BIOSYNTHESIS OF PHOSPHATIDIC ACID FROM DIHYDROXYACETONE PHOSPHATE

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Summary: Phosphatidic acid is synthesized by a particulate preparation of of guinea pig liver from dihydroxyacetone phosphate, acyl CoAs and NADPH via acyl dihydroxyacetone phosphate. Glycerol-3-phosphate is also converted to phosphatidate in this system. The phosphatidate formed via acyl dihydroxyacetone phosphate has more saturated fatty acid in the 1-position than in the 2-position, while the fatty acid distribution in phosphatidate from glycerophosphate is more random.

Acyl dihydroxyacetone phosphate was recently characterized by us to occur in guinea pig liver (1) and to be formed biosynthetically by acylation of dihydroxyacetone phosphate (DHAP)* with acyl CoA (2). This lipid is then reduced in mitochondria by NADPH to lysophosphatidate (3). Acylation of lysophosphatidate to phosphatidate (PA)* has been demonstrated (4,5). These reactions, taken together, indicate the presence of an alternate pathway for the biosynthesis of PA from DHAP without its prior reduction to GP (6). Though acylation of GP is known to occur (7), the distribution of fatty acids in the 1- and 2-position of the resulting PA does not correspond to the specific distribution of saturated and unsaturated fatty acids in natural glycerides and phosphoglycerides (8,9). In this report, the formation of PA from DHAP is demonstrated and the fatty acid distribution pattern of the resulting lipid is compared to that derived from GP.

Most of the materials and methods were described previously (1,2,3). Lipids from the incubation mixtures were extracted with acidic chloroform-

*Abbreviations used: DHAP - dihydroxyacetone phosphate; PA - phosphatidic acid; GP - glycerol-3-phosphate.

methanol (10). The dried lipids were put on a column (0.5 cm. i.d.) containing 0.5 g of silicic acid (Unisil, Clarkson Chemical Co., Pa.) with chloroform-methanol (99:1). The unconverted radioactive fatty acids and other non-polar lipids were eluted with 15 ml of the above solvent. The radioactive phospholipids were eluted with 15 ml of chloroform-methanol (8:2). This fraction contained acyl DHAP, lysophosphatidate, PA, and cardiolipin. The remaining phospholipids could be eluted by using more polar solvent mixtures, but very little radioactivity was found in these fractions. Thin layer chromatography was performed on commercial pre-coated silica gel plates (E. Merck, Darmstadt, obtained through Brinkmann, N.Y.). Radioactive PA was treated with snake venom phospholipase A (Crotalus adamanteus) in ether with added lecithin as described by Lands and Hart (8). The reaction mixture was stirred vigorously with Teflon coated magnetic bars. After 4 hr of this treatment, the reaction mixture was dried under a flow of nitrogen, put on TLC plates and developed with chloroform-methanol-acetic acid-water (50:20:6:1). The fatty acids, PA and lysophosphatidate were separated well in this solvent. The radioactive spots were located by autoradiography and scraped into counting vials. After adding 0.5 ml of water and 10 ml of scintillation mixture containing toluene-BBS-3 (Beckman) (9:1) and scintillators (10) to the powder, the vials were subjected to sonic oscillation treatment to disperse the particles and then counted.

Fig. 1 shows the biosynthesis of radioactive PA from either 1-^{14}C-palmitate or 1-^{14}C-palmitoyl CoA in the presence of non-radioactive linoleate or its CoA derivative and other cofactors. The identity of the major radioactive lipid as PA was also confirmed by chromatography on paper and other TLC systems (1). The presence of DHAP or GP was essential for the formation of PA in the washed cell fractions. When NADPH was omitted, only acyl DHAP was formed from DHAP, supporting the formation of PA by the pathway postulated above. As expected, PA was formed from GP in the presence or absence of NADPH. Small amounts of lysophosphatidate were always formed along with PA.

