Distribution of lecithin-retinol acyltransferase activity in different types of rat liver cells and subcellular fractions

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It is now well documented that lecithin-retinol acyltransferase (LRAT) is the physiologically important enzyme activity involved in the esterification of retinol in the liver. However, no information regarding the cellular distribution of this enzyme in the liver is presently available. This study characterizes the distribution of LRAT activity in the different types of rat liver cells. Purified preparations of isolated parenchymal, fat-storing, and Kupffer + endothelial cells were isolated from rat livers and the LRAT activity present in microsomes prepared from each of these cell fractions was determined. The fat-storing cells were found to contain the highest level of LRAT specific activity (383 ± 54 pmol retinyl ester formed $\min^{-1} \cdot mg^{-1}$ versus 163 ± 22 pmol retinyl ester formed $\min^{-1} \cdot mg^{-1}$ for whole liver microsomes). The level of LRAT specific activity in parenchymal cell microsomes (158 ± 53 pmol retinyl ester formed $\min^{-1} \cdot mg^{-1}$) was very similar to LRAT levels in whole liver microsomes. The Kupffer + endothelial cell microsome fractions were found to contain LRAT, at low levels of activity. These results indicate that the fat-storing cells are very enriched in LRAT but the parenchymal cells also possess significant levels of LRAT activity.

Fat-storing cell; Hepatic parenchymal cell; Retinoid; Cellular retinol-binding protein; Retinol esterification; LRAT

1. INTRODUCTION

The liver is the site of storage of the majority of the body's retinoid (vitamin A) reserves [1]. Within the liver, more than 80% of this retinoid is stored as retinyl ester in fat-storing cells (also called lipocytes, Ito cells and stellate cells) [2-5]. This small and relatively unabundant non-parenchymal cell, which accounts for about 1% of hepatic protein and less than 10% of the total cells present in the liver, is dynamically involved in hepatic retinoid storage and metabolism (see [6] for recent review). Fat-storing cells have been shown to be greatly enriched in the important retinoid-related parameters of cellular retinol-binding protein (CRBP), cellular retinoic acid-binding protein (CRABP), and retinyl ester hydrolase (REH), in addition to containing very high levels of retinyl esters [3,4].

The parenchymal cells are also very important in hepatic retinoid metabolism. These cells, which take up dietary retinoid in the form of retinyl esters in chylomicron remnants [7], contain relatively low levels of retinyl esters but substantial levels of CRBP and REH [3,4] and are the hepatic site for the synthesis of the serum transport protein for retinol, retinol-binding protein (RBP) [8]. Although much information regar-

ding hepatic retinoid metabolism has become available in recent years, many questions regarding this topic remain to be answered. Amongst these, is the question of the metabolic origin of the retinyl esters found in the parenchymal and fat-storing cells.

Since the first description of the enzyme catalyzed acyl CoA-independent synthesis of retinyl ester by Ong et al. [9] in 1987, evidence has accumulated that an acyl CoA-independent enzymatic activity is the physiologically important process involved in retinol esterification [9-16]. This enzymatic activity has been shown to utilize phosphatidyl choline as the acyl donor for retinol esterification and has been termed lecithin-retinol acyltransferase (LRAT) by MacDonald and Ong [11]. At present the LRAT activity has been shown to be the physiologically important activity for retinol esterification in rat small intestine [9], rat liver [11-13], human liver [11], bovine eye [10,14], and rat testis [15,16].

The studies described in this manuscript aim to explore the distribution of hepatic LRAT activity in the different types of liver cells. We now report information concerning the question of whether the large quantities of retinyl ester present in fat-storing cells can arise from the actions of LRAT activity present in these cells. We also explore the question of whether the relatively low levels of retinyl esters present in parenchymal cells arise solely from the uptake of chylomicron remnant retinyl ester or, alternatively, through the enzymatic esterification of retinol within the parenchymal cells.

