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LIPASES AND CARBOXYLESTERASES: Possible Roles in the Hepatic Metabolism of Retinol

Earl H. Harrison
Department of Biochemistry, MCP-Hahnemann School of Medicine, Allegheny University of Health Sciences, Philadelphia, Pennsylvania 19129;
e-mail: harrison@auhs.edu

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ABSTRACT

The formation and hydrolysis of retinyl esters are key processes in the metabolism of the fat-soluble micronutrient vitamin A. Long-chain acyl esters of retinol are the major chemical form of vitamin A (retinoid) stored in the body. Retinyl esters are found in a variety of tissues and cell types, but most of the total body retinoid is accounted for by the retinyl esters stored in the liver. Thus, these esters represent the major endogenous source of retinoid that can be delivered to peripheral tissues for conversion to biologically active forms. This review summarizes current knowledge about the identity, function, and regulation of the hepatic enzymes potentially involved in catalyzing the hydrolysis of retinyl esters. These enzymes include several known and characterized lipases and carboxylesterases. Although there is accumulating evidence that these enzymes function as retinyl ester hydrolases in vitro, it is not clear which play important physiological roles in hepatic retinyl ester metabolism.

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INTRODUCTION

The formation and hydrolysis of retinyl esters are key processes in the metabolism of the fat-soluble micronutrient vitamin A. Vitamin A is essential for the growth and general health of higher animals, including humans. The vitamin is needed for vision, reproduction, and the development and maintenance of differentiated tissues. The vitamin's role in vision is fulfilled by its metabolic conversion to 11-*cis* retinaldehyde, which functions as the active chromophore in rhodopsin (70, 76). Its role in differentiation and development is likely fulfilled by its conversion to either all-*trans* or 9-*cis* retinoic acid. Both interact with a number of nuclear receptors (of the RAR or RXR families) that function as hormone-activated, *trans*-activating factors in the regulation of gene expression (21). The role of retinoic acid in gene expression is likely the basis of the profound role of retinoids in preventing or reversing certain neoplasms (56). Thus, understanding the basic biochemistry of retinoid metabolism is not only of fundamental scientific interest, it also is needed to better understand the roles that vitamin A nutriture and the retinoids play in health and disease.

As described in more detail below, long-chain acyl esters of retinol are the major chemical form of vitamin A (retinoid) stored in the body. Retinyl esters are found in a variety of tissues and cell types, but most of the total body retinoid is accounted for by the retinyl esters stored in the liver. Thus, these esters are the major endogenous source of retinoid that can be delivered to peripheral tissues for conversion to biologically active forms. This review summarizes current knowledge about the identity, function, and regulation of the hepatic enzymes potentially involved in catalyzing the hydrolysis of retinyl esters.

VITAMIN A METABOLISM AND TRANSPORT—AN OVERVIEW

De novo synthesis of compounds with vitamin A activity is an ability limited to plants and microorganisms (32, 33). Thus, higher animals must obtain vitamin A from the diet, either as the preformed vitamin or as a provitamin carotenoid such as β-carotene. In the intestinal mucosa, carotene is converted (via two enzymatic steps) to retinol (22, 31). The major dietary forms

of preformed vitamin A are long-chain fatty acid esters of retinol (63). These esters must be hydrolyzed prior to intestinal absorption. Hydrolysis of the esters can be catalyzed by enzymes secreted by the pancreas into the intestinal lumen and by enzymes associated directly with intestinal cells.

Erlanson & Borgstrom (20) showed that rat pancreatic juice contains two enzymes capable of catalyzing the hydrolysis of retinyl palmitate in vitro. Which enzyme was active depended on the physical form of the substrate presented. Emulsified retinyl palmitate was mostly hydrolyzed by pancreatic lipase. In the presence of millimolar concentrations of bile salt (conditions that inhibited pancreatic lipase), pancreatic carboxylester lipase was the enzyme responsible for the observed activity. When the two forms of substrate were compared, the absolute activity due to the latter enzyme was about two times that of the former. The ability of both crude intestinal preparations and purified pancreatic carboxylester lipase to hydrolyze retinyl esters in the presence of millmolar concentration of trihydroxy bile salts was confirmed in subsequent reports (36, 48, 58, 67). Pancreatic carboxylester lipase has often been studied as a cholesteryl ester hydrolase. There is evidence that the bile salt–dependent cholesteryl ester hydrolase activity of intestine is due to the binding and uptake of the pancreatic enzyme by intestinal cells (27–29, 34, 46). This enzyme has broad substrate range for naturally occurring lipids, including triacylglycerols, cholesteryl esters, retinyl esters, phospholipids, and lysophospholipids (for a comprehensive review, see 77).

