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 different 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 \(^{32}P\)-P, or \(^{14}C\)-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 (microbodies) rather than in lysosomes (9). However, the possibility of the presence of this enzyme in lysosomes was not completely ruled out, especially when it was also found that, in the membrane-bound form, the enzyme had a low pH optimum (pH 5.5) (8, 9). To establish the presence of dihydroxyacetone-P acyltransferase in liver peroxisomes (microbodies), it proved necessary to separate lysosomes from peroxisomes. These two organelles are normally separable from each other by differential or gradient centrifugation due to similarities in size and density. However, Trouet (10) and Leighton et al. (11) have developed a method which changes the density of liver lysosomes by injecting Triton WR 1339 into rats. This detergent is slowly taken up by liver lysosomes with a resulting decrease in density so that on density gradient centrifugation those lysosomes (sometimes termed as tritosomes) can be separated from peroxisomes (12). In the present work, this technique was employed to prepare peroxisomes free of lysosomes, and it was found that in rat liver the enzyme acyl-CoA:dihydroxyacetone-P acyltransferase (EC 2.3.1.42) is primarily 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 (acyl/alkyl 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 \(\beta\)-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). \(^{14}C\)-NADPH, dihydroxyacetone-\(^{3}P\), and glycerol-3-\(^{14}C\) 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 livers were homogenized in 0.25 M sucrose containing 0.1% ethanol (11). Subcellular fractions were obtained by differential centrifugation using essentially the method 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 in density from 1.04 to 1.25 g/ml as described by Baudhuin (12). The light mitochondrial fraction, obtained by differential centrifugation (9, 11), was layered on top of the gradient, which was centrifuged in a Spinco SW 40 rotor at 30,000 rpm (112,000 \(\times\) 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 230). 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 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 (13) or Peters et al. (16). Acid phosphatase for lysosomes was assayed by measuring the release of P\(_\text{i}\) from \(\beta\)-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

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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 noted in one subfraction 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 106%, 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 peroxisomal enzymes is about 1.5 times that of the corresponding enzyme in the homogenate.

![Diagram](https://via.placeholder.com/150)

**Fig. 1.** Distribution pattern of different enzymes in normal rat liver subcellular fractions. Relative specific activity with respect to that of homogenate is plotted as a function of per cent protein according to de Duve et al. (14). The fractions are represented according to the order in which they are isolated, i.e. from left to right: a, nuclear fraction (0 to 6,000 x g-min); b, mitochondrial fraction (6 x 10^5 x g-min to 3.3 x 10^5 x g-min); c, light mitochondrial or "L" fraction (3.3 x 10^5 x g-min to 2.5 x 10^5 x g-min); d, microsomal fraction (2.5 x 10^5 x g-min to 3 x 10^5 x g-min); e, cytosol (3 x 10^5 x g-min supernatant). The enzymes are assayed as described in the text. The activities of different enzymes/mg of protein (specific activity) in the homogenate are A, urate oxidase, 45 nmol/min; B, dihydroxyacetone-P acyltransferase, 0.62 nmol/min; C, catalase, 386 units; D, acid phosphatase, 26 nmol/min; E, glycerophosphate acyltransferase, 1.5 nmol/min; F, NADPH-cytochrome C reductase, 0.65 nmol/min; G, lactate dehydrogenase, 988 nmol/min; H, acyl dihydroxyacetone-P reductase, 2.42 nmol/min; I, glucose-6-phosphatase, 197.7 nmol/min; J, succinate-cytochrome C reductase, 1.29 units.
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 x 10^4 x g-min to 2.5 x 10^5 x 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 of the 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 enriched 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.

Peroxisomal Localization of Dihydroxyacetone Phosphate Acyltransferase

oxisomal marker enzymes is higher than the relative specific activity of dihydroxyacetone-P acyltransferase. It can also be seen that contaminating mitochondria (succinate-cytochrome c reductase) and microsomes (NADPH-cytochrome c reductase) show distribution patterns which are distinctly different from the dihydroxyacetone-P acyltransferase. These marker enzymes show patterns similar to those described by Leighton et al. (11, 23) who used the same method for isolating peroxisomes.

For purposes of comparison, the activity of a similar enzyme, glycero-3-P-acyl-CoA acyltransferase, was also measured in all the fractions. In Fig. 1, it is seen that glycero-3-P acyltransferase is present in mitochondrial, light mitochondrial (L), and microsomal fractions with relatively higher specific activity in the mitochondrial than in the microsomal fraction. This distribution is similar to that described by Bremer et al. (25). The glycero-3-P acyltransferase activity present in the "L" fraction of the Triton-WR 1339-treated rat liver follows the microsomal NADPH-cytochrome C reductase distribution pattern rather than the peroxisomal catalase or urate oxidase after density gradient centrifugation (Fig. 3). Occasionally, a second small peak of this enzyme is seen which coincides with the mitochondrial succinate-cytochrome C reductase activity peak.

Schlossman and Bell (7) have recently suggested that in rat liver microsomal fraction, dihydroxyacetone-P acyltransferase and glycero-3-P acyltransferase are the same enzyme. The distribution pattern of these enzymes given here shows that these enzymes are distinctly different. However, it may be that the microsomal glycero-3-P acyltransferase is nonspecific, and also accepts dihydroxyacetone-P as substrate, as has been suggested for the rat brain microsomal enzyme (8). We have also found that the properties of peroxisomal dihydroxyacetone-P acyltransferase are quite different from those of glycero-3-P acyltransferase present in the same fraction. (C. L. Jones and A. K. Hajra, manuscript in preparation.)

The relative absence of glycero-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 glycero-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 is seen to be associated with the “L” fraction (Figs. 1 and 2), 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, testes, 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 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 short 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 (glycrophosphate 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 (1, 42–44), the quantitative role of the acyl dihydroxyacetone-P pathway compared to that of the glycerol-3-P pathway 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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