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2. MATERIALS AND METHODS

Hepatic cells were isolated from 12 month-old female BN/BiRij rats. For parenchymal cell isolations, the liver was digested with collagenase and the parenchymal cells were purified by centrifugal elutriation as described previously [2,3]. The parenchymal cell preparations were greater than 99% pure as judged by light microscopy. Hepatic fat-storing cells were isolated using the pronase E digestion-Nycodenz centrifugation procedure described by Hendriks et al. [2]. Each of the fat-storing cell preparations used in this study was greater than 80% pure as judged by light microscopy and contained to detectable parenchymal cell contamination (the remaining cells present in these preparations were primarily hepatic Kupffer and endothelial cells). The preparations of Kupffer + endothelial cells were prepared as described by Hendriks et al. [2] and contained approximately 10–15% contamination with fat-storing cells.

Microsomes were obtained from whole livers of 12 month-old BN/BiRij rats according to the procedure described by Ong et al. [12]. The microsomal pellet derived from this procedure was resuspended, using a Dounce homogenizer, in 0.2 M potassium phosphate, pH 7.2, containing 1 mM DTT to a final microsomal protein concentration of 10 mg/ml. Microsomes were prepared from purified parenchymal, fat-storing, and Kupffer + endothelial cells by first resuspending a cell pellet in 5 ml (for the parenchymal cells) or 2 ml (for fat-storing and Kupffer + endothelial cells) of 0.2 M potassium phosphate, pH 7.2, containing 1 mM DTT followed by homogenizing with 20 strokes of a tight-fitting Dounce homogenizer. Microsomes were isolated from these cell homogenates using exactly the same procedures employed for the whole liver homogenates [12]. All microsomal preparations were either used for LRAT assay or were frozen in 100 μl aliquots at $-70°C$ prior to assay. LRAT activity remained stable for at least 1 month under these storage conditions.

Fat-storing cell lipid droplets were isolated from purified fat-storing cells as described by Yamada et al. [8].

The standard LRAT assay incubation mixture consisted of 10 μM retinol bound to purified CRBP at 30 μg microsomal protein in an assay buffer consisting of 0.2 M potassium phosphate, pH 7.2, and 1 mM DTT. The final assay volume was 0.2 ml and all incubations were carried out for 5 min at 37°C in a darkened room. All LRAT assays were carried out under appropriate conditions (time, temperature, substrate concentrations, and protein concentration) to ensure that linear enzyme reaction rates were measured. This assay procedure is identical to the LRAT assay described by Ong et al. [12] except for the retinol-CRBP concentration (10 μM in our study as opposed to 5 μM as used by Ong et al. [12]). Our basis for choosing this higher retinol-CRBP concentration is given below in sections 3 and 4. The retinyl esters produced in the assay were extracted into hexane and separated from the substrate retinol, either by chromatography on alumina [10] or by reverse phase HPLC [17]. These alternative separation procedures gave identical measures of LRAT activity.

3. RESULTS

Our initial experiments were designed to set up and characterize the assay for LRAT in whole rat liver microsome. The assay we employed was originally described by Ong et al. [12] and later by Yost et al. [13]. It was our intent, before characterizing the distribution of LRAT in different kinds of isolated liver cells, to verify that we were indeed observing the same LRAT activity in rat liver microsomes that has been reported by these investigators [12,13]. Like these authors, we found that retinol bound to CRBP was the preferred substrate for LRAT in liver microsomes. The rate of retinyl ester formation was approximately 5-fold larger when 20 μM retinol was added as a retinol-CRBP com-