Rigtrup et al (66, 67) have shown that retinyl ester hydrolase (REH) activities associated with the brush border membrane of the intestines are distinct from the pancreatic carboxylester lipase described above. They studied in vitro the hydrolysis of retinyl esters by purified rat intestinal brush border membranes. The purified membranes contained two neutral REH activities that were distinguished by a number of criteria. One of these REH activities was later purified and shown to be identical to brush border phospholipase B (65).

The work outlined above indicates that three or more enzymes might be involved in the digestion of dietary retinyl esters: pancreatic lipase, pancreatic carboxylester lipase, phospholipase B, and perhaps other REHs associated with the brush border membrane. The relative roles of these enzymes in the digestion of retinyl esters remain to be determined. However, given their abilities to interact with substrate retinyl esters in the different physico-chemical forms these esters might adopt in the complex mixtures found in the intestinal lumen (i.e. emulsions, mixed micelles, liquid-crystalline vesicles), it seems reasonable to speculate that they each play a role (42).

Regardless of which enzyme(s) is(are) responsible for the hydrolysis of dietary retinyl esters, the free retinol is then taken up by the mucosal cell (19). There it is reesterified with long-chain, mainly saturated, fatty acids by

the enzyme(s) lecithin:retinol acyltransferase (LRAT) and/or acyl-coenzyme A:retinol acyltransferase (ARAT), both membrane-bound enzymes (41, 49, 61). The resulting retinyl esters are incorporated with other neutral lipid esters (i.e. triacylglycerols and cholesteryl esters) into chylomicrons and absorbed via the lymphatics (43). In the vascular compartment, much of the chylomicron triacylglycerol is hydrolyzed by lipoprotein lipase in extrahepatic tissues. This results in the production of a "chylomicron remnant," which contains most of the newly absorbed retinyl esters (40). In the rat, the chylomicron remnants are rapidly and almost quantitatively taken up by the liver, and there is evidence that the retinyl esters are rapidly hydrolyzed and reesterified during this process (8, 37, 45). The reesterification in liver is also thought to be catalyzed by LRAT and/or ARAT enzymes (62, 68, 79).

Under conditions of adequate vitamin A nutriture, the main site of vitamin A storage is the liver, where over 95% of the total neutral retinoid is present as retinyl esters, predominately retinyl palmitate and stearate (10, 24, 35, 47). Although chylomicron remnants (and the retinyl esters they contain) are initially taken up exclusively by the hepatocytes in liver, the retinyl esters are then transferred largely to the perisinusoidal stellate cells (8, 9). In vitamin A–adequate rats, the stellate cells account for approximately 80% of the total retinyl ester store, with the remainder in hepatocytes (3, 10, 52). In both cell types, the retinyl esters are stored in cytoplasmic lipid droplets along with other neutral lipids. Prior to mobilization from the liver, the retinyl esters are hydrolyzed, and free retinol is complexed to serum retinol-binding protein for secretion from the liver (71).

As indicated by this overview, the hydrolysis of retinyl esters plays a major role in the metabolism of vitamin A. The process is especially crucial in the digestion and intestinal absorption of dietary vitamin A and in the hepatic uptake, storage, and mobilization of the vitamin. The remainder of this review focuses on enzymes potentially involved in the latter processes.

SUBSTRATE SPECIFICITY AND KINETICS—A CAUTION

The title of this review emphasizes the fact that lipases and carboxylesterases may function in hepatic retinyl ester hydrolysis. All such known enzymes hydrolyze a variety of substrates, and no purified enzymes have been shown to be absolutely specific for long-chain retinyl esters, or indeed even to hydrolyze them at faster rates than other esters. And, of course, the substrate specificity of an enzyme in crude preparations cannot be fully assessed. It can be argued that the lack of purified and specific enzymes reflects the primitive state of the

art in this area of enzymology, and that the isolation of specific retinyl ester hydrolases will be forthcoming. This may occur, but it also may not. That it will not is supported by what is known about the general nature of lipases, enzymes that hydrolyze water-insoluble substrates.