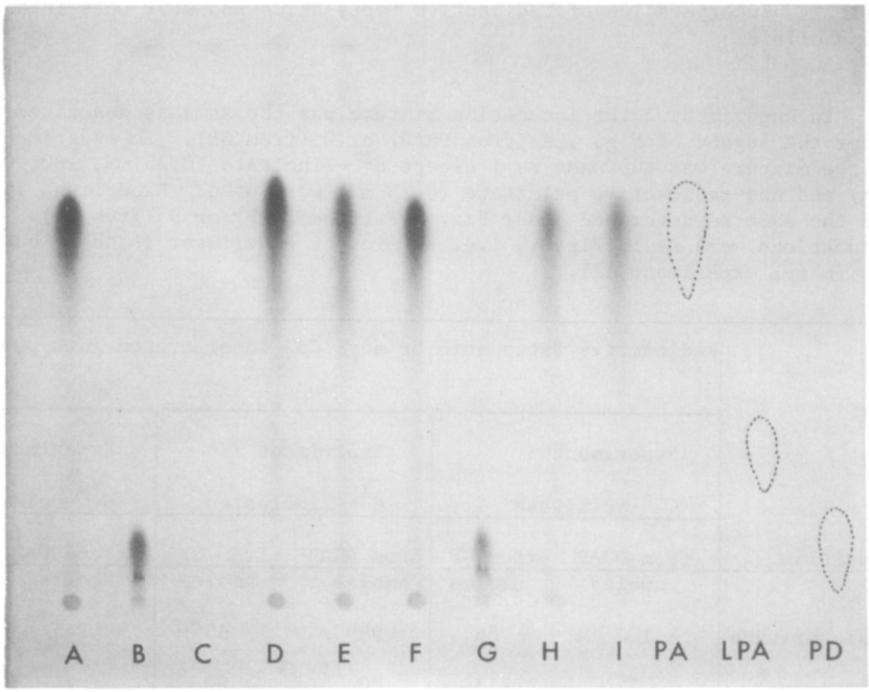

Fig. 1. Autoradiogram of lipids formed from labeled acyl precursors in the presence of DHAP or GP. Fraction 2 (see text) of the lipid extracted from each incubation mixture was applied to TLC plate which was developed with chloroform-methanol-acetic acid-5% aqueous sodium bisulfite (50:20:6:2). Incubations A-E contained K phosphate buffer (30 mM, pH 7.4), ATP (0.7 mM), CoASH (0.035 mM), glutathione (0.35 mM), $MgCl_2$ (0.35 mM), NaF (0.7 mM), 1-^{14}C-K palmitate (0.25 mM, 200,000 cpm), K linoleate (0.25 mM), mitochondria (0.8 mg protein) and microsomes (1.0 mg protein) from 50 mg guinea pig liver, in a total volume of 1.2 ml. The other components were as follows: A, NADPH (0.6 mM) and DHAP (1.8 mM). B, as A minus NADPH. C, as A minus DHAP. D, NADPH (0.6 mM) and GP (DL, 3.6 mM). E, as D minus NADPH. The mixtures were incubated at 37° for 30 min. Incubations F-I contained the same amount of phosphate buffer, NaF, mitochondria and microsomes but instead of the acyl CoA generating system, 1-^{14}C palmitoyl CoA (0.05 mM, 45,000 cpm) and unlabeled linoleoyl CoA (0.05 mM) were added. Other components were as follows: F, NADPH (0.6 mM) and DHAP (1.8 mM). G, as F minus NADPH. H, NADPH (0.6 mM) and GP (DL, 3.6 mM). I, as H minus NADPH. The mixtures were incubated at 37° for 10 min. The position of known lipids are also shown: PA - phosphatidic acid, LPA - lysophosphatidic acid, PD - palmitoyl dihydroxyacetone phosphate.

Table 1 shows the effects of addition or omission of various components on the incorporation of radioactive fatty acids or fatty acyl CoAs into phospholipids. As stated above, the main phospholipid in the

Table 1. Incorporation of radioactive fatty acids and acyl CoAs into phospholipid

In experiment I the incubation mixture was the same as described under the legend of Fig. 1,A (from DHAP) or D (from GP). In experiment II the mixture was the same as I except C^{14}-linoleate (0.25 mM, 180,000 cpm) and non-radioactive palmitate (0.25 mM) were used. Experiment III was the same as described under Fig. 1,F (from DHAP) or H (from GP). Incubations were as in Fig. 1, i.e. 30 min for experiment I and II but 10 min for experiment III.