plex to the assay mixture containing the liver microsomes, as opposed to adding (at the same concentration) either retinol bound to β-lactoglobulin (a protein which binds retinol and may be important for the intestinal uptake of dietary retinol [18]) or retinol solubilized in a small volume of ethanol. In our hands, the liver microsomal LRAT activity showed properties, with regards to microsomal protein-, pH, and time-dependence, similar to those reported by Ong et al. [12] and Yost et al. [13]. Our studies explored the dependence of the rate of retinyl ester formation on retinol-CRBP concentration over a wider range of substrate (retinol-CRBP) concentrations than either of these two earlier studies [12,13]. Fig. 1 shows the results from our studies where retinol-CRBP concentration was varied from 0.5 μM to 20 μM. From Fig. 1, Panel A, it can be seen, that at retinol-CRBP concentrations of 10 μM, the rate of reaction appears to be only starting to approach saturation and saturation seems nearly reached at 20 μM. Fig. 1, Panel B, shows a plot of the Lineweaver-Burk transformation of the data presented in Panel A. The calculated K_m of the LRAT activity for
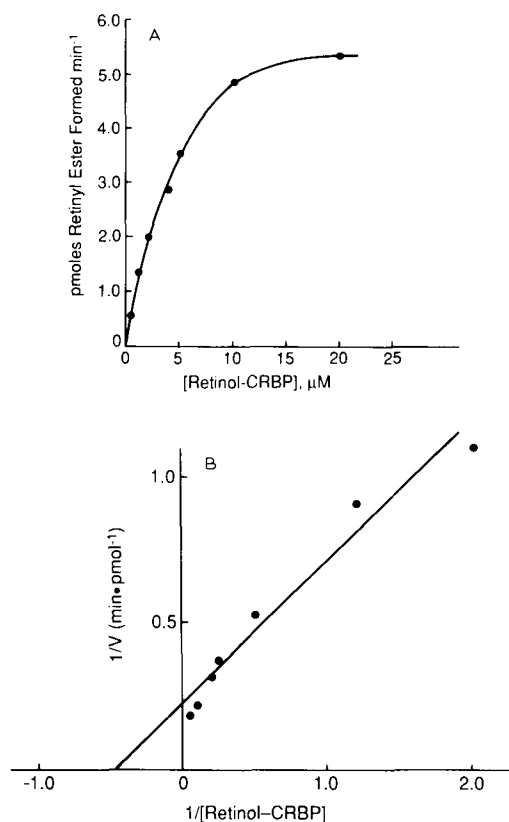

Fig. 1 Dependence of the rate of retinyl ester formation on retinol-CRBP concentration in the LRAT incubation mixture. Panel A shows the relationship between the rate of retinyl ester formation by 30 μg of liver microsomes and retinol-CRBP concentration. Panel B presents a Lineweaver-Burk plot of the data presented in Panel A. All assays were carried out under conditions which provided linear reaction rates for 5 min at 37°C. Each plotted value represents the average of at least 3 independent assays.

Table I

Apparent LRAT activity levels in isolated liver cell microsomes and fat-storing cell lipid droplets

Fractions	(n)	Activity[a]
Whole liver microsomes	4	163 ± 22
Parenchymal cell microsomes	7	158 ± 53
Fat-storing cell microsomes	7	383 ± 54
Fat-storing cell lipid droplets	4	124 ± 50
Kupffer + endothelial cell microsomes	3	134 ± 21

[a]All LRAT assays were carried out under appropriate conditions (time, temperature, substrate concentrations, and protein concentration) to ensure that linear enzyme reaction rates were measured. LRAT activities are expressed as pmol of retinyl ester formed/min/mg protein and are given as mean ± 1 SD

retinol-CRBP is 2.2 μM and for this set of experiments a V_{max} of 144 pmol retinyl ester formed min^{-1} · mg^{-1} was obtained. These values are very similar to those reported by Ong et al. [12] and Yost et al. [13]. In addition, as these authors [12,13] have reported for the LRAT activity present in whole liver microsomes, retinyl palmitate, retinyl stearate, and retinyl oleate account for greater than 95% of the retinyl esters produced by all of the cellular microsomal preparations examined.

The overall goal of our studies was to determine the levels of LRAT activity present in different types of rat liver cells. We have measured LRAT activity levels in whole rat liver, parenchymal cell, fat-storing cell, and Kupffer + endothelial cell microsomes and in isolated fat-storing cell lipid droplets. The LRAT activity levels for each of these preparations is given in Table I. The fat-storing cell microsomes were found to possess the highest specific activities of LRAT of any of the liver cell fractions examined. Whole liver microsomes and parenchymal cell microsomes displayed approximately the same levels of LRAT activity but these levels were only about 42% of the LRAT activity level measured in fat-storing cell microsomes. By considering the estimated number of parenchymal cells, fat-storing cells, and Kupffer + endothelial cells present in one gram of liver and the recoveries of microsomal protein from known numbers of each cell preparation, it is possible to estimate the relative pools of hepatic LRAT present in each of these cell fractions. Such estimates indicate that approximately 85% of hepatic LRAT is present in parenchymal cells and approximately 10% in fat-storing cells. This distribution is quite similar to the cellular distribution of CRBP in the different cell types of the rat liver [3].