The fundamental distinction between esterases and lipases is the involvement of a lipid-water interface in the catalytic process for the latter class of enzymes. Esterases function on water-soluble substrates and, hence, catalyze reactions in which the enzyme, substrate, and products are homogeneously distributed in a single (aquaeous) phase. With lipases and water-insoluble substrates (such as retinyl esters), this is not the case. The presence of heterogeneous phases per se, and the fact that they change in composition during the course of the lipolytic reaction, makes the interpretation of enzyme kinetic data more complicated than for homogeneous catalysis. A full discussion of lipase kinetics is beyond the scope of this review, and the reader is referred to the excellent monograph edited by Borgstrom & Brockman (13). A few points of particular relevance to the study of the hydrolysis of highly apolar lipids such as retinyl esters should be made, however. The composition and packing of nonsubstrate molecules at the interface (the "quality" of the interface) plays a large role in the binding of the enzyme and the rates of substrate hydolysis observed. Obviously, the availability of substrate molecules is also important. The point is that the observed kinetic "preference" for one substrate over another may reflect more on the substrate molecule's interactions with lipids that allow it to achieve a high concentration at the interface than on any preferential binding to the enzyme itself. In other words, the apparent specificity of a lipolytic enzyme may be a reflection of the physical availability of the substrate at the interface.

Studies of the enzymatic hydrolysis of retinyl esters generally have been conducted under conditions where the interfacial concentration of substrate (and other lipids or detergents) is undefined. Although this does not preclude making certain operational comparisons of the rates of hydrolysis of different potential substrates, it does mean it is problematic to conclude that higher rates of hydrolysis of one ester over another tell much about the enzyme's specificity. This is especially true when one considers that even fairly well-characterized substrate forms (i.e. micelles, liposomes, or monolayers of defined composition) probably do not closely resemble the physical forms adopted by retinyl esters in vivo (i.e. the complex emulsions, micelles, and vesicles in the intestinal lumen, the chylomicrons and remnants delivered to the liver, and the mixed lipid droplets found within various sorts of cells). Most, if not all, lipases catalyze the hydrolysis of water-soluble esters in solution, and in this sense, no lipase shows a strict substrate specificity.

HEPATIC RETINYL ESTER HYDROLASES

Bile Salt–Dependent Retinyl Ester Hydrolase (Carboxylester Lipase)

In 1966, a retinyl palmitate hydrolyzing activity was found in rat liver homogenates that required the addition of bile salts for in vitro activity (50). The investigators partially characterized this activity in extracts of acetone powders of pooled rat livers. In 1979, these findings were confirmed, and it was demonstrated that rat liver homogenates contained a lipid ester hydrolase activity with a neutral pH optimum and several unusual properties (39). The enzyme activity, which appeared to hydrolyze both retinyl palmitate and cholesteryl oleate, required millimolar concentrations of cholate or taurocholate for maximal activity. Almost no activity was observed in the absence of bile salts or in the presence of several other detergents. The activity had an unusual distribution among subcellular fractions of liver homogenates, being equally distributed between the nuclear fraction and the high-speed supernatant, with little activity associated with microsomes. The activity varied markedly (over a 50-fold range) among the livers of individual rats. All these unusual properties were observed for the hydrolysis of retinyl palmitate and cholesteryl oleate but were not observed for the hydrolysis of eight other lipid and non-lipid ester substrates. Subsequent studies confirmed these observations and demonstrated further that the triolein hydrolase activity shared these unusual properties (5, 64). Particularly striking was the covariation in the three hydrolase activities over a 50-fold range among 66 individual rat liver homogenates (5).

Following the initial description of the properties and subcellular localization of the bile salt–dependent retinyl ester hydrolase (BSDREH) activity in rat liver, a series of papers was published on the spacial (anatomic) distribution of the enzyme activity in the liver and its distribution among different liver cell types. In the first study, livers of 11 vitamin A–sufficient and 11 vitamin A–deficient rats were dissected into 11 distinct anatomical sections, and each was homogenized and assayed for BSDREH (6). The results showed a significant section-to-section variation in activity that did not show any consistent anatomic pattern from rat to rat. Although the biological basis and possible physiological relevance of these observations are not known, they indicate that it is not possible to accurately measure the total liver levels of enzyme activity in a homogenate made from a small section. In two independent studies on the distribution of enzyme activity in different liver cell types, BSDREH activity was mostly associated (80–90% of total activity) with hepatic parenchymal cells, with the remainder in the fat-storing (stellate) cells (3, 10). However, the specific activity, expressed on either a per cell or a per milligram of protein

basis, was higher in the smaller and less-numerous stellate cells. Endothelial and Kupffer cells had low activities.

