Subcellular Localization of Acyl Coenzyme A: Dihydroxyacetone-Phosphate Acyltransferase in Rat Liver Peroxisomes (Microbodies)*

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Upon differential centrifugation of rat liver homogenate, the enzyme acyl-CoA:dihydroxyacetone-phosphate acyltransferase (EC 2.3.1.42) was found to be localized in the light mitochondrial (L) fraction which is enriched with lysosomes and peroxisomes. Peroxisomes were separated from lysosomes in a density gradient centrifugation using rats which were injected with Triton WR 1339. By comparing the enzyme distribution with the distribution of various marker enzymes, it was concluded that dihydroxyacetone phosphate acyltransferase is primarily localized in rat liver peroxisomes (microbodies). Similarly, the enzyme acyl dihydroxyacetone-phosphate: NADPH oxidoreductase (EC 1.1.1.101) was shown to be enriched in the peroxisomal fraction, although a portion of this reductase is also present in the microsomal fraction.

Acyl dihydroxyacetone phosphate has been shown to be an important intermediate in the biosynthesis of glycerolipids containing ester and ether bonds (1, 2). This lipid was first characterized as a rapidly labeled lipid formed in guinea pig liver mitochondria from $^{14}P$, or $^{14}P$-ATP (3, 4). Later, it was shown that acyl dihydroxyacetone-P is biosynthesized in different organs of guinea pig and rat by the enzymatic acylation of dihydroxyacetone-P with acyl-CoA (5–8). The subcellular distribution of the enzyme was somewhat unusual in the sense that it was found to be localized in both mitochondrial and microsomal fractions, depending on the tissue, with different relative activities in these organelles (6, 8). However, a thorough quantitative study of the subcellular distribution of this enzyme was not performed before. Recently, a careful examination of the subcellular localization of this enzyme in guinea pig liver showed that the enzyme was localized neither in mitochondria nor in microsomes, but in an intermediate fraction which contains lysosomes and peroxisomes (light mitochondrial or “L” fraction) (9). By comparing its distribution with the distribution of various marker enzymes, it was concluded that this acyltransferase is probably localized in peroxisomes rather than in lysosomes. During the course of this work, the distribution of the enzyme which catalyzes the reduction of acyl dihydroxyacetone-P and alkyl dihydroxyacetone-P to dihydroxyacetone-P: NADPH oxidoreductase (EC 1.1.1.101) to the corresponding glycerophosphate derivatives was studied and it was found that this reductase is also concentrated in the peroxisomal fraction. These results are reported here.

MATERIALS AND METHODS

Palmityl-CoA and β-glycerophosphate were obtained from Sigma Chemical Co. (St. Louis, MO). Triton WR 1339 came from Ruggers Chemical Co. (Irvington, NJ), and bovine serum albumin (Fraction V, fatty acid-poor) is a product of Miles Laboratories (Elkhart, IN). $^{3}H$-NADPH, dihydroxyacetone-3-P, and glycerol-3-P were prepared as described previously (6, 8, 12). All other materials have been described previously (6, 8).

Experimental female Sprague-Dawley rats (350 g) were injected intraperitoneally with 1 ml of 20% (w/v) Triton WR 1339 in 0.9% NaCl solution as described by Baudhuin (12) and Leighton et al. (11). They were fed ad libitum for 5 days, and killed by decapitation. The control rat liver and Triton WR 1339-treated rat liver were homogenized in 0.25 M sucrose containing 0.1% ethanol (11). Subcellular fractions were obtained by differential centrifugation using essentially the same technique as described by de Duve et al. (14), as slightly modified by Jones and Hajra (9). The Bio-Rad Gradient-Former (model 230) was used to generate a linear sucrose-0.1% ethanol density gradient ranging from 1.04 to 1.25 g/ml as described by Baudhuin (12). The light mitochondrial fraction, obtained by differential centrifugation (5, 11), was layered on top of the gradient, which was centrifuged in a Spinco SW 40 rotor at 30,000 rpm (112,000 X g) for 2.5 h (12). Successive 1-ml fractions were collected by puncturing the bottom of the tubes with a gradient collecting device (ISCO model 184). The density of each fraction was measured using a refractometer (Fisher Scientific Co.), and fractions of equal density from different gradient tubes were pooled. All were stored frozen at –20°C and thawed only once before assaying.