	Radioactive fatty acid or acyl CoA incorporated into phospholipid					
	Experiment I C^{14}-palmitate		Experiment II C^{14}-linoleate		Experiment III C^{14}-palmitoyl CoA	
Condition	from DHAP	from GP	from DHAP	from GP	from DHAP	from GP
	nmoles	nmoles	nmoles	nmoles	nmoles	nmoles
Whole mixture	102	66	98	65	26	21
- acceptor*	11	11	20	20	2	2
- NADPH	45	63	42	69	14	23
- unlabeled acyl derivative**	210	280	115	120	31	24
- ATP	15	18	18	17	-	-

*DHAP or GP

**linoleate in experiment I, palmitate in experiment II and linoleoyl CoA in experiment III.

incubation mixture was PA (> 80% of the radioactivity) and in the absence of added NADPH, acyl DHAP was the product from DHAP. In the presence of both palmitate and linoleate, the amount of PA formed from DHAP was more than that from GP. The same results were found when GP was used instead of the DL-mixture. However, at a low fatty acid concentration (0.1 mM) or with fatty acyl CoAs, the yields of PA from DHAP and GP were similar. Omission of linoleate from the incubation mixture was found to stimulate the incorporation of ^{14}C-palmitate into PA, even after corrections had been made for

Table 2. Hydrolysis of radioactive phosphatidic acid by snake venom phospholipase A

PA was prepared from radioactive fatty acids (or acyl CoA) by incubation as described in the legend of Fig. 1. NADPH was included when DHAP was the substrate but not with GP. The substrates and acceptors used are indicated below. The lipid fraction, after extraction and chromatography, containing PA was treated with snake venom and the products were separated on TLC and counted (see text). The activity in the lysophosphatidate and fatty acids reported here, were the difference between the enzymatically hydrolyzed sample and control sample. The controls were treated the same way as above but the snake venom was omitted. About 80-90% of the phosphatidic acid was hydrolyzed by the snake venom treatment.

Substrates in incubation mixture			Radioactivity after snake venom treatment	
Radioactive precursor	Non-radioactive precursor	Acceptor	Fatty acid cpm	Lysophosphatidate cpm
Palmitate (0.25 mM)	Linoleate (0.25 mM)	DHAP	3,498	13,118
Palmitate (0.25 mM)	Linoleate (0.25 mM)	GP	4,474	5,630
Linoleate (0.25 mM)	Palmitate (0.25 mM)	DHAP	1,636	892
Linoleate (0.25 mM)	Palmitate (0.25 mM)	GP	418	350
Palmitoyl CoA (0.05 mM)	Linoleoyl CoA (0.05 mM)	DHAP	1,308	6,416
Palmitoyl CoA (0.05 mM)	Linoleoyl CoA (0.05 mM)	GP	2,431	3,357

the replacement of non-radioactive linoleate by radioactive palmitate. This was in part due to a lowering of total fatty acid concentration in the incubation mixture. However, this did not explain completely the stimulation observed. When the amount of linoleate in the incubation mixture was replaced by non-radioactive palmitate, a stimulation of the formation of PA (120%) was observed, but similar inhibition was found when oleate was used instead of linoleate. When the acyl CoA generating system was replaced by the acyl CoAs, no such inhibition by unsaturated acyl CoA was observed (Table 1).

The selectivity of the distribution of fatty acids in the PA biosynthesized from different precursors was studied by hydrolysis with snake venom phospholipase A. Phospholipase A hydrolyzes the ester bond from the 2-positions of different phosphoglycerides (11). Table 2 shows that PA formed from DHAP and ^{14}C-palmitate and non-radioactive linoleate, released some radioactivity in the fatty acids after hydrolysis. The resulting lysophosphatidate, however, contained much more radioactivity. The ratio of radioactivity in lysophosphatidate to that in released fatty acid varies from 3:1 to 4:1, showing that most of the radioactivity was in the 1-position of glycerol. Similar results were found when ^{14}C-palmitate and acyl CoA generating system was replaced by ^{14}C-palmitoyl CoA (Table 2). When ^{14}C-linoleate was used and the resulting PA was treated with snake venom, more radioactivity was found in the fatty acid than in lysophosphatidate. This proved that linoleate was located predominantly in the 2-position of the PA. In contrast, the distribution of fatty acids in the 1- and 2- position of PA formed from GP under identical condition was found to be more random. This has been previously shown by other workers (8,9).

The above results indicate that PA is rapidly formed from DHAP via the reduction of acyl DHAP. The fatty acid distribution shows a pattern similar to that of natural glycerides and phosphoglycerides, i.e. predominantly saturated fatty acid at the 1-position and unsaturated fatty acid at the 2-position. It cannot be determined at present whether the acyl DHAP pathway accounts for the natural positional specificity. While saturated fatty acids may be preferred for the acylation of DHAP in mitochondria (2) and reduction to lysophosphatidate has also been found (3), the second acylation to form PA is not very specific for unsaturated fatty acyl CoAs (4,5). It does appear that in a combined mitochondrial and microsomal preparation, greater selectivity is found via acyl DHAP than via GP.

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