The rat hepatic bile salt–dependent hydrolytic activities against retinyl esters, cholesteryl esters, and triacylglycerols copurified through ammonium sulfate precipitation and chromatography on phenyl-Sepharose, resulting in a partial purification (about 25x) of the three activities (64). Further work on the purification of the enzyme(s) was reported by Blaner et al (5). These investigators achieved a partial purification (about 200x) by sequential chromatography of acetone powder extract on columns of phenyl-Sepharose, DEAE-Sepharose, and heparin-Sepharose. The three hydrolase activities copurified during each of the chromatographic steps. In addition, the properties of the three copurifying activities were similar with regard to stimulation of catalysis by bile salts and in neutral pH optimum. However, despite these similarities, the authors presented evidence that the activities against the three substrates are differentially sensitive to a number of inhibitors. They also claimed that the cholesteryl ester hydrolase activity could be separated from the other two activities by extraction of rat liver acetone powders with pH 5 acetate buffer. The report by Ghosh et al (30) on the separation and differential activation of rat liver cytosolic retinyl ester, cholesteryl ester, and triacylglycerol hydrolase activities is probably not relevant to the present discussion because the assays were carried out in the absence of bile salts.

Much recent work on the hepatic, bile salt–dependent retinyl ester hydrolase has focused on the possibility that most or all of this activity is due to the bile salt–activated carboxylester lipase, an enzyme that has been purified from the pancreata and milks of several mammalian species (77). The basis for considering this possibility was the fact that, like the bile salt–dependent lipid ester hydrolase of rat liver, the bile salt–activated carboxylester lipase required millimolar concentrations of trihydroxy bile salts for activity in bulk-phase assay systems and showed a broad substrate range, including cholesteryl esters and retinyl esters (20, 23, 26, 48). Experiments in my laboratory demonstrated the close similarity in enzymatic properties between purified rat pancreatic carboxylester lipase and the bile salt–dependent lipid ester hydrolase activities of rat liver cytosol for the hydrolysis of both cholesteryl esters and retinyl esters (34, 36). Moreover, monospecific anti–pancreatic hydrolase immunoglobulin G specifically and completely inhibited both the bile salt–dependent cholesteryl ester and the retinyl ester hydrolase activities of rat liver cytosol (34, 36).

Further support for the suggestion that the bile salt–dependent liver hydrolase is highly related to the pancreatic enzyme came from Camulli et al (14). They isolated a bile salt–stimulated cholesteryl ester hydrolase from rat liver cytosol using chromatography on DEAE-Sepharose, gel filtration, and

an immunoaffinity column of anti–porcine pancreatic cholesterol esterase. On the basis of N-terminal sequence analysis and reaction with anti–pancreatic enzyme antibodies, they concluded that liver and pancreatic enzymes are identical. However, the hepatic enzyme isolated by Camulli et al (14) has a much lower specific activity than does the pancreatic enzyme (77). It was suggested that the enzymes are related but not identical and that they perhaps differ in post-translational modifications (77). Nonetheless, recent analysis of the nucleotide sequence of the cDNA for the hepatic enzyme also indicates it is identical to the pancreatic enzyme (15, 44).

Much of the early work on the hepatic, bile salt–dependent carboxylester lipase was motivated by the search for an intracellular enzyme in rat liver that might be involved in the mobilization of stored retinyl esters. However, evidence suggests that the hepatic enzyme (like that of the pancreas and breast) is largely secreted from the tissue that makes it. Thus, both rat hepatoma cells and intact rat livers secrete more enzyme than they retain in the cell (78). Consistent with the idea that the enzyme is secreted by the liver is the demonstration of enzyme activity in rat serum (34). Thus, the hepatic carboxylester lipase may function as a retinyl ester hydrolase to hydrolyze chylomicron remnant retinyl esters after the enzyme is secreted into the space of Disse. Whether or not this enzyme, or other lipases that share these features, are involved physiologically in hepatic retinyl ester metabolism is unclear. Studies on retinoid metabolism in mice rendered deficient in carboxylester lipase by gene knockout techniques will likely shed light on this point.

Bile Salt–Independent Retinyl Ester Hydrolases

INTRODUCTION Most early studies of the hydrolysis of retinyl esters in liver and other tissues focused on the bile salt–dependent hydrolase described above. More recently, reports have documented the existence of bile salt–independent retinyl ester hydrolases that are distinct from the bile salt–dependent hydrolase (11, 25, 36–38, 59).

Rat liver homogenates contain a neutral, bile salt–independent REH activity that differs from the BSDREH (i.e. carboxylester lipase) in that (*a*) its absolute activity does not vary widely among individual rats, (*b*) it is not inhibited by antibodies to pancreatic carboxylester lipase, and (*c*) it is localized in the microsomal fraction of liver homogenates, with almost no activity in the soluble fraction (36). Subfractionation of microsomes demonstrated that the enzyme activity is specifically enriched in plasma membranes and/or endosomes. This localization would allow the enzyme to play a role in the initial hydrolysis of retinyl esters delivered to the liver in association with cylomicron remnants.