Urate oxidase, a marker for liver peroxisomes, was assayed using the method of Leighton et al. (11), and catalase was determined either by the method of Luck (15) or Peters et al. (16). Acid phosphatase for lysosomes was assayed by measuring the release of $^{3}H$ from β-glycerophosphate at pH 5 (acetate buffer) (12, 17). Succinate-cytochrome c reductase for mitochondria (18), NADPH-cytochrome c reductase (19) and glucose-6-phosphatase for microsomes (20), and lactate dehydrogenase for cytosol (21) were assayed in each gradient and
subcellular fraction. Protein was determined using the method of Lowry et al. (22). Dihydroxyacetone-P acyltransferase at pH 5.5, glycerol-3-P acyltransferase, and acyl dihydroxyacetone-P:NADPH oxidoreductase were assayed as described previously (8).

RESULTS AND DISCUSSION

Fig. 1 shows the relative specific activities of different enzymes in various subcellular fractions of normal rat liver. Relative specific activity (the specific activity in the fraction compared to that in whole homogenate) is plotted against per cent protein as described by de Duve et al. (14). Fig. 1 shows the results from an experiment which is typical of six experiments performed in our laboratory. Some quantitative variations were observed from one fractionation to another; however, the enzyme distribution pattern in all these experiments is the same as shown in Fig. 1. The recoveries of various marker enzymes varied between 90 and 105%, similar to what has been described by other workers (12, 23). The recovery of dihydroxyacetone-P acyltransferase was 89 to 111% (mean 98 ± 10 S.D., n = 4); recovery of glycerol-3-P acyltransferase was 73 to 103% (89 ± 11) and acyl dihydroxyacetone-P reductase recovery was 89 to 109% (96 ± 9).

As was found in guinea pig liver (9), the dihydroxyacetone-P acyltransferase in normal rat liver is seen to be mainly localized in the light mitochondrial or "L" fraction which contains lysosomes and peroxisomes (microbodies) (Fig. 1). Comparison with the marker enzymes shows that this acyltransferase follows closely the distribution pattern of peroxisomal enzymes (urate oxidase and catalase) and lysosomal enzyme (acid phosphatase). From these results alone, however, it is not possible to determine whether the dihydroxyacetone-P acyltransferase is localized in peroxisomes or lysosomes. To show unequivocally that dihydroxyacetone-P acyltransferase is mainly localized in peroxisomes and not in lysosomes, it was necessary to separate these two organelles from one another. Rats were injected with Triton WR 1339 as described above, and the subcellular fractions were isolated using the same method as was employed for the normal rat liver. The distribution profiles of the marker enzymes in these fractions from Triton WR 1339-treated rats (Fig. 2) are very similar to those of normal rats (Fig. 1). Contrary to the report of Ishii et al. (24), the specific activity of the peroxisomal marker enzymes, i.e. catalase and urate oxidase, in the Triton WR 1339-treated rats did not significantly decrease (or increase) compared to that of normal rats. This was probably because much less Triton WR 1339 was injected here (55 to 60 mg/100-g rats body weight) than was injected by Ishii et al. (200 mg/100-g rat). A small increase in the dihydroxyacetone-P acyltransferase activity (10 to 30%) was observed in the Triton WR 1339-treated rats. However, it is not clear at present whether this increase in activity is statistically significant, since a large variation (+15%) in the specific activity of this enzyme in normal rat liver was also observed.

