THE SUBCELLULAR DISTRIBUTION OF ACYL CoA:DIHYDROXYACETONE PHOSPHATE ACYL TRANSFERASE IN GUINEA PIG LIVER

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Summary: Upon differential centrifugation, the enzyme acyl CoA:dihydroxyacetone phosphate acyl transferase (EC 2.3.1.42) in guinea pig liver is shown to sediment in a lysosomal-peroxisomal fraction. Comparison of the distribution of the marker enzymes and of DHAP acyl transferase indicates that the acyl transferase is localized in peroxisomes (microbodies).

Acyl dihydroxyacetone phosphate (acyl DHAP[1]) has been shown to be an important intermediate in the biosynthesis of glycerolipids containing ester and ether bonds (1,2). This lipid is formed by a direct acylation of DHAP with a long chain acyl CoA, and this acyl transferase (EC 2.3.1.42) has been shown to be widely distributed in both the mitochondrial and microsomal fractions of different mammalian tissues (2-6). The activity of this enzyme is very high in guinea pig liver, (3) and the acyl transferase has been solubilized and partially purified from this organ (7). During this work the distribution of this acyl transferase in different subcellular fractions of guinea pig liver was rigorously investigated, and it was found that this enzyme was highly enriched in a particulate fraction which contained mainly lysosomes and peroxisomes. These results are presented here.

MATERIALS AND METHODS

Palmityl CoA, DHAP, G-3-P[1], cytochrome C, and D-glucose-6-phosphate were obtained from Sigma (St. Louis, Mo.). Fatty acid poor bovine serum albumin was from Miles Laboratories (Elkhart, Ind.). [^{32}P]DHAP and [^{32}P]G-3-P were prepared by the enzymatic phosphorylation of dihydroxyacetone and glycerol respectively with [γ-^{32}P]ATP as described previously (3,5). The O-nitrophenyl glycoside of N-acetyl-β-D glucosamine was a kind gift of Dr. Otto Sellinger.

[1] Abbreviations used: DHAP - dihydroxyacetone phosphate; G-3-P - sn-glycerol-3-phosphate.

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The subcellular fractionation of guinea pig liver was performed according to de Duve and coworkers (8) with some modifications. Livers from unstarved guinea pigs were homogenized in 3 vol. of 0.25 M sucrose - 1 mM EDTA, and the homogenate was centrifuged (10,000 g-min) to sediment the crude nuclear fraction. This nuclear fraction was rehomogenized twice as above and centrifuged (6,000 g-min) (8). The post-nuclear combined supernatant was centrifuged for 10 min at 8,000 x g to obtain a crude mitochondrial fraction. After washing once (80,000 x g-min) the pellet was suspended in 0.25 M sucrose- 1 mM EDTA and centrifuged at 3,300 x g for 10 min to isolate the mitochondria. The combined supernatant was centrifuged at 25,000 x g for 10 min to isolate the lysosomes and peroxisomes (microbodies), and the microsomes were obtained from the resulting supernatant by further centrifugation (3×10^6 g-min) (8).

Uricase was assayed according to Leighton et al (9), succinate-cytochrome C reductase according to Schnaitman et al (10), N-acetyl-β-D-glucosamindase according to Sellinger et al (11), and glucose-6-phosphatase according to Nordlie et al (12). Acyl CoA:DHAP acyl transferase and acyl CoA:G-3-P acyl transferase activities were determined by measuring the radioactive lipid formed from either [^{32}P]DHAP or [^{32}P]G-3-P and palmityl CoA in the presence of different subcellular fractions. The reaction mixture contained buffer (0.1 M 2-(N-morpholino)ethane sulfonic acid buffer at pH 5.5 or Tris-HCl buffer at pH 7.5), NaF (8.3 mM), palmityl CoA (60 μM), $MgCl_2$ (4.2 mM), bovine serum albumin (1 mg), [^{32}P]DHAP or [^{32}P]G-3-P (0.32 mM, 1-2 x 10^6 cpm), and enzyme (50 to 250 μg protein) in a total volume of 0.6 ml. After incubation at 37° for 15 min, the lipids were extracted by an acidic Bligh and Dyer method (13). The radioactive lipid in the lower layer was washed twice with acidic upper phase to remove the water-soluble radioactive compounds and then an aliquot of the extract was used to determine the radioactivity (14). Other methods and materials were the same as previously described (5,14).

RESULTS

The DHAP acyl transferase activity in guinea pig liver was found only in the particulate fractions, with the highest specific activity in the fraction which sedimented down between mitochondria and microsomes. This is clearly seen in Fig. 1 where the results are plotted as the relative specific activity vs. percent of protein according to de Duve et al (8) to indicate the enrichment of different enzymes in the various subcellular fractions. Marker enzymes, such as succinate-cytochrome C reductase for mitochondria (10), N-acetyl-β-D-glucosaminidase for lysosomes (11), uricase for peroxisomes (9) and D-glucose-6-phosphatase for microsomes (12) were measured to identify and check the purity of each subcellular fraction. The recovery and the relative distribution of these marker enzymes were found to be in good agreement with the results reported by de Duve and coworkers (8). It is seen in Fig. 1 that the DHAP acyl transferase activity at two different pH's[2] is primarily localized in the

[2] The DHAP acyl transferase has a broad pH optima from pH 5 to pH 8.5 with a peak activity at pH 5.5.- A.K. Hajra, C. Burke and C.L. Jones.- Manuscript in preparation.