The potential specificity and importance of the bile salt–independent, neutral REH activity in retinoid metabolism is also indicated by evidence that

the reaction is specifically activated by apo-cellular retinol-binding protein (apoCRBP) (11). These elegant studies demonstrated that the hydrolysis of endogenous retinyl esters in rat liver microsomes was stimulated by apoCRBP in a concentration-dependent and saturable fashion. The lack of inhibition of the apoCRBP-stimulated hydrolysis by anti–pancreatic cholesterol esterase or by bis-p-nitrophenyl phosphate demonstrated that the reaction under study was, in fact, the bile salt–independent REH. Moreover, the concentrations of apoCRBP used in this study were close to the concentrations of the binding protein found in rat liver cytosol (35). Thus, apoCRBP may be an important regulator of retinyl ester hydrolysis in vivo.

Other studies have demonstrated that the microsomal, neutral, bile salt–independent REH is distinct from microsomal cholesteryl esterases (25). Activities against the two ester substrates were markedly differentially sensitive to heat inactivation, protease treatments, and active site-directed inhibitors. This same study demonstrated that rat liver plasma membrane/endosome fractions also contain bile salt–independent REH activity active at acid pH. The acid REH is distinct from the neutral activity on the basis of their differential sensitivities to n-alkyl carbamates and diethylphosphates. Evidence suggests that chylomicron retinyl esters delivered to the liver are initially associated with endosomes but are not transferred to lysosomes (7, 37). Thus, the presence of neutral and acid REHs in plasma membranes and endosomes could allow for the efficient hydrolysis of retinyl esters newly delivered to the liver.

More recent studies were directed at discovering whether neutral and acid, bile salt–independent retinyl ester hydrolases associated with plasma membrane and endosome fractions of rat liver homogenates are involved in hepatic retinyl ester metabolism (37). Chylomicrons containing tritium-labeled retinyl esters were injected intravenously into rats to study the initial metabolism of retinyl esters during and following uptake into the liver. At various times after chylomicron injection, plasma was obtained and the liver was homogenized and subjected to analytical subcellular fractionation. Labeled retinyl esters were rapidly cleared from plasma (half-time $\sim$10 min) and appeared in the liver. Within the liver, label first appeared in plasma membrane/endosomal fractions that were also enriched in both neutral and acid, bile salt–independent retinyl ester hydrolase activities. At no time were the labeled esters significantly associated with fractions enriched in lysosomes. Rather, it appeared that the labeled esters were hydrolyzed and/or transferred to fractions enriched in endoplasmic reticulum. These studies demonstrated the colocalization of newly delivered retinyl esters and bile salt–independent retinyl ester hydrolase enzyme activities and, thus, suggested a probable role for these enzymes in the initial hepatic metabolism of chylomicron retinyl esters. This conclusion was further supported by the observation that plasma membrane/endosomal fractions were

active in catalyzing the hydrolysis of chylomicron remnant retinyl esters in vitro.

Analysis of the cellular distribution of the membrane-bound, bile salt–independent acid and neutral REHs in hepatocytes and nonparenchymal cell fractions of rat liver revealed that there was no preferential enrichment of either activity in either cell fraction (52). Thus, these enzymes are present in hepatocytes, the cell type that is almost exclusively involved in chylomicron uptake in liver.

It was also demonstrated that the activities of the neutral and acid, bile salt–independent REHs were unaffected by vitamin A (retinoid) status (52). Thus, rats sufficient in vitamin A, rats deficient in vitamin A, and rats deficient in vitamin A and treated with retinoic acid or with N-(4-hydroxyphenyl)-retinamide had similar hepatic BSIREH activities.

CARBOXYLESTERASES The older biochemical literature refers to more than 30 rat liver carboxylesterases, but it is now appreciated that almost all of these enzyme activities are manifestations of the gene products of five major loci in linkage group V (54). These esterases are referred to as ES-2 (serum esterase), ES-3 (pI 5.6 esterase), ES-4 (pI 6.2/6.4 esterase or microsomal hydrolase), ES-10 (pI 6.0/6.1 esterase), and ES-15 (pI 5.0/5.2 esterase). All these enzymes have polypeptide monomer molecular weights of 58,000–65,000 and all function catalytically as monomers, except for esterase ES-10, which exists as a homotrimer in the native state (54). All these enzymes function as carboxylesterases (on a wide variety of oxyester substrates), whereas the microsomal hydrolase, ES-4, also functions as a thioesterase and catalyzes the hydrolysis of long-chain acyl-coenzyme As (2, 54).

