ENZYMATIC TRANSFER OF THE ACYL GROUP FROM ACYL DIHYDROYXACETONE PHOSPHATE TO DIFFERENT SUBSTRATES

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Summary: The acyl group of acyl dihydroxyacetone phosphate was shown to be enzymatically transferred in guinea pig liver mitochondria to various acceptors such as lysolecithin, lysophosphatidyl ethanolamine and sn-glycerol-3-phosphate to form lecithin, phosphatidyl ethanolamine and phosphatidate, respectively. Coenzyme A and Mg^{++}, but not ATP, were required for this reaction. A rapid exchange of acyl group between acyl dihydroxyacetone phosphate and dihydroxyacetone phosphate was also observed.

Acyl dihydroxyacetone phosphate (acyl DHAP[1]) which was first discovered in guinea pig liver mitochondria (1) was shown to be biosynthesized by the acylation of DHAP with long chain acyl CoAs (2). This lipid is an important intermediate in the biosynthesis of glycerolipids containing ester and ether bonds (3,4). During investigations on the metabolism of [^{14}C]palmitoyl DHAP to different glycerolipids it was discovered that in the presence of CoA the acyl group of this lipid could be transferred to different substrates in guinea pig liver mitochondria or in other systems. This communication reports the evidence for such a transfer of acyl group from acyl DHAP.

MATERIALS AND METHODS

[^{32}P]DHAP was enzymatically prepared by the phosphorylation of dihydroxyacetone and purified as described previously (5). [1-^{14}C]Palmitoyl DHAP was prepared by incubating [1-^{14}C]palmitate, DHAP, ATP, CoA and Mg^{++} with guinea pig liver mitochondria and the labeled lipid was purified by chromatography (2). Lysolecithin and lysophosphatidyl ethanolamine were obtained from

[1]Abbreviation used: DHAP -dihydroxyacetone phosphate.

Sigma Chemical Co. (St. Louis, Mo.). On thin layer chromatography, lysolecithin was found to be pure but lysophosphatidyl ethanolamine was found to contain a small amount ($\sim$ 20%) of additional phospholipid which migrated with lysophosphatidic acid. Guinea pig liver mitochondria were suspended in 0.25 M sucrose (10 mg protein/ml) and then were disrupted by sonic oscillation (Branson Sonifier, Heat System Co., Great Neck, N. Y.) for four 15-sec periods and then centrifuged at 100,000 x g for 30 min. The particles were then suspended in 0.25 M Sucrose and used for the incubation. This procedure removed most of the water soluble compounds present in the mitochondria. The incubation procedure, extraction of lipid, thin-layer chromatography, radioautography and other methods were the same as described previously (6).

RESULTS AND DISCUSSION

When [1-^{14}C]palmitoyl DHAP was incubated with different substrates and guinea pig liver mitochondria in the presence of CoA, different radioactive lipids were formed depending on the nature of the substrate used. Fig. 1 shows the formation of different radioactive lipids from [1-^{14}C]palmitoyl DHAP. When the radioactive lipid was incubated with mitochondria in the presence of CoA (lane A, Fig. 1) a small amount of labeled fatty acid (R_f = 0.94 and acyl dihydroxyacetone (R_f = 0.87 and 0.84 for monomer and dimer) were seen to be present on the chromatogram in addition to the parent radioactive palmitoyl DHAP (R_f = 0.34). When lysolecithin was present in the same incubation mixture (lane C, Fig. 1) a new radioactive lipid was formed (R_f = 0.18) which had the same migration rate as lecithin (lane J). No such lipid was formed from lysolecithin if CoA was absent from the incubation mixture (lane D). Similarly when lysophosphatidyl ethanolamine was used with CoA in the incubation mixture a radioactive lipid was formed (lane E) which migrated with phosphatidyl ethanolamine (R_f = 0.53). A small amount of another radioactive lipid was also seen which migrated with phosphatidic acid (lane E). This was probably formed from contaminating lysophosphatidic acid