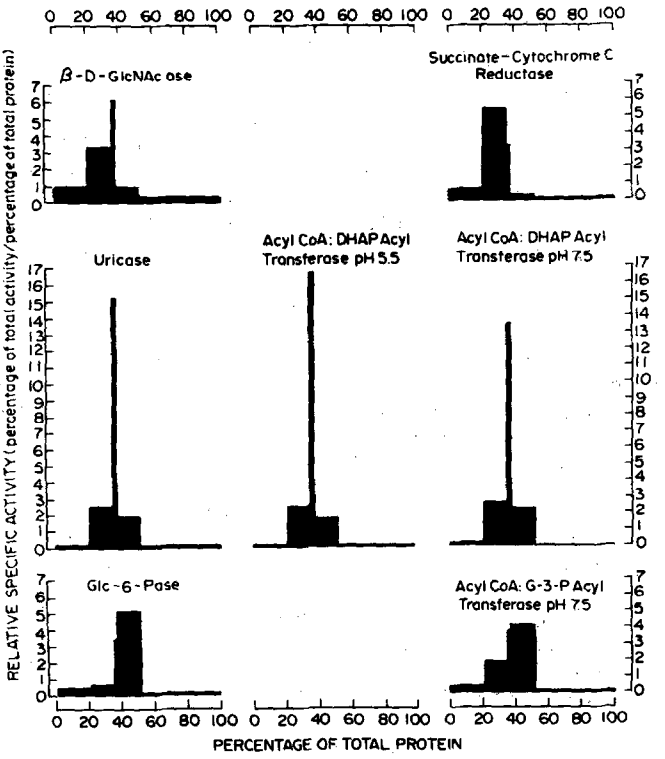

Fig. 1 Distribution pattern of different enzymes in the guinea pig liver upon subcellular fractionation. Abscissa: fractions are represented by their relative protein content in the order in which they are isolated, i.e. from left to right, nuclear fraction (23.4% protein), mitochondrial fraction (12.7% protein), lysosomal-peroxisomal fraction (2.2% protein), Microsomal fraction (12.6% protein) and cytosol (49.1% protein).

particulate fraction which is enriched with lysosomes and peroxisomes. This fraction generally contains between 2 - 2.5% of the total protein but 45-50% of the total DHAP acyl transferase activity. On the other hand, a similar enzyme, the G-3-P acyl transferase (EC 2.3.1.15) activity, was found to have the largest enrichment in the microsomal fraction (Fig. 1).

DISCUSSION

The results show that the enzyme acyl CoA:DHAP acyl transferase is localized neither in the mitochondria nor in the microsomes in the guinea pig liver but mainly in an intermediate fraction. It seems from Fig. 1 that the DHAP acyl-transferase is primarily located in the peroxisomes (microbodies) rather than

in lysosomes because the distribution of the acyl transferase closely follows the distribution of the peroxisomal marker enzyme uricase (9) rather than that of the lysosomal marker N-acetyl-β-D-glucosaminidase (11). Unfortunately, lysosomes and peroxisomes cannot be separated from each other by simple differential centrifugation and further work will be required to prove that DHAP acyl transferase in guinea pig liver is solely localized in peroxisomes. Preliminary work in our laboratory indicates that the DHAP acyl transferase in rat liver has the same distribution profile as that obtained in guinea pig liver. Assays done on the same subcellular fractions show that the G-3-P acyl transferase has the greatest enrichment in microsomes with some activity appearing also in the mitochondrina (Fig. 1).

These results give a probable explanation of the anomalous subcellular distribution pattern observed for the DHAP acyl transferase in different organs (2-6). In liver and kidney where the activity has been shown to be mainly present in mitochondria, the peroxisomes are relatively larger and generally sediment down with mitochondria on conventional fractionation by differential centrifugation (6). In other organs such as in brain, adipose tissues, testis etc., the peroxisomes are relatively smaller ("microperoxisomes") (15) and mostly appear in the microsomal fraction on differential centrifugation (16). In these tissues, DHAP acyl transferase activity has been localized in the microsomes (2-6).

Although the exact function of peroxisomes in tissues is not clear (17), these organelles have been postulated, on the basis of histochemical studies, to play a major role in lipid metabolism (15, 18-21). However, previous to this report, no lipid synthesizing enzyme has been described in peroxisomes. Recent works from our laboratory[3] show that two other enzymes which use acyl DHAP as substrate, i.e. alkyl DHAP synthase (22) and acyl/alkyl DHAP:NADPH oxidoreductase, are also highly enriched in the lysosomal-peroxisomal fraction of guinea pig liver.

[3] P.A. Davis and A.K. Hajra - unpublished results.

By a variety of methods it has been demonstrated that a major fraction of the glycerolipids in different tissues are synthesized via the acyl DHAP pathway (23,24). However, the results are somewhat controversial and one of the main arguments against the significant participation of the acyl DHAP pathway (and in favor of G-3-P pathway) in lipid synthesis has been the fact that the concentration of G-3-P in tissues is much higher than the DHAP concentration (25). But recent work by Tolbert and coworkers indicates that the local concentration of DHAP in peroxisomes may be high because a large fraction of the liver NAD-linked G-3-P dehydrogenase is present in the peroxisomes (26) and that this peroxisomal enzyme mainly catalyzes the oxidation of G-3-P to DHAP (27). Therefore, it seems that the enzymes of acyl DHAP pathway for glycerolipid biosynthesis are localized in the peroxisomes and are segregated from the microsomal G-3-P pathway of glycerolipid biosynthesis. The physiological significance of this compartmentalization is not clear, and future investigations should be directed towards the elucidation of the relationship between peroxisomes and tissue lipid metabolism.

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