The "L" fraction of the Triton WR 1339-treated rat liver is subjected to further fractionation by density gradient centrifugation, and the dihydroxyacetone-P acyltransferase activity is found to sediment with catalase and urate oxidase at the bottom of the gradient (density, 1.23 g/ml) rather than with the acid phosphatase which is present in the lower density fractions near the top of the gradient (density, 1.1 g/ml) (Fig. 3). Dihydroxyacetone-P acyltransferase follows closely the distributions of catalase and urate oxidase except in one fraction at the very bottom of the gradient (Fig. 3). In this particular fraction, the relative specific activity of these per-
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FIG. 2. Subcellular distribution pattern of different enzymes in the liver of rat which has been injected with Triton WR 1339. The data are presented the same way as described in the legend of Fig. 1. The specific activities of the enzymes (activity/mg of protein) in the homogenate are as follows: A, urate oxidase, 40.2 nmol/min; B, dihydroxyacetone-P acyltransferase, 0.84 nmol/min; C, catalase, 396 units; D, acid phosphatase, 29 nmol/min; E, glycero-phosphate acyltransferase, 1.56 nmol/min; F, NADPH-cytochrome c reductase, 0.71 nmol/min; G, succinate-cytochrome c reductase, 1.17 units; H, acyl dihydroxyacetone-P reductase, 2.22 nmol/min; I, glucose-6-phosphatase, 21.5 nmol/min.

FIG. 3. Distribution of different enzymes present in the light mitochondrial or "L" fraction (3.3 × 10⁴ × g-min to 2.5 × 10⁶ × g-min residue) of Triton WR 1339 injected rat liver on centrifugation in a linear sucrose density gradient (see text for details). Successive fractions from the bottom of the tube were collected (from left to right) and the density of each fraction determined (upper right). Different enzyme activities and protein content (milligrams/ml; lower right) are determined as described in the text. The relative specific activity (compared to that in the original "L" fraction) of each enzyme is plotted against the cumulative per cent protein to emphasize the enrichment of the enzymes in different fractions (11, 12, 14). The specific activity of dihydroxyacetone-P acyltransferase and acyl dihydroxyacetone-P oxidoreductase in the starting "L" fraction were 3.46 and 5.46 nmol/min/mg of protein, respectively.

The relative absence of glycerol-3-P acyltransferase in the density gradient fractions enriched with mitochondrial marker enzyme (succinate-cytochrome c reductase) was somewhat surprising because on differential centrifugation this acyltransferase is found to be mostly enriched in the mitochondrial fraction (Fig. 1). It is possible that the "L" fraction of Triton WR 1339-treated rats contained mainly mitochondria with stripped off outer membranes (mitoplasts) as seen morphologically by Leighton et al. (11). Mitochondrial glycerol-3-P acyltransferase is localized on the outer membrane (26).
The acyl dihydroxyacetone-P:NADPH oxidoreductase activity is seen to be associated with the “L” fraction (Figs. 1 and 2) and with the peroxisomes (Fig. 3), similar to what was found for dihydroxyacetone-P acyltransferase. However, a considerable amount of activity also appears to be present in the microsomal fraction. Previous work has shown that this enzyme has considerable activity in the microsomal fraction of rat liver (13). The maximum enrichment of this enzyme in the peroxisomal fraction (Figs. 1 and 3) is 6- to 10-fold which is lower than that of dihydroxyacetone-P acyltransferase in peroxisomes (20- to 30-fold). However, because the specific activity of the reductase in the homogenate is at least 3 to 4 times higher than the acyltransferase, the actual activity of acyl dihydroxyacetone-P reductase in the peroxisomal fraction is more than the dihydroxyacetone-P acyltransferase.

