The Hydrolysis of Cholesterol Esters in Plasma Lipoproteins by Hormone-sensitive Cholesterol Esterase from Adipose Tissue*

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SUMMARY

Adipose tissue contains a high level of neutral esterase active against emulsions of cholesteryl olate. The present studies show that this enzyme can also effectively hydrolyze the cholesterol esters in native rat plasma high density lipoproteins (HDL) and low density lipoproteins (LDL). The hydrolysis of lipoprotein cholesterol esters by a pH 5.2 isoelectric precipitate fraction from the freshly prepared 100,000 x g supernatant of chicken adipose tissue was low, but increased more than 50-fold on activation with cyclic AMP-dependent protein kinase. Rat adipose tissue homogenates were also very active against lipoprotein cholesterol esters, hydrolyzing as much as 60% of the total labeled cholesterol ester in HDL or LDL in 1 h. Activity was optimal at pH 7 and very low at pH 4. No protease activity was detected at pH 7 and, since assays were done in 2 mM EDTA, phospholipase A activity was presumably negligible. The results show that hormone-sensitive cholesterol esterase of adipose tissue has ready access to the neutral lipid core of plasma lipoproteins, either because the enzyme penetrates the polar shell or because the cholesterol ester in the core is exposed, at least intermittently, to allow enzyme-substrate complex formation. Whether or not this enzyme activity plays a role in lipoprotein degradation by adipose tissue remains to be determined.

Previous studies in this laboratory have shown that adipose tissue contains a neutral cholesterol esterase that can be activated by cyclic AMP-dependent protein kinase (1, 2). This cholesterol esterase activity is closely associated with the hormone-sensitive triacylglycerol hydrolase. In fact, attempts to resolve the two activities have been unsuccessful to date (1, 2). The functional significance of the high levels of cholesterol esterase activity found in adipose tissue is not known. It might play a role in the mobilization of cholesterol esters stored in adipose tissue, especially during extended periods of triglyceride mobilization, as suggested previously (1, 2). In addition, it might play a role in the degradation of lipoprotein cholesterol esters taken up from the plasma. The present studies were undertaken to explore the latter possibility.

EXPERIMENTAL PROCEDURES

Adipose tissue pH 5.2 precipitate (5.2 P) fractions were prepared from the 100,000 x g supernatant fraction as described previously (1). Protein kinase inhibitor was purified from skeletal muscle through the DEAE-cellulose step by the method of Walsh et al. (3). Activation of cholesterol esterase with cyclic AMP-dependent protein kinase was carried out in a final volume of 0.1 ml as described elsewhere (1). Briefly, 5.2 P fraction, which contains endogenous protein kinase, was incubated 5 to 10 min at 30°C with 5 mM Mg++, 0.5 mM ATP, and 0.01 mM cyclic AMP. Following activation, activity against [3H]cholesterol esters in lipoproteins was assayed in a mixture containing 2 mM EDTA, 20 mg/ml of bovine serum albumin, and 40 mM sodium phosphate at pH 7.0. EDTA was included to inhibit phospholipase A activity (4) that might be present in the 5.2 P fraction and also to terminate the protein kinase activation. The assay mixture (0.5 ml) was extracted with 10 ml of chloroform:methanol (2:1, v/v) (5); the chloroform phase was evaporated to dryness, the residue was redissolved in 0.15 ml of hexane, and an aliquot was applied to silica Gel G thin layer plates (Analtech) and developed in hexane-diethyl ether:acetic acid (60:20:1, v/v/v). The free and esterified cholesterol were localized by iodine vapor and the radioactivity was determined in a liquid scintillation counter as described by Drevon et al. (6).

Acid phosphatase activity was determined as described previously (1). Acid phosphatase activity was assayed with beta-glycerolphosphate as substrate (12).

RESULTS AND DISCUSSION

As shown in Fig. 1, cholesterol esterase from chicken adipose tissue was able to hydrolyze the LCAT-labeled [3H]cholesterol esters in HDL (Panel A), LDL (Panel B), and VLDL (Panel C) very effectively after activation with protein kinase. Prior to activation, activity was very low but increased 7- to 50-fold after protein kinase activation. The protein kinase-stimulated activity was blocked by the addition of an inhibitory protein specific for the catalytic subunit of cyclic AMP-dependent protein kinase. The activity of cholesterol esterase against lipoprotein cholesterol esters was linear as a function of time (Fig. 1) and as a function of enzyme concentration (data not shown). When native HDL or LDL from rat plasma (10 times in excess) was added to the assay mixture containing LCAT-labeled HDL or LDL, cholesterol esterase activity was inhibited by 50%.