4. DISCUSSION

LRAT has now been demonstrated by a number of laboratories to be the physiologically important enzyme involved in retinol esterification in liver [11-13], small intestine [9], testis [15,16], and the eye [10,14]. Although it is known that the majority of the body's retinoid reserves are stored as retinyl ester in hepatic fat-storing cells [3-6], nothing is known about the cellular distribution of LRAT in the liver. We set out to ask if LRAT activity is present solely in the fat-storing cells of the liver or, alternatively, if it is also present within other cell types of the liver. It is well documented that hepatic parenchymal cells contain low levels of retinyl esters [3,4] and hence, it might be postulated to possess LRAT. However, these cells receive dietary retinoid in the form of retinyl ester in chylomicron remnants and although it is well established that the bulk of this retinyl ester is hydrolyzed before it is transported to the fat-storing cells for storage as retinyl ester [19], some of this chylomicron remnant retinyl ester may remain unhydrolyzed in the parenchymal cells. Thus, it is unclear if the retinyl ester present in parenchymal cells is of dietary origin or if it results from the action of LRAT activity within parenchymal cells. Our findings, presented in Table I, clearly demonstrate that the fat-storing cell microsomes are especially enriched in LRAT activity, and in addition, the parenchymal cells also contain a substantial level of LRAT activity. This indicates that parenchymal cells, along with the fat-storing cells, possess the enzymatic ability to esterify retinol and suggests that at least some of the retinyl ester present in these cells arises from esterification of retinol within these cells.

Interestingly, LRAT activity was not enriched in the isolated fat-storing cell lipid droplet preparations. These lipid droplets have been shown to be the site of retinyl ester storage in fat-storing cells [8, 20-22]. The lipid droplets also have associated with them substantial levels of REH [21], which is thought to be necessary for the hydrolysis of retinyl esters to retinol during the mobilization of retinol from the liver. The relative absence of LRAT activity in the lipid droplet fraction (as compared to the fat-storing cell microsomes) suggests that retinyl ester is not synthesized directly at its site of storage in the lipid droplets but rather, is made at some cellular site which is not directly associated with the lipid droplets.

The Kupffer + endothelial cell microsomes also were found to contain some LRAT activity. Although the Kupffer + endothelial cell fractions did contain some contamination with fat-storing cells, the level of LRAT activity in the fraction can not be fully accounted for by the fat-storing cell contamination. The level of fat-storing cell contamination, as determined by light microscopy, for each of the 3 Kupffer + endothelial cell fractions employed in this study was always less than 15% of the total cells present in the preparation. Thus, it would seem likely that either the hepatic Kupffer or endothelial cells (or perhaps both) possess some LRAT activity.

Our study also raises an interesting technical question

which should be considered in future studies of hepatic LRAT activity. While carrying out our characterizations of the LRAT assay described by Ong et al. [12], we observed that retinol-CRBP concentrations as large as 20 μM were only beginning to be rate saturating for retinyl ester formation. Ong et al. [12] standardly used 5 μM retinol-CRBP for their assays and these investigators provided no information indicating if they observed full substrate saturation at this concentration. Similarly, Yost et al. [13], employed 5 μM retinol-CRBP in their standard assay, but these authors also failed to indicate whether this substrate concentration was rate saturating. Our observations indicate that the measured rate of the LRAT reaction is not independent of substrate concentration at concentrations of retinol-CRBP below 20 μM (see Fig. 1). Thus, if 5 μM retinol-CRBP is standardly used as an assay substrate concentration, extreme care must be taken in making the measurement of this concentration, in order to eliminate the possibility that different reaction rates might arise from small differences in substrate concentrations. The use of more nearly saturating concentrations of retinol-CRBP ($\geq$ 20 μM) however, presents a practical dilemma regarding the amount of purified retinol-CRBP (which is very time consuming and difficult to purify) to be used in each assay. For our studies, we have chosen to use 10 μM retinol-CRBP as our standard substrate concentration. We feel that this concentration represents a good compromise between wanting to measure assay rates which are independent of substrate concentration and the need to conserve precious retinol-CRBP. For future more sophisticated studies of hepatic LRAT activity, we suggest that careful consideration be given to the choice of the assay retinol-CRBP concentration. Such consideration will do much to render appropriate comparisons of data obtained by different laboratories investigating hepatic retinoid storage.

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