The results presented here indicate that most of the rat liver dihydroxyacetone-P acyltransferase and a part of acyl dihydroxyacetone-P:NADPH oxidoreductase are localized in peroxisomes (microbodies). Previously, these enzymes were believed to be present mainly in liver mitochondria (5, 6) because a higher speed of centrifugation (80,000 × g-min instead of 33,000 × g-min used here) was used to isolate the mitochondria with the result that the mitochondrial fraction was heavily contaminated with peroxisomes. However, by the use of a more discriminating differential centrifugation method and by the inclusion of density gradient separation when marker enzymes for other organelles are assayed, it is seen that these enzymes are not associated with mitochondria, but with peroxisomes. Preliminary results also show that in guinea pig liver, another enzyme of the acyl dihydroxyacetone-P pathway, i.e. alkyl dihydroxyacetone-P synthase, which was previously believed to be localized in mitochondria, is also concentrated in peroxisomes (27).

Previous reports have stated that the enzymes of the acyl dihydroxyacetone-P pathway are localized in mitochondria in kidney and liver and in the microsomal fraction of brain, heart, testis, and adipose tissue (5-8). The results presented here offer a probable explanation of these anomalous distribution patterns in different organs if it is assumed that these enzymes are present in the peroxisomes (microbodies). In liver and kidney, the peroxisomes are larger (0.3 to 0.9 µm diameter), and on conventional differential centrifugation, are sedimented down with mitochondria (28). However, in other organs the microbodies or microperoxisomes (catalase containing particles) are smaller (0.15 to 0.25 µm), and upon differential centrifugation sediment mainly with the microsomal fraction (29-32). Preliminary results from this laboratory indicate that subfractionation of the rat brain microsomal fraction, the acyl dihydroxyacetone-P pathway enzymes are associated with catalase (microperoxisomal marker enzyme) containing particles rather than with NADPH-cytochrome c reductase (endoplasmic reticulum marker enzyme) containing particles (33).

The presence of acyl dihydroxyacetone-P pathway enzymes for glycerolipid metabolism in peroxisomes raises some intriguing questions about the role of peroxisomes in cellular lipid metabolism. Though the role of peroxisomes in cells is not clear, it is generally believed that these organelles are associated with cellular catabolic activity because of the presence of a number of oxidative enzymes which generate and utilize H₂O₂ (34, 35). This report is probably the first example of the presence of biosynthetic enzymes in animal peroxisomes, although anabolic enzymes are known to be present in plant peroxisomes (glyoxysomes) (34, 35).

In recent years, some lipid-metabolizing enzymes such as long chain carnitine acyltransferase (36) and enzymes for β-oxidation of fatty acids (37) have been described in liver peroxisomes. From the histological observation of a close association of lipid droplets with peroxisomes, Novikoff and co-workers (29, 38) have postulated a relationship between lipid metabolism and peroxisomes. Peroxisomes have been implicated in lipid metabolism from a variety of other lines of evidence (34). The strongest evidence for this association of peroxisomes with cellular lipid metabolism is the observation that a number of hypolipidemic drugs cause a proliferation of peroxisomes in liver and other tissues (39, 40).

The results presented here point towards a specific role of peroxisomes in lipid metabolism which is associated with the acyl dihydroxyacetone-P pathway. Peroxisomes have also been shown to contain enzymes which will generate dihydroxyacetone-P (glycerophosphate dehydrogenase) (41) and NADPH (isocitrate dehydrogenase) (11), the precursors of the lipid formed via the acyl dihydroxyacetone-P pathway. Though it is fairly well established now that all the glycerol-ether lipids and a part of triglyceride and phosphoglycerides are formed via the acyl dihydroxyacetone-P pathway compared to that of the glycerol-3-P pathway, it is still somewhat controversial. It seems now that the enzymes of these two pathways are segregated in two different cellular compartments which may have different roles in glycerolipid metabolism. It is possible that other enzymes of glycerolipid metabolism are also present in the peroxisomes. Further studies on the role of peroxisomes in cellular metabolism will probably clarify the role of the acyl dihydroxyacetone-P pathway in overall glycerolipid metabolism.

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