The hydrolysis of retinyl palmitate by four purified rat liver microsomal carboxylesterases—ES-3, ES-10, and esterases of pIs 6.2 and 6.4 (isozymic forms of a single protein coded by the ES-4 locus)—was studied (53). The latter enzyme (ES-4) hydrolyzed retinyl palmitate that was comixed with various emulsifiers. Emulsification with either 0.1 mM bovine serum albumin, 10 mM taurocholate, or 0.2% Triton X-100 all supported hydrolysis, with the latter giving the highest rates (2x higher than the others). Thus, this assay is not measuring the BSDREH but rather a neutral bile salt–independent REH. ES-10 showed lower activity with some substrate forms. ES-3 was inactive under any of the assay conditions employed.

Recently, a neutral, bile salt–independent retinyl ester hydrolase was purified from a rat liver microsomal fraction (73). The purification procedure involved detergent extraction, DEAE-Sepharose ion exchange, phenyl-Sepharose hydrophobic interaction, Sephadex G-100 and Sephacryl S-200 gel filtration chromatographies, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The isolated enzyme has an apparent molecular mass of approximately 66,000 under denaturing conditions on SDS-PAGE. Analysis of

the amino acid sequences of four peptides isolated after proteolytic digestion revealed that the enzyme is highly homologous with other rat liver carboxylesterases. In particular, the sequences of the four peptides of the NREH (60 amino acids total) were identical to those of a rat serum carboxylesterase (ES-2) expressed in the liver (1). Antibodies against ES-2 also reacted with the purified neutral REH. Purified NREH showed a substrate preference for retinyl palmitate over triolein and did not catalyze the hydrolysis of cholesteryl oleate. With retinyl palmitate as substrate, the enzyme had a pH optimum of 7 and showed apparent saturation kinetics, with half-maximal activity achieved at substrate concentrations (K_m) of about 70 μM. In the same study, evidence showed that a nearly homogeneous preparation of ES-10 also functioned as a neutral BSIREH. Thus, it appears that three known carboxylesterases (viz ES-2, ES-4, and ES-10) can function effectively as REHs in vitro.

ACID HYDROLASES Some studies in the literature indicate that hepatic lysosomal acid hydrolases can catalyze the hydrolysis of retinyl esters in vitro, but these enzymes have yet to be characterized in detail (55). Moreover, there is an apparent lack of involvement of lysosomes per se in the metabolism of chylomicron retinyl esters (7, 25, 37). Thus, cell fractionation experiments failed to demonstrate the accumulation of retinoid in fractions enriched in lysosomes. In these experiments, when labeled asialoglycoproteins were used as positive controls, accumulation of label was observed in lysosome-rich fractions. Thus, chylomicron remnants and other ligands delivered by receptor-mediated endocytosis appear to take different intracellular pathways after initial uptake. This is not surprising in light of recent evidence that even different lipoproteins undergo different fates after uptake into cells (74).

If lysosomal enzymes are not involved in the hepatic metabolism of chylomicron retinyl esters, then other enzymes must be responsible for the rapid hydrolysis that takes place during or shortly after their hepatic uptake (8, 37, 45). Colocalization of retinoid taken up by endocytosis and bile salt–independent retinyl ester hydrolases provides evidence that these enzymes are important in this process. Furthermore, membrane fractions enriched in the retinyl ester hydrolases catalyze the hydrolysis of chylomicron remnant retinyl esters in vitro at both acid and neutral pH. Thus, acid and neutral hydrolases present in hepatic endosomes may play a role in the hydrolysis of chylomicron remnant retinyl esters in vivo. Endosomal hydrolases are also thought to play a role in the hydrolysis of cholesteryl esters delivered to the hepatocyte in association with chylomicron remnants (51).

OTHER RETINYL ESTER HYDROLASES IN LIVER *Lipoprotein lipase and hepatic lipase* The first step in the hepatic metabolism of chylomicron remnants seems to be their sequestration in the space of Disse by the binding of apolipoprotein

E to heparin sulfate proteoglycans on the cell surface (51). It is also now appreciated that secreted lipases, namely lipoprotein lipase (LPL) and hepatic lipase (HL), are also found in the space of Disse. Both can potentiate the cellular uptake of cholesteryl ester from chylomicron remnants or other lipoproteins by mechanisms that may involve anchoring the lipoprotein to the cell surface and/or hydrolysis of the esters themselves (12, 60, 69, 72).