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1 The abbreviations used are: LCAT, lecithin:cholesterol acyltransferase; VLDL, LDL, and HDL, very low density, low density, and high density lipoproteins, respectively.

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activity was suppressed (data not shown), showing that the native lipoproteins competed with the LCAT-labeled lipoproteins. The calculated absolute rate of cholesterol ester hydrolysis in HDL under the conditions described in legend to Fig. 1 was about 20 nmol/mg of protein of 5.2 P fraction/h. This is to be compared with 36 nmol/mg of protein of 5.2 P fraction/h produced when the same concentration of cholesteryl [1-14C]oleate in ethanolic emulsion was used, indicating that lipoprotein cholesterol esters are readily accessible substrates for hormone-sensitive cholesterol esterase. To further validate the data using LCAT-labeled lipoproteins, biologically labeled lipoproteins were prepared by feeding [3H]cholesterol to rats as described above. As shown in Fig. 2, similar to those presented in Fig. 1, there was no degradation of the apoprotein moieties.

The experiments in Figs. 1 and 2 were performed under conditions similar to those used to activate hormone-sensitive lipase (1). When 125I-labeled HDL or 125I-LDL were incubated with chicken adipose tissue 5.2 P under the conditions described in Fig. 1, there was no degradation of the apoprotein moieties. The activity of the chicken enzyme against lipoprotein cholesterol esters was optimal at pH 7; activity at pH 4 was 30% of that at pH 7.

Rat adipose tissue also contained a neutral cholesterol esterase which was very active against lipoprotein cholesterol esters. However, this activity was increased very little by protein kinase treatment in comparison to the large activation found in chicken adipose tissue. To determine what fraction of adipose tissue cholesterol esterase was accounted for by the neutral activity, a 1,000 x g supernatant fraction was prepared from epididymal fat pads. The fat pads had been first subjected to freezing and thawing (10 times) in order to disrupt the lysosomes. As shown in Fig. 3, the hydrolysis of cholesterol esters in LCAT-labeled HDL and LDL was optimal between pH 6.5 and 7.0. Note that fully 60% of the labeled cholesterol ester was hydrolyzed in 1 h. Activity at pH 4 to 5 was extremely low, indicating very little cholesterol esterase activity of lysosomal origin. These findings with regard to the hydrolysis of lipoprotein cholesterol esters parallel observations previously reported using emulsions of cholesterol esters as substrate (1, 2, 13). The finding of a significant level of acid phosphatase, as shown in Fig. 3, indicates that the lysosomes from the adipose tissue had indeed been disrupted. The very low level of lysosomal cholesterol esterase activity in adipose tissue stands in contrast to the predominantly lysosomal activity.
location of cholesterol esterase in other cells, such as fibroblasts and smooth muscle cells (13–16). Other tissues in which high levels of neutral cholesterol esterase activity are found are the adrenals and the testis (13). These represent tissues with significant stores of cholesterol esters available as potential precursors for steroidogenesis (17, 18). Adipose tissue also contains significant levels of stored cholesterol, mostly in free form but some in ester form (19, 20) and, as has been previously suggested, the cholesterol esterase could play a role in mobilization of stored cholesterol esters concurrent with mobilization of depot triglyceride fatty acids (1, 2). While other tissues have much lower levels of neutral cholesterol esterase activity (13), they may have analogous enzymes that simply function at a much lower level of activity in nonspecialized tissues. If the differences are primarily quantitative rather than qualitative, further characterization of the adipose tissue enzyme studied here could provide information generally applicable to understanding of cholesterol ester metabolism.

D’Costa and Angel have reported that human adipocytes take up and degrade the apoprotein moiety of LDL and that the uptake is stimulated by norepinephrine and dibutyryl cyclic AMP (21). Preliminary studies in swine, using a new technique for determining sites of plasma lipoprotein degradation (22, 23), show that more than 10% of the LDL degradation in vivo occurs in adipose tissue. \(^1\) Taken together, these findings suggest the possibility that the neutral cholesterol esterase may play a role in the degradation of lipoprotein cholesterol esters during uptake in adipose tissue.

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