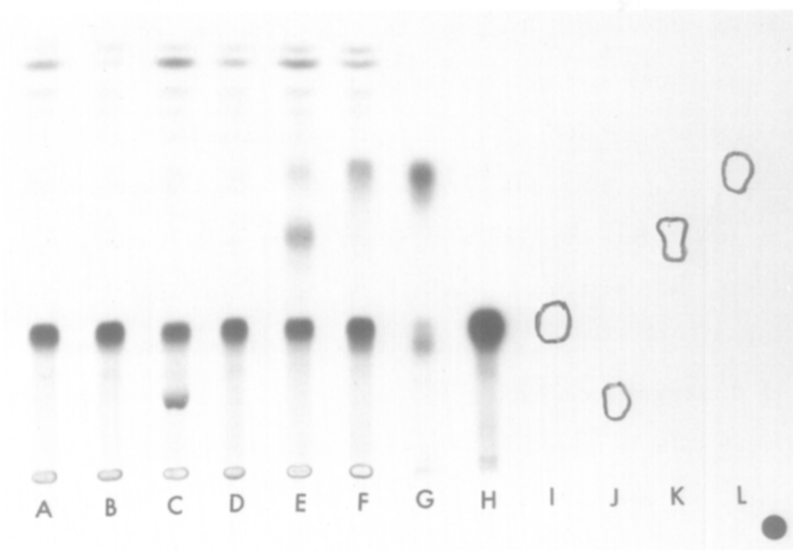

Fig. 1. Radioautogram of lipids extracted from incubation mixture after separation by thin-layer chromatography. All incubations contained the following: Tris-HCl (pH 7.4, 75 mM), NaF (16.6 mM), $MgCl_2$ 3.3 mM), guinea pig liver mitochondrial particles (0.9 mg protein) and CoA (40 µM except in D) in a total volume of 1.2 ml. In addition, respective incubation contained: (A) [1-^{14}C]palmitoyl DHAP (4.7 nmoles, 8.0 x 10^4 cpm). (B) Same as A but heated (10 min, 100°) mitochondria were used. (C) Same as A but in addition lysolecithin (50 µg) was present during the incubation. (D) As in C but no CoA was added. (E) Same as A plus lysophosphatidyl ethanolamine (50 µg). (F) Same as A plus sn glycerol-3-phosphate (5 mM). (G) Palmitoyl DHAP (30 µg) and sn [^{32}P]glycerol-3-phosphate (0.25 mM 8.3 x 10^5 cpm). (H) Palmitoyl DHAP (30 µg) and [^{32}P]DHAP (0.25 mM 1.2 x 10^6 cpm). The mixtures were incubated at 37° for 15 min and the reaction was stopped by adding 4.5 ml $CHCl_3$-methanol (1:2). The lipid was extracted under acidic conditions (5) and an aliquot (20-50%) was applied to a silica gel thin-layer chromatographic plate along with standard lipids (I) Palmitoyl DHAP, (J) Lecithin, (K) Phosphatidyl ethanolamine and (L) Phosphatidic Acid. The plate was developed with $CHCl_3$-methanol-acetic acid-water (100:40:12:4) and the radioautogram of the plate along with the positions of standard lipids are shown here.

in the lysophosphatidyl ethanolamine. The identities of [^{14}C]labeled lecithin and phosphatidyl ethanolamine were verified by running the lipids in two other silica gel G thin-layer chromatographic systems (I. $CHCl_3$-methanol-water 24:7:1 and II. $CHCl_3$-methanol-30% aqueous methylamine 65:25:8). In all the systems the lipid formed from lysolecithin migrated with lecithin and the one formed from lysophosphatidyl ethanolamine migrated with phosphatidyl

ethanolamine. In different experiments 25-35% (1.2-1.8 nmoles) of the labeled acyl DHAP was converted to these phospholipids under the conditions described in Fig. 1.