Recent work strongly suggests that LPL catalyzes the hydrolysis of retinyl esters in both chylomicrons and artificial emulsions (4). Moreover, through this stimulation of hydrolysis, the enzyme facilitated retinoid uptake by cultured adipocytes. Thus, evidence suggests that three secreted lipases may be found in the space of Disse [viz carboxylester lipase (CEL), LPL, and HL] and that at least two of these (CEL and LPL) can function as retinyl ester hydrolases. There is no published information on whether hepatic lipase catalyzes retinyl ester hydrolysis. Thus, although CEL, LPL, and HL do not show an absolute specifity for retinyl esters (and indeed are more active in catalyzing the hydrolysis of cholesteryl esters and/or triacylglycerols), they nonetheless may play an important role in hepatic retinyl ester metabolism. The enzymes' ability to catalyze the hydrolysis of a number of lipid esters and the fact that they are found in the space of Disse argue that they may play roles in the hydrolysis of a number of components of the chylomicron remnant, including retinyl esters.

Other enzyme activities Several reports have appeared in which the assay for neutral REH first described by Cooper & Olson (17) has been used. This assay differs prominently from those used in the studies described above by the much higher concentrations of substrate and enzyme protein used. In addition, it utilizes 100–300 mM 3-[(3-cholamidopropyl)dimethylammonio]-1-propansulfonate, a bile salt analog, and 0.2% Triton X-100 in the assay mixture. This assay has been applied to the study and partial characterization of the activity in the livers of pigs (17, 18), rats (16, 75), and humans (57). On the basis of these initial characterizations it would appear that the activities detected with this assay do not correspond to either the bile salt–dependent REH or the bile salt–independent REHs described above (38).

SUMMARY AND PROPOSED MODEL OF THE ROLES OF VARIOUS RETINYL ESTER HYDROLASES IN HEPATIC RETINYL ESTER METABOLISM

Figure 1 outlines a possible pathway for the hepatic metabolism of retinyl esters that is consistent with the available experimental data and that emphasizes the possible role of various retinyl ester hydrolases. It is now thought that the first step in the hepatic metabolism of chylomicron remnants is their sequestration

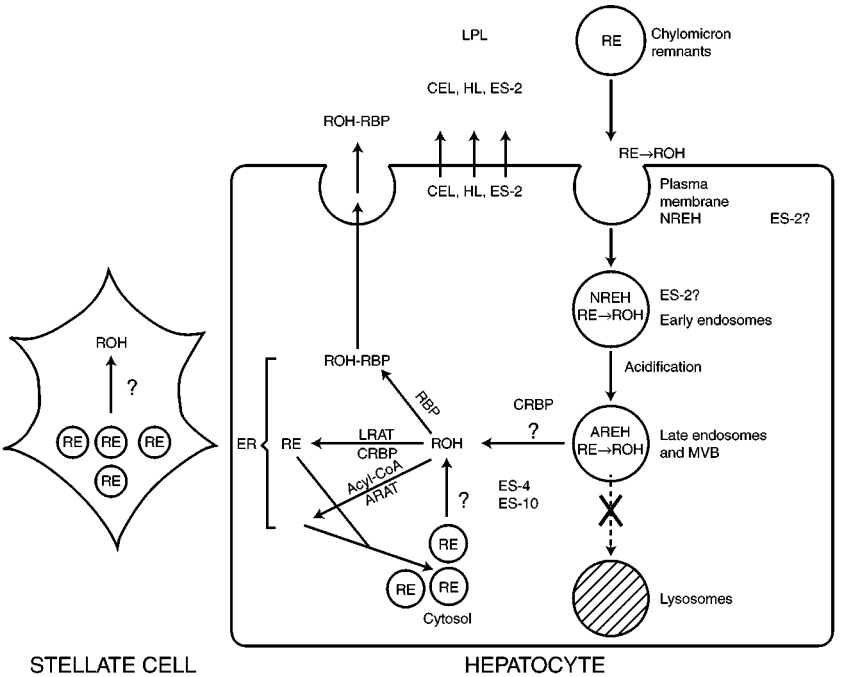

Figure 1 Proposed scheme for the involvement of lipases and carboxylesterases in the hydrolysis of retinyl esters (RE) during the uptake and mobilization of vitamin A in liver. Chylomicron remnants containing RE are first sequestered in the sinusoidal space of Disse outside of hepatocytes. Carboxylester lipase (CEL), hepatic lipase (HL), and carboxylesterase ES-2 (ES-2) are secreted by the liver and could occur in the space of Disse along with lipoprotein lipase (LPL) bound to sinusoidal endothelial cells. Any of these enzymes might function to hydrolyze RE to free retinol (ROH). During endocytosis, the bile salt–independent, neutral retinyl ester hydrolase (NREH) and acid retinyl ester hydrolase (AREH) found in plasma membranes, endosomes, and multivesicular bodies (MVB) could continue to hydrolyze RE. The free retinol so produced is transferred to the endoplasmic reticulum (ER) likely in association with cellular retinol-binding protein (CRBP). In the ER, the ROH can be complexed with plasma retinol-binding protein (RBP) for secretion from the liver or reesterified by the enzymes lecithin:retinol acyltransferase (LRAT) or acyl-coenzyme A (Acyl-CoA):retinol acyltransferase (ARAT). RE found in the ER or stored in cytoplasmic lipid droplets also can be hydrolyzed enzymatically. The ER-localized carboxylesterases ES-10 and ES-4 may play a role in this process. The identity of the hydrolases involved in the hydrolysis of stored RE in stellate cells is not known.

