K_m and V_{max} values were calculated from linear Lineweaver Burk plots. The liver enzyme was purified on a heparin Sepharose column by elution with a NaCl gradient[11]. TG, MG and PL are the abbreviations for triglyceride, monoglyceride and phospholipid respectively.

Enzyme source	K_m (mM)			V_{max} (mU/mg protein)		
	TG	MG	PL	TG	MG	PL
Liver perfusate	6.2	0.5	0.1	10	83	0.7
Purified liver perfusate	6.2	0.6	0.2	2390	19833	116

higher when monooleate is sonicated with gum acacia (present study) than with phosphatidylcholine as emulsifyer[11].

In conclusion: The heparin-releasable neutral liver lipase activities measured (triglyceridase, monoglyceridase, palmitoyl-CoA hydrolase and phospholipase A_1) are probably catalyzed by one enzyme. It may attack both 1- and 2-monoglycerides[2] and may be involved in the breakdown of 2-monoglyceride-rich remnants of chylomicrons and very low density lipoproteins produced by (extra hepatic) lipoprotein lipase, which has exclusively α-lipolytic activity. Another activity may be triglyceride breakdown especially when blood levels are high, then having an overflow function in hypertriglyceridemia. The non-releasable enzyme(s) are at least partly located within the liver cells, beyond the plasma membrane barrier for heparin. The non-releasable neutral monoglyceridase activity has recently been purified from post mitochondrial supernatant 140-fold, using ammoniumsulphate fractionation and molecular sieving. It also has both 1- and 2-monoglyceridase activities and may therefore play a role when remnants of lipoprotein particles are further degraded within the liver cells[16] or if monoglycerides tend to accumulate otherwise. The properties of this enzyme, that has no activity towards di- and triglycerides, and therefore should

be denominated a monoglyceridase or glycerolester esterase, will be described elsewhere.

ACKNOWLEDGEMENTS

Miss Ina Kalkman and Mrs. Tiny Geelhoed-Mieras are thanked for their expert technical assistance. Miss Cecile Hanson is thanked for typing the manuscript.

This investigation was carried out under the auspices of the Netherlands Foundation for Chemical Research (S.O.N.) and with financial aid from the Netherlands Organization for the Advancement of Pure Scientific Research (Z.W.O.).

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