When glycerol-3-phosphate was present in the incubation mixture (lane F, Fig. 1), [^{14}C]phosphatidate (R_f = 0.68) and small amount of [^{14}C]lysophosphatidate (R_f = 0.29, just below acyl DHAP) were formed from [1-^{14}C] palmitoyl DHAP and CoA. Labeled phosphatidate (and lysophosphatidate) was also formed from sn [^{32}P]glycerol-3-phosphate and non-radioactive palmitoyl DHAP plus CoA (lane G, Fig. 1).

These results show that the acyl group of acyl DHAP is transferred to different acceptors by guinea pig liver mitochondria in the presence of CoA. This transfer of the acyl group from acyl DHAP was clearly observed in the exchange reaction between radioactive DHAP and non-radioactive acyl DHAP. [^{32}P]-labeled acyl DHAP was formed when non-radioactive acyl DHAP was incubated with [^{32}P]DHAP in the presence of guinea pig liver mitochondria and CoA (lane H, Fig. 1). This exchange reaction in guinea pig liver mitochondria between DHAP and palmitoyl DHAP was found to be completely dependent on the presence of CoA (Table I). Magnesium ion stimulated the enzymatic transfer of the acyl group. This was especially seen when EDTA was found to inhibit the reaction (Table I). It should be pointed out here that Mg^{++} is also required for the biosynthesis of acyl DHAP from acyl CoA and DHAP (2). The rate of the exchange reaction (1.3 nmoles/min/mg protein) was about 10 times more than the transfer of acyl group to acceptors other than DHAP.

These results suggest that the biosynthesis of acyl DHAP (2) is reversible and that acyl DHAP can react with CoA to form acyl CoA, the acyl group of which then can be transferred to different acceptors. An investigation of this reverse reaction produced evidence that acyl CoA could be formed from acyl DHAP and CoA in guinea pig liver mitochondria. [1-^{14}C]palmitoyl DHAP was incubated with CoA, Mg^{++} and guinea pig liver mitochondria and the lipid after precipitation with perchloric acid was washed with ether and acetone (7).

Table I

Enzymatic Exchange of Palmitoyl DHAP and DHAP

The whole system contained Tris-HCl buffer (75 mM, pH 7.4), NaF (8.3 mM), $MgCl_2$ (3.3 mM), CoA (40 μM), palmitoyl DHAP (50 μg), [^{32}P]DHAP (0.25 mM, 4 x 10^6 cpm) and guinea pig liver mitochondrial particles (1.05 mg protein) in a total volume of 1.2 ml. When hexokinase and glucose were used the mitochondria in Tris-buffer and Mg^{++} were incubated with hexokinase (0.28 unit) and glucose (8.3 mM) for 10 min at 37° before adding other ingredients. The mixture was incubated at 37° for 15 min and lipids were extracted, washed and the radioactivity of an aliquot was determined.

System	Radioactivity in lipid cpm x 10^{-4}
a) whole system	25.0
b) whole system, minus CoA	1.8
c) whole system, minus palmitoyl DHAP	2.5
d) as c) plus 1-acyl, glycerol-3-P (50 μg)	3.0
e) whole system but precinbuated with hexokinase and glucose	22.0
f) as c) plus palmitic acid (50 μg)	3.5
g) as f) preincubated with hexokinase and glucose	1.5
h) whole system minus Mg^{++}	12.5
i) as h) plus Na_2H_2 EDTA (1.6 mM)	2.0
j) as i) + Mg^{++} (3.3 mM)	21.8