in the space of Disse by the binding of apolipoprotein E to heparin sulfate proteoglycans on the cell surface (51). While in the space of Disse, some of the neutral lipid ester may be hydrolyzed by secreted lipases such as hepatic lipase and lipoprotein lipase. Because the bile salt–dependent carboxylester lipase is also secreted from liver cells (78), as is serum carboxylesterase ES-2 (1), these enzymes may play some role in the metabolism of remnants in the space of Disse, although there is no evidence that directly supports this possibility.

Regardless of possible metabolism in the space of Disse, chylomicron remnant retinyl esters also could be hydrolyzed at the cell surface by the bile salt–independent, neutral retinyl ester hydrolase (perhaps an intracellular form of ES-2; see 73). The same enzyme could continue to catalyze the hydrolysis of retinyl esters after internalization of the remnant in early endosomes. As the endosomal pH gradually falls to <6 during acidification, the neutral hydrolase would be less active and the acid retinyl ester hydrolase that is also present in these vesicles might play a greater role. Thus, perhaps both the acid and neutral REHs function similarly in the hydrolysis of chylomicron retinyl esters during initial hepatic uptake. The relative contribution of each enzyme activity to the total activity might change as the endosomal pH drops.

Evidence suggests that after uptake and hydrolysis of retinyl esters, the unesterified retinol is transferred to the endoplasmic reticulum for further metabolism. The mechanism of this transfer is not known, but it may involve cellular retinol-binding protein (CRBP), if transfer occurs through the cytoplasm. Given the limited aqueous solubility of retinol, it is unlikely to exist in cytoplasm in true solution. There is sufficient cytosolic CRBP in liver to bind all the unesterified retinol (35). Regardless of the mechanism of transfer to endoplasmic reticulum, it is also clear that, in the steady state, a significant fraction (about one third) of the unesterified retinol is localized there (35). So, too, are the enzymes that can reesterify the retinol for storage in cytoplasmic lipid droplets (LRAT and ARAT) and the binding protein (retinol-binding protein) necessary for its secretion from the liver. It is unclear what enzyme(s) may play a role in the hydrolysis of stored retinyl esters formed in the endoplasmic reticulum, but maybe the two carboxylesterases that are known to be localized there and to function in vitro as REHs (viz ES-4 and ES-10) are good potential candidates. However, it is also possible that as yet unrecognized lipases or esterases may be involved.

All the hydrolases mentioned above are known to be found in hepatocytes. An important area that requires further investigation is the retinyl ester hydrolases present in hepatic stellate cells. Although it is clear that both BSDREH and BSIREH activities are found in stellate cell-enriched fractions, it is important to further define the specific enzymes expressed in these cells. It is likely that one or more stellate cell REHs play a major role in the mobilization of the bulk of hepatic retinyl esters stored in them.

PROSPECTIVE

The literature reviewed above suggests that the hydrolysis of retinyl esters plays an important role in hepatic retinoid metabolism and that a sometimes bewildering number of enzymes and activities catalyze this process. It should be apparent that the large number of activities described by various authors, and some of the discrepancies among their reports, may relate to the differing compositions of reactants used in different assays. If it is accepted that different enzymes hydrolyze retinyl esters presented to the same preparation in different partially defined physical forms in vitro, it is reasonable to assume that different enzymes act on different physical forms in vivo. Thus, caution is urged in attempts to define which "one" enzyme is the physiologically relevant one. Several of the activities studied in vitro may be relevant; alternatively, none may be relevant. In the future, techniques such as overexpression of specific enzymes or gene ablation might ultimately lead to a definition of which enzymes play which roles in hepatic retinyl ester hydrolysis. In addition, development of highly specific enzyme inhibitors could also shed light on these issues. To develop these tools, more work of an enzymological and biochemical nature needs to be done. Retinyl ester hydrolase activities need to be purified to homogeneity so that more detailed knowledge of their substrate kinetics, specificity, and relationship to other lipases can be assessed. This work could, in turn, be extended to the development of specific inhibitors. Finally, the cDNAs and genes for these enzymes need to be isolated so that the regulation and physiological consequences of specific enzyme expression can be studied.

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