[^{14}C]Acyl CoA was extracted from the remaining residue by water at pH 5 (7) and then identified by thin-layer chromatography (silica gel-oxalate plate, $CHCl_3$-methanol-ammonia-water 90:90:22:8, ref. 8,9) and radioautography. A radioactive spot corresponding to acyl CoA (R_f = 0.3) was present on the chromatogram. One other radioactive compound was present on the chromatogram which migrated with acyl carnitine (R_f = 0.43) probably formed from endogenous precursor. The identity of acyl CoA was further confirmed by its conversion to acyl hydroxamate with hydroxylamine at neutral pH (10). Use of hydroxylamine in the incubation mixture to show the continuous formation of acyl hydroxamate from acyl CoA (10) was not successful because hydroxylamine completely inhibited the transfer reaction by chemically reacting with acyl DHAP to form the oxime. The equilibrium concentration of acyl CoA and acyl DHAP in the incubation mixture could not be determined because of the presence

of thiol ester hydrolase activity and other endogenous substrate in the crude mitochondrial system. The possibility that acyl DHAP is hydrolyzed to form fatty acid which is activated by CoA in the presence of ATP (possibly present in the mitochondrial system) to form acyl CoA was completely ruled out when it was found that the reaction was not inhibited by preincubation with hexokinase and glucose (Table I). Under the identical condition palmitate cannot replace acyl DHAP for the acyl transfer reaction (Table I). The transfer and exchange reactions were also observed in guinea pig liver microsome, rat brain microsome and Ehrlich ascites cell microsomes systems where acyl DHAP is also biosynthesized (2,5).

The physiological significance of this enzymatic reaction is not clear. As mentioned above, the results indicate that the enzymatic formation of acyl DHAP from acyl CoA and DHAP is reversible. This also suggests that the acyl ester bond in acyl DHAP may have a higher free energy of hydrolysis than ordinary ester bonds. Most of the acyl transferase reactions involving the formation of O-ester from long chain acyl thio-esters are not reversible probably because of the large difference in free energy of hydrolysis between them (11). The only notable exception is the formation of acyl carnitine from acyl CoA and carnitine (12,13) and the physiological significance of the reversibility of that reaction is well established (14). There are other similarities between the biosynthesis of acyl carnitine and acyl DHAP. For example, like acyl carnitine, acyl DHAP is biosynthesized in both mitochondria and microsomes and also a rapid CoA dependent exchange reaction between carnitine and acyl carnitine similar to the reaction described above, has already been described (13,15). It is possible that acyl DHAP may act as a carrier of acyl groups between intracellular compartments, where the transport of fatty acids and acyl CoAs is restricted.

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REFERENCES

1. Hajra, A. K. and B. W. Agranoff (1968) J. Biol. Chem., 243, 1617.
2. Hajra, A. K. (1968) J. Biol. Chem., 243, 3458.
3. Hajra, A. K. (1968) Biochem. Biophys. Res. Commun., 33, 929.
4. Hajra, A. K. (1968) Biochem. Biophys. Res. Commun., 39, 1037.
5. LaBelle, E. F., Jr. and A. K. Hajra (1972) J. Biol. Chem., 247, 5835.
6. LaBelle, E. F., Jr. and A. K. Hajra (1972) J. Biol. Chem., 247, 5825.
7. Seubert, W. (1960) Biochem. Prep., 7, 80.
8. Masiarz, F. R., A. K. Hajra, and B. W. Agranoff (1972) Biochem. Biophys. Res. Commun., 46, 992.
9. Ullman, M. D. and N. S. Radin (1972) J. Lipid Res., 13, 422.
10. Kornberg, A. and W. E. Pricer, Jr. (1953) J. Biol. Chem., 204, 329.
11. Mahler, H. R. and E. H. Cordes (1971) Biological Chemistry, 2nd Edition, p. 24, Harper & Row, New York.
12. Fritz, I. B., S. K. Schultz, and P. A. Srere (1963) J. Biol. Chem., 238, 2509.
13. Norum, K. (1964) Biochim. Biophys. Acta, 89, 95.
14. Bressler, R. (1970) in Lipid Metabolism, p. 49, S. J. Wakil, Ed., Academic Press, New York.
15. Bremer, J. (1963) J. Biol. Chem